Biochemical markers of myocyte injury in heart failure

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This review discusses the role of biochemical markers of myocyte injury in patients with chronic congestive heart failure. Heart specific assays have been developed for the measurement of cardiac troponin T (cTnT), cardiac troponin I (cTnI), heart type fatty acid binding protein (H-FABP), and myosin light chain 1 (MLC-1). Concentrations of these biochemical markers increase in the absence of ischaemic events in the subset of patients with heart failure whose long term outcomes are most adverse. The markers are easy to measure serially and it is therefore easy to follow patients without inter-observer variability. The serial clinical use of these markers, separately or in combination, will sharpen our understanding of the state of heart failure.

Chronic congestive heart failure is associated with a dismal long term prognosis and remains a major worldwide health concern. While various management strategies have become available, clinical tools to stage chronic heart failure remain few. New York Heart Association (NYHA) functional classification, along with several tests, including chest x ray, echocardiogram, myocardial scintigraphy, cardiopulmonary exercise, and haemodynamic measurements are useful to estimate the degree of heart failure, though are subject to inter-observer variations in interpretation.1 2

The loss of cardiac function in patients with chronic heart failure may be caused by ventricular remodelling, a process by which ventricular size, shape, and function are altered by mechanical, neurohormonal, and genetic factors. Ventricular remodelling consists of myocyte hypertrophy and slippage, loss of myocytes, decrease in myofibril content, and myocardial interstitial fibrosis.3 4 Serial measurements of reliable biochemical markers of myocyte injury in patients with heart failure would be helpful to monitor their long term progress, without inter-observer variability. This review discusses our understanding, and the significance, of current biochemical markers detected in patients with chronic heart failure.

BIOCHEMICAL MARKERS OF MYOCYTE INJURY

Cardiac enzymes have long been used as frontline diagnostic tools in the detection of myocardial injury caused by myocardial ischaemia.5 However, the most commonly used enzymes, including creatine kinase (CK) and its myocardial fraction CK myocardial band (MB), aspartate aminotransferase, and lactate dehydrogenase, are limited in their ability to detect myocardial injury by short diagnostic windows, limited sensitivities, and lack of specificity because of their presence in skeletal muscle. Furthermore, studies performed as long as two decades ago found no correlation between serum concentrations of CK or CK-MB and heart failure.6 Myoglobin, an 18 kD cytosolic protein, also lacks specificity because its release from skeletal muscle cannot be distinguished from its release from heart muscle.7

These limitations, as well as the known unique amino acid sequence of myofibrillar cardiac proteins, prompted the development of monoclonal antibodies for the detection of cardiac troponins by immunoassay. The subunits I, T, and C of the troponin complex on the actin filament regulate the force and velocity of muscle contraction. The 37 kD T subunit is responsible for binding the troponin complex to tropomyosin. The first generation of cTnT assays were flawed by spuriously increased values in patients with severe skeletal muscle or renal disorders,8 9 perhaps from cross-reactivity of the cTnT assay with skeletal muscle troponin T, or the expression of cTnT by skeletal muscles during regenerative processes, particularly in patients with neuromuscular disorders and nephropathies. However, the latest cTnT assay is a sensitive and specific marker of myocyte injury, even in the presence of these disorders.10 11 cTnI (21 kD) prevents contraction in the absence of calcium by inhibiting the adenose triphosphatase activity of the actin–myosin interaction. cTnI is highly cardiac specific because of the dissimilarity of a 31 amino acids sequence on the N-terminus compared to that of skeletal troponins; it is also absent during human skeletal muscle regeneration.12 cTnT and cTnI are highly sensitive and specific markers of myocardial injury in acute coronary syndromes. In addition, increased serum concentrations of these markers have been associated with adverse short and long term outcomes in patients with unstable angina or acute myocardial infarction.13 14 Moreover, the background concentration of cTnT and cTnI is very low while the background level of CK, aspartate aminotransferase, and lactate dehydrogenase is significant even in normal conditions. cTnT and cTnI

Abbreviations: BNP, brain natriuretic peptide; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CK, creatine kinase; DCM, dilated cardiomyopathy; H-FABP, heart type fatty acid binding protein; MB, myocardial band; MLC-1, myosin light chain 1; NYHA, New York Heart Association
therefore, have replaced CK-MB as the standard marker in acute coronary syndromes, and a new definition of acute myocardial infarction has been developed, based on increases in cardiac troponins in the blood.\textsuperscript{15} \textsuperscript{16}

The mechanisms of release and clearance of cTnT and cTnI are incompletely understood. Although both are structural proteins, it has been suggested that cytosolic pools of these proteins are released into the circulation after cell injury. The cytosolic pool for cTnT was estimated at 6--8\%,\textsuperscript{11} and that for soluble cTnI at 2.8\%.\textsuperscript{14} The release of cTnT in ischaemic myocardial injury may be because of transient leakage from the cytosolic component from loss of sarcolemmal integrity during reversible ischaemia,\textsuperscript{17} or from its continuous release when ischaemic injury is irreversible.\textsuperscript{18}

Other biochemical markers of myocyte injury have also been described. H-FABP, a 15 kDa cytoplasmic protein involved in lipid homeostasis, is abundant in heart muscle.\textsuperscript{19} \textsuperscript{20} It has recently been reported to detect early myocyte injury in patients with acute myocardial infarction.\textsuperscript{22} Myosin is a structural protein of the sarcosome; a heart specific assay using monoclonal antibodies against MLC-1 has been previously described in patients with acute myocardial infarction.\textsuperscript{23}

**Biochemical markers of myocyte injury in patients with heart failure**

cTnT

Congestive heart failure is a clinical syndrome which may develop from a variety of diseases. Dilated cardiomyopathy (DCM) is a primary myocardial disorder of unknown aetiology characterised by ventricular dilatation and depressed myocardial contractility, which leads to chronic heart failure without apparent myocardial ischaemia. While ongoing myocyte injury has been documented by \textsuperscript{131}Indium anti-myosin antibody imaging in patients with DCM,\textsuperscript{23} this technique requires radioisotopes and cannot be used to follow patients serially in the long term.

In an earlier study, persistently high serum concentrations of cTnT were observed over several years of follow up in approximately 30\% of our DCM patients.\textsuperscript{24} \textsuperscript{25} These patients had a significantly greater decrease in left ventricular ejection fraction and higher rates of long term adverse outcomes than patients without increased cTnT concentrations. It is particularly noteworthy that, in most patients, cTnT concentrations remained elevated after the patients were stabilised clinically by conventional therapy, free of dyspnoea, and without radiographic and auscultatory signs of pulmonary congestion. These observations indicate that cTnT is a marker of subclinical myocyte injury even when heart failure is compensated. In that study of patients with DCM, we chose 0.02 ng/ml as the upper normal limit of serum concentration, a relatively low value compared with patients with ischaemic heart disease.

Cardiomyopathic disorders are associated with predominantly systolic or diastolic dysfunction, or with both. In hypertrophic cardiomyopathy, which is initially associated with predominant diastolic dysfunction,\textsuperscript{20} we recently reported increased concentrations of cTnT in 50\% of patients during the non-dilated phase of the disease, when systolic function was preserved, and in the absence of ischaemia.\textsuperscript{26} Some patients had increased cTnT concentrations persisting over several years of follow up, during which fractional shortening and intraventricular septum thickness decreased significantly. These observations indicate that cTnT is a marker of myocyte injury in patients with hypertrophic cardiomyopathy. In a univariate analysis, Dispenzieri and colleagues found cTnT, cTnI, septal thickness, left ventricular ejection fraction, urine M spike, age, and symptoms of congestive heart failure to be significant predictors of overall survival in patients with cardiac amyloidosis, while in multivariate analysis, the detection of cTnT was the most reliable predictor.\textsuperscript{27}

Though we initially studied the significance of cTnT as a marker of myocyte injury in patients with heart failure and non-ischaemic disorders, ischaemic heart disease remains the predominant cause of chronic heart failure. We and others have reported the presence of increased cTnT concentrations in patients with heart failure, old myocardial infarctions, and without ongoing ischaemic events.\textsuperscript{28} \textsuperscript{29} Ventricular remodelling after myocardial infarction may occur over several weeks or months, while other factors may also contribute to the progression of left ventricular dysfunction, including current ischaemia with myocardial stunning, hibernation of the myocardium caused by a sustained reduction in myocardial blood flow, and the vascular and myocardial effects of endothelial dysfunction.\textsuperscript{3}

While more studies of cTnT during cardiac remodelling after myocardial infarction should further promote its acceptance as a monitoring tool in patients with heart failure, the interpretation of results in patients with ischaemic heart disease is not without ambiguity. Since patients with heart failure after a healed myocardial infarction may have asymptomatic stenoses of one or more, large or small, coronary arteries, increased concentrations of cTnT in patients with heart failure may be a manifestation of ischaemic myocardium in the territory of the stenotic artery. Therefore, patients with chronic heart failure after an old myocardial infarction may have to undergo serial coronary angiograms to clarify the mechanism of cTnT release.\textsuperscript{21}

We have collected preliminary data in patients with old myocardial infarctions who have undergone coronary revascularisation, and had persistently elevated cTnT and progression of heart failure, in the absence of increased CK or ischaemic events (unpublished data). Although cTnT is usually elevated in patients with unstable angina or acute coronary syndrome,\textsuperscript{22} \textsuperscript{23} the persistently high concentrations, measured for several months or years after myocardial infarction, in our patients with chronic heart failure could not be attributed to unstable angina. Further studies are needed to clarify the significance of persistently elevated cTnT in the process of chronic ventricular remodelling after myocardial infarction.

Valvar and congenital heart diseases are other major causes of chronic heart failure, which may progress despite successful surgical interventions.\textsuperscript{30} \textsuperscript{31} We and others have found that some patients with valvar and congenital diseases also have elevated cTnT concentrations in the absence of cardiac ischaemia.\textsuperscript{29} \textsuperscript{30} \textsuperscript{31} Our preliminary observations of persistently high cTnT concentrations in some patients after surgical repair may be useful for the post-operative monitoring of chronic myocyte injury (unpublished data).

cTnI

The first description of a biochemical marker of myocyte injury in patients with heart failure was offered in 1995 by Missov and colleagues, who reported increased cTnI concentrations in patients with NYHA class III and IV heart failure caused by DCM, or secondary to ischaemic disease.\textsuperscript{32} Cardinale and associates reported that the elevation of cTnI in patients treated with high doses of chemotherapeutic agents for aggressive malignancies predicted the subsequent evolution of left ventricular function.\textsuperscript{33} They concluded that cTnI is a sensitive and reliable marker of myocardial injury caused by high dose chemotherapy. Schulz and colleagues reported an exercise induced increase in cTnI concentrations in patients with heart failure, though the prognostic value of this finding should be further investigated.\textsuperscript{34}
A recent study evaluated 238 advanced heart failure patients referred for cardiac transplantation evaluation who had a cTnT assay drawn at the time of initial presentation. Patients with acute myocardial infarction were excluded. Detectable cTn was associated with subsequent cardiac events in patients with chronic heart failure caused by DCM, old myocardial infarction, hypertensive heart disease, valvar heart disease, or congenital heart disease. While both cTnT and H-FABP were associated with subsequent cardiac deaths or rehospitalisation for the management of worsening heart failure, H-FABP was much more detectable among patients in NYHA functional class II. H-FABP is a small protein abundant in the cytosol which is readily released into the circulation following myocardial damage. In contrast, most troponins are components of the myofibrillar contractile apparatus, present in small amounts in the cytosol. This may explain the different patterns of increase of these two markers following myocyte injury.

**H-FABP**

Recently, Setsuta and colleagues, who previously reported the elevation of cTnT in patients with heart failure, reported that H-FABP was associated with subsequent cardiac events in patients with chronic heart failure caused by DCM, old myocardial infarction, hypertensive heart disease, valvar heart disease, or congenital heart disease. While both cTnT and H-FABP were associated with subsequent cardiac deaths or rehospitalisation for the management of worsening heart failure, H-FABP was much more detectable among patients in NYHA functional class II. H-FABP is a small protein abundant in the cytosol which is readily released into the circulation following myocardial damage. In contrast, most troponins are components of the myofibrillar contractile apparatus, present in small amounts in the cytosol. This may explain the different patterns of increase of these two markers following myocyte injury.

**MLC-1**

Studies of MLC-1, a 27 kD protein, as a biochemical marker of myocyte injury in patients with heart failure are few. Hansen and colleagues reported that circulating MLC-1 was elevated in some patients in NYHA functional class III and IV, and this increase was associated with a poor prognosis in a clinical trial of flosequinan. Studies are needed to further characterise this marker in patients with heart failure and to distinguish it from the other biochemical markers of myocyte injury described earlier.

**USE OF BIOCHEMICAL MARKERS TO MEASURE THE DEGREE OF HEART FAILURE**

Since heart failure is a complex clinical syndrome, a single biomarker may not reflect all of its characteristics. The serial and combined measurements of biochemical markers of myocyte injury may open new perspectives in heart failure. Brain natriuretic peptide (BNP) is an amino acid peptide chiefly secreted by the ventricular myocardium in response to strain. The plasma measurement of BNP is being used increasingly in the diagnosis, prognosis, and monitoring of patients with congestive heart failure. BNP may be viewed as a marker of myocardial load and cTnT as a marker of myocyte injury. Combining these biochemical markers may provide new insight in the management of heart failure. In our small study of patients presenting with decompensated heart failure, approximately one third had initial concentrations of cTnT within normal limits. While BNP decreased significantly after treatment in all patients, cTnT remained elevated in some patients in NYHA functional class II. H-FABP was much more detectable among patients in NYHA functional class II. H-FABP is a small protein abundant in the cytosol which is readily released into the circulation following myocardial damage. In contrast, most troponins are components of the myofibrillar contractile apparatus, present in small amounts in the cytosol. This may explain the different patterns of increase of these two markers following myocyte injury.

At this time, the relative contributions of cTnT, cTnI, H-FABP, and MLC-1 in patients with heart failure remain unclear. The different half-lives, molecular sizes, and intracellular distributions of these markers may provide detailed information regarding the process of myocyte injury by monitoring the markers in combination. Combinations of markers of myocyte injury and markers of interstitial matrix collagen turnover may also add new information on the process of cardiac remodelling in patients with chronic heart failure.

**FUTURE APPLICATIONS OF BIOCHEMICAL MARKERS IN HEART FAILURE**

**Mechanisms of myocyte injury and biochemical markers**

Although these biochemical markers indicate the presence of ongoing myocyte injury in patients with heart failure, the mechanisms of that injury remain unclear. In our study of DCM, the presence of active myocarditis was excluded by endomyocardial biopsies using the Dallas criteria. Furthermore, transverse sections of postmortem cardiac specimens from three patients with DCM with persistently elevated cTnT showed no significant mononuclear cellular infiltration (unpublished data). The mechanism of myocyte injury without cellular infiltration needs to be studied. Adrenergic stimulation, calcium handling abnormalities, the renin–angiotensin system, endothelin, inflammatory cytokines, nitric oxide, oxidative stress, and mechanical stress have been explored as potential contributors to myocyte injury in the setting of heart failure. The existence of correlations among these factors with biochemical markers of myocyte injury should be examined in clinical studies to provide important information applicable to the management of heart failure.

**Biochemical markers as surrogate end points in heart failure**

Since heart failure is a life threatening condition, survival was chosen as the primary end point in the clinical trials which proved the effectiveness of angiotensin–converting enzyme inhibitors, aldosterone antagonists, and β adrenergic blockers. However, large study populations and long study periods are usually required to show a significant effect of treatment on survival. Therefore, the interest in surrogate end points has recently increased, since their use may allow the successful completion of controlled clinical trials with smaller patient populations, within shorter observation periods. Combinations of certain biochemical markers described earlier may represent surrogate endpoints suitable for the design of such trials.

**CONCLUSIONS**

No guidelines have been issued regarding the monitoring of biochemical markers of myocyte injury as part of the management of chronic heart failure. Recent technological advances will allow the rapid application of these assays in the near future. The real time detection of myocyte injury will render the management of heart failure more precise and effective. It is our expectation that these assays will become the new standards in the monitoring of patients with heart failure.
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