BIOCHEMICAL MARKERS IN CARDIAC DISEASE

The correct interpretation of biochemical markers in acute coronary syndromes is important.

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This article reviews current biochemical markers in cardiac disease, with the emphasis on markers in acute coronary syndromes (ACS). It also highlights developments in the early detection of cardiac ischaemia, the diagnosis and monitoring of myocardial dysfunction and the future role of candidate gene analysis for cardiac disease or coronary risk factors.

PATHOPHYSIOLOGY OF ACS AND BIOMARKER RELEASE

ACS represent an acute or subacute primary reduction of myocardial oxygen supply, provoked by disruption of atherosclerotic plaque, associated with inflammation, thrombosis, vasoconstriction and microembolisation. This is a progressive process, comprising reversible and irreversible phases, and it takes at least 4 - 6 hours for cellular necrosis to develop. *Reversible* arterial occlusion produces anoxia and myocardial ischaemia, with leakage of ions (e.g. K^+) and metabolites (e.g. lactate). *Irreversible* cell death and tissue necrosis are associated with leakage of intracellular proteins and enzymes.

Acute myocardial infarction (AMI) poses clinical challenges, with a 10 - 15% early mortality. However, inappropriate ICU admission occurs in up to 50% of chest pain patients, and inappropriate early discharge in up to 5% of AMI patients. Early, reliable diagnosis of AMI is needed for prompt initiation of myocardium-sparing treatment, and to assess the risk of recurrent cardiac events. Historical WHO diagnostic criteria for AMI were the presence of 2 of the triad: typical ischaemic chest pain, ECG changes consistent with AMI, and elevated serum cardiac enzymes.

CARDIAC BIOMARKERS

Cardiac biomarkers may be enzymes, other intracellular proteins or metabolites. They are released into the circulation during cardiac injury (ischaemia, inflammation, or necrosis). Smaller, lower molecular weight markers, and those located in the cytosol, are released more rapidly and may be associated with minimal myocardial damage.

Cardiac markers should benefit patient management. High myocardial concentration, rapid release after injury (early diagnosis) and persistence of elevation (later diagnosis) confer **sensitivity**. Absence from non-myocardial tissue and non-detectability in blood of non-diseased subjects determine **specificity**. Markers should be measurable by cost-effective, accessible methods, with rapid turnaround time and acceptable precision and accuracy.

Risk stratification in apparently healthy persons is not done with cardiac markers, but by the measurement and assessment of cardiac risk factors, such as total, LDL and HDL cholesterol, triglycerides, sensitive C-reactive protein (CRP), homocysteine and lipoprotein (a).

BIOCHEMICAL MARKERS IN MYOCARDIAL NECROSIS/ISCHAEMIA

Current biomarkers are CK-MB, cardiac troponins (I or T) and myoglobin. Markers of myocardial ischaemia (reversible phase) are under development. The time course of cardiac markers is important (Fig. 1) (Table I). Specimens drawn too early may result in false negative clinical decisions.

Table I. **Time course of cardiac biomarkers**

Marker	Detection (hours)	Peak (hours)	Return to normal
Myoglobin	1 - 4	6 - 7	24 hours
CK-MB	3 - 12	12 - 18	2 - 3 days
Total CK	4 - 8	12 - 30	3 - 4 days
cTnI	4 - 12	12 - 24	5 - 7 days
cTnT	4 - 12	12 - 48	5 - 15 days

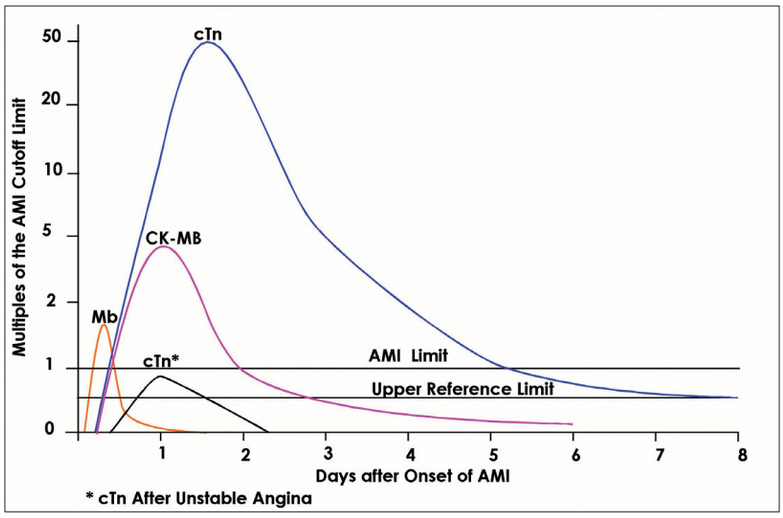

Fig. 1. Appearance of cardiac biomakers in blood v. time from onset of symptoms (modified from Wu et al.*)*

CREATINE KINASE MB FRACTION (CK-MB)

Creatine kinase (CK) is an enzyme associated with striated muscle (skeletal or myocardial). Blood levels vary with age, sex, race and muscle mass. Pathological increases occur in myocardial and skeletal muscle pathology, as well as in hypothyroidism, generalised convulsions, malignant hyperpyrexia, hypothermia and blood-brain barrier compromise.

CK-MB is the most cardiac-specific CK isoenzyme. The proportion of CK-MB to total CK differs for skeletal and cardiac muscle. Cardiac muscle CK-MB usually comprises more than 6% of total CK. However this varies considerably for different skeletal muscles, and relating CK-MB to total CK levels may be unreliable.

CK-MB is a sensitive marker, with rapid rise and fall. It has been the gold standard cardiac marker for more than 2 decades. Blood levels should be measured by more specific concentration (mass) immunoassay methods, rather than activity assays, which are subject to interferences.

Serial measurements detect rising CK-MB levels in an evolving AMI.

MYOGLOBIN (Mb)

Myoglobin is a low molecular weight protein and currently the earliest rising marker of myocardial damage. Serum levels increase rapidly following muscle damage. Skeletal and cardiac muscle myoglobin is similar and it is thus a nonspecific marker for acute muscle damage. It is an excellent negative predictor for myocardial injury. No increase in Mb levels in specimens collected 2 - 4 hours apart virtually excludes AMI.

CARDIAC TROPONINS (cTN)

Striated and cardiac muscle filaments consist of actin, myosin and a troponin regulatory complex comprising three sub-units: troponin C, troponin T (TnT) (MW 37 000), and troponin I (TnI) (MW 24 000). The complex is involved with calcium binding and regulation of muscle contraction.

Immunoassays specific for cTns have high specificity for detection of myocardial injury. Levels are almost undetectable in a normal population. Early release from the cytosolic pool (3 - 4 hours) and prolonged release due to myofilament degradation (5 - 14 days) results in biomarkers which are both sensitive to minor myocardial damage and useful for late AMI detection.

Troponins are consistently shown to be elevated in a group of unstable angina (UA) patients in the absence of classic ECG or CK-MB changes typifying AMI. These patients show increased and sustained risk for subsequent major cardiac events (myocardial infarction or death).

Minor myocardial injury detected by cTns can stratify ACS patients at increased risk for progression to AMI. Modern understanding of the pathophysiology of ischaemic heart disease suggests a continuum, with progression of ischaemia through phases of reversible to irreversible injury. Non-occlusive plaques may produce sufficient ischaemia for release (possibly transient) of low molecular weight cardiac biomarkers.

A joint committee of the European Society of Cardiology (ESC) and the American College of Cardiologists (AACC) released a consensus document in 2000 redefining myocardial infarction. Key recommendations are that:

- AMI is diagnosed when blood levels

of sensitive and specific biomarkers, such as cTns, are increased and ischaemic chest pain is present. The best alternative marker is CK-MB_{mass}.

- Absence of classic ECG changes does not exclude AMI.
- Serial marker determinations are recommended – on admission, and after 6 - 9 and 12 - 24 hours. Ruling in/out AMI should not be based on a single result.
- An evidence-based cut-off equal to the higher of the 99th percentile or the 10% imprecision level for the specific troponin assay used was recommended.
- Diagnostic criteria for an acute/evolving/recent MI were redefined as the concurrence of typical myocardial necrosis-associated rise and fall of troponin or CK-MB **plus** one of: ischaemic chest pain, classic ECG changes or imaging evidence of coronary artery stenosis/obstruction.

About 30% of patients presenting with chest pain without ST-segment elevation or CK-MB elevation, who would historically have been diagnosed as having UA, have non S-T elevation MI (NSTEMI) when assessed with cTn assays. A new subgroup of high-risk, poor-prognosis ACS patients is thus defined (Fig. 2), with diagnostic, therapeutic and social implications.

Troponins classify ACS patients into high-and low-risk UA groups for appropriate acute monitoring and management (conservative or aggressive) and follow-up. Low molecular weight heparin or GPIIb/IIIa inhibitors may benefit NSTEMI patients with cTn elevations.

In older patients without chest pain, but with anginal equivalents (dyspnoea or weakness), troponins may detect ACS.

Alternative myocardial pathology should be considered in patients where cTns are elevated in the absence of clinical ischaemia (Table II).

Troponins do not replace ECGs or clinical investigation. Laboratory results **must** be interpreted within their clinical context.

GUIDELINES FOR THE USE OF CARDIAC MARKERS IN PATIENTS WITH CHEST PAIN (TABLE III)

Troponins are effective early and retrospective biomarkers for MI, particularly in the presence of skeletal muscle pathology caused by, for example, surgery or trauma. Levels correlate with infarct size. They are important in the risk stratification of UA patients.

Mb and/or CK-MB may be useful in patients with recent onset of symptoms (< 6 hours).

Serial levels of TnI and CK-MB may assist in the detection of reinfarction (15 - 20% of AMIs); confirmation of thrombolytic reperfusion and monitoring of ischaemic complications of percutaneous coronary artery intervention (PCI).

Table II. **Non-ischaemic causes of elevated troponins**

Congestive cardiac failure	Mechanical injury (defibrillation)
Hypertension with LVH	Myocardial toxins (e.g. 5-fluorouracil)
Haemodynamic compromise ('shock')	Hypotension (arrhythmias)
Myocarditis and pericarditis	Heart transplantation (rejection)
Cardiac trauma	Critically ill patients (sepsis, diabetes mellitus)
Right ventricular injury in pulmonary embolism	End-stage renal failure – possibly associated with increased cardiac morbidity and mortality
	Hypothyroidism

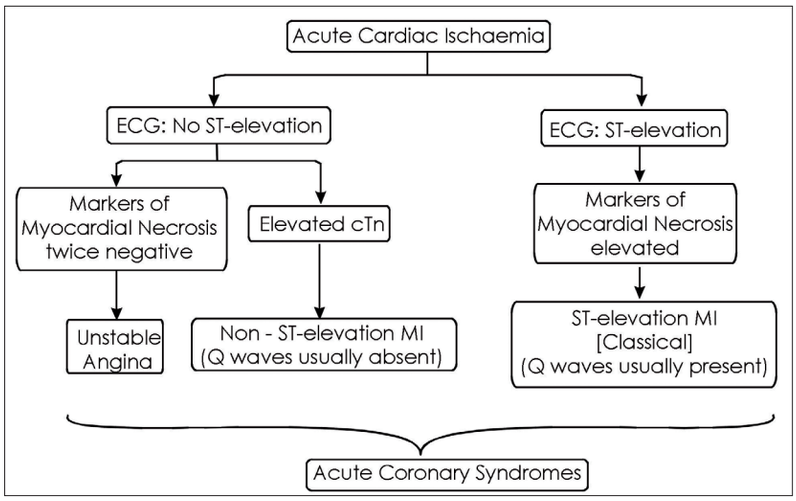

Fig. 2. Role of cardiac biomakers in evaluation of acute coronary syndromes.

The following points are particularly important in the diagnosis of AMI:

- Serial sampling is important for accurate diagnosis. Repeat the markers after 4 - 6 and 9 - 12 hours.
- The use of marker combinations enhances diagnostic sensitivity and specificity. Only cTn is required if chest pain onset is more than 9 hours earlier.
- Do not discharge/dismiss patients on the basis of a single negative/normal result.
- Biomarker results should not delay appropriate intervention in **overt** AMI patients.
- Appropriate laboratory-specific reference ranges should be used.
- Positive results should be interpreted with care if other severe cardiac or

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systemic disease is present. Rising serial biomarker levels may assist in AMI diagnosis.

Laboratory considerations for cardiac biomarkers:

- Instrumentation should allow rapid and reliable measurement of biomarkers.
- Point of care (POC) and laboratory analytical platforms should give comparable results and diagnostic cut-offs.
- Heparin interference in some assays may cause false negative results. Troponin tests should, if possible, be heparinate (plasma)-compatible to improve turnaround time.
- Cut-off values must be selected to accommodate an analytical imprecision of less than 10%.
- Susceptibility of immunoassays (including new-generation cardiac markers) to interferences by heterophile antibodies or rheumatoid factors may produce false positive cTn elevations. Manufacturers must select assay parameters to minimise such interferences. They are, however, unlikely to be eliminated and should be considered if unexpected or disproportionate positive results are obtained.

MARKERS OF MYOCARDIAL ISCHAEMIA

There is currently much interest in the development of biomarkers for the detection of early (reversible) ischaemia. Examples include ischaemia-modified albumin; fatty acid or CD 40 ligand-binding proteins; cytokines (including IL6 and TNF) and markers of fibrinolytic or coagulation function.

These metabolite markers may confirm ischaemia within minutes of onset. Most will be nonspecific and may be used essentially as **rule-out** markers.

Ischaemia-modified albumin (IMA)

Tissue ischaemia alters the N-terminal binding region of circulating albumin. Angioplasty studies have shown that this change occurs within minutes of onset of ischaemia. IMA levels rise rapidly (5 minutes), remain elevated for 2 - 4 hours and return to baseline within 6 hours. Measurement may allow clinical detection of **reversible** myocardial ischaemic damage. IMA is, however, not specific for myocardial ischaemia because other conditions, e.g. stroke, some neoplasms, hepatic cirrhosis and end-stage renal disease may also be associated with elevated IMA levels. Its value will thus be as a negative predictor (rule-out marker). Failure of IMA levels to rise within 4 hours of onset of chest pain will virtually exclude acute myocardial ischaemia.

Commercial assays are under evaluation.

BIOMARKERS OF MYOCARDIAL DYSFUNCTION

Cardiac natriuretic peptides

Atrial (ANP) and brain (BNP) natriuretic peptides and their pro-peptide forms are a family of peptide hormones secreted by the cardiac atria and ventricles, with potent diuretic, natriuretic and vasodilator properties. BNP, originally described in porcine brain, is present in high concentrations in human cardiac ventricular tissue.

Myocyte stretching in failing myocardium stimulates release of these neuro-hormonal factors, which can be measured in blood.

The clinical usefulness of BNP/pro-BNP lies primarily in their sensitivity in detecting left ventricular (LV) dysfunction and, thus, as a screening test in the differential diagnosis of dyspnoea. They are useful as rule-out markers for cardiac failure. Natriuretic peptides have produced a clinical paradigm shift as an adjunct to invasive and non-invasive procedures in the assessment of the presence, severity and management of cardiac

Table III. **Cardiac markers in patients with ischaemic chest pain**

Mb, CK-MB, cTn	Positive	AMI
Mb only	Positive	Early infarction/skeletal muscle injury Repeat markers Mb is a negative predictor
Mb + CK-MB	Positive	Possible early infarction Repeat markers, Rising CK-MB/cTn confirms possible AMI
cTn	Negative	Unstable angina
2 specimens > 6 hrs apart		
cTn CK-MB }	Positive Negative	ACS/non-ischaemic cardiac disease These patients require follow-up!!

Table IV. **Pathological causes of increased natriuretic peptides**

Cardiac disease	
Heart failure	↑↑
AMI (early, first 2 - 3 days)	↑↑
Essential hypertension with LVH	↑
Pulmonary disease	
Acute dyspnoea	↑
Obstructive pulmonary disease	↑
Endocrine and metabolic disorders	
Hyperthyroidism	↑
Hypothyroidism	↓
Cushing's syndrome	↑
Primary aldosteronism	↑
Diabetes mellitus	N-↑
Liver cirrhosis (with ascites)	↑
Renal failure (acute or chronic)	↑↑

failure. They have recently also been promoted as markers to assess acute and residual (on discharge) LV dysfunction in AMI patients. Recent publications suggest applications in the prediction of coronary events in patients with stable coronary artery disease, ACS or type 2 diabetes mellitus. Causes for elevated natriuretic peptide elevation are shown in Table IV.

Use of BNPs as rule-in markers requires refinement of reference ranges in terms of age, gender, physiological and pharmacological factors.

Pro-BNP levels below 300 pg/ml exclude left ventricular failure in patients with acute dyspnoea. Levels greater than 450, 900 or 1 800 pg/ml in patients < 50, 50 - 75 or > 75 years of age, respectively, suggest the presence of acute congestive cardiac failure.

Standardisation of commercial natriuretic peptide immunoassays will improve analytical performance and clinical reliability. Reliable POC procedures will also increase the clinical application of these relatively expensive markers.

GENETIC ANALYSIS FOR CANDIDATE GENES IN CARDIOVASCULAR DISEASE/RISK

Micro-array technology in genetic analysis will, within the decade, allow comprehensive screening for hereditary risk factors for cardiac disease. Single gene defects may be responsible for hereditary metabolic diseases such as familial hypercholesterolaemia and some cardiomyopathies, or inherited cardiac arrhythmias. Common cardiovascular diseases are polygenic, with multiple susceptibility loci interacting with lifestyle and environment. Responsible use of genetic screening for cardiac disease/risk factors will allow proactive lifestyle modification.

Further reading

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IN A NUTSHELL

Cardiovascular disease represents a continuum from UA to AMI. This has required a redefinition of clinical criteria for myocardial infarction.

Cardiac troponins play a pivotal role in the diagnosis of AMI and the risk stratification of ACS patients.

Elevated troponin levels may identify patients without ECG changes/CK-MB elevation to be at risk for significant cardiac events.

Serial sampling is important for accurate diagnosis.

'Cardiac enzymes' (CK, AST, LDH) are obsolete.

Other causes of cardiac pathology should be considered where troponin levels are elevated in the absence of clinical ischaemia.

The increased role of cardiac markers in the diagnosis of AMI/ACS requires reliable biomarkers with rapid turnaround time. Clinically and analytically appropriate cut-off values must be used.

Biomarker studies should not delay appropriate intervention in overt AMI.

The advent of markers for myocardial ischaemia will facilitate earlier exclusion of AMI.

There is an evolving role for the laboratory in the evaluation of cardiac disease, particularly in the areas of cardiac dysfunction (BNP, pro-BNP) and general biochemical or genetic risk factors.