Troponin T: a diagnostic marker for myocardial infarction and minor cardiac cell damage


University Hospital, Department of Internal Medicine III, Heidelberg, Germany

The diagnosis of acute myocardial infarction is straightforward when anginal pain is accompanied by typical ECG changes and in these patients measurements of cardiac markers are unnecessary in deciding whether thrombolytic therapy is appropriate. Cardiac markers in patients with acute ischaemic coronary syndromes, however, may serve to identify a high risk subgroup of patients with small acute infarctions or minor myocardial damage. In many patients with chest pain a valid diagnosis of myocardial cell injury depends on the result of biochemical assays. In 30% of patients with unstable angina, troponin T is elevated although myocardial infarction was ruled out by cardiac enzymes and ECG recordings. The outcome of these patients at 4 weeks and 6 months follow-up is not different from that of patients with definite myocardial infarction. To guide therapeutic decisions on these patients a troponin T test result needs to be available rapidly.

The rapid troponin T test strip assay, which allows the determination of troponin T levels in whole blood at the patient's bedside, can be performed conveniently in the emergency room or in laboratories with less sophisticated equipment and has the potential to aid in the triage of chest pain patients and the selection of therapeutic strategies.

Key Words: Troponin T, acute myocardial infarction, minor cell damage, unstable angina.

When the WHO criteria are used to classify patients with suspected myocardial infarction, only 50% of all chest pain sufferers admitted to coronary care units are finally confirmed as having the condition. In the emergency room the prevalence of acute myocardial infarction (AMI) in chest pain patients is even lower, ranging between 10 and 20%. In a 1992 survey on final diagnoses of 6 million patients with chest pain seen in U.S.A. emergency rooms, AMI was confirmed in 17% of all patients. A similar number of patients (1-08 millions, 18%) were classified as suffering from unstable angina. The most frequent diagnosis (65%), however, was non-cardiac chest pain, with AMI ruled out by cardiac enzyme measurements and ECG recordings. This highlights the diagnostic problem of myocardial necrosis in patients with chest pain.

Role of cardiac enzyme markers in clinical decision making

Massive AMI

In patients with massive AMI, thrombolytic treatment can be selected without recourse to biochemical assays.

Correspondence: Hugo A. Katus, MD, Medizinische Klinik II, University of Lübeck, Ratzeburger Allee A60 23538 Lübeck, Germany.

Yusuf and co-workers reported on confirmation of AMI by biochemical methods in 99.7% of all chest pain patients with significant ST-segment elevations in the limb or precordial leads. In the Multicentre Investigation for Limitation of Infarct Size (MILIS) trial, the corresponding proportion was 91-100% in patients with chest pain, ST-segment elevation and depression in corresponding leads.

While ST-segment elevations on the ECG develop within minutes, cardiac enzymes take longer to become elevated in blood. This is also the case for the so-called early markers, e.g. subforms of creatine kinase MB, which have been found to be elevated in some 60% of patients with AMI 4 h after onset of symptoms. Thus measurements of cardiac enzymes in the blood are not needed to decide whether thrombolytic therapy is appropriate in patients with ECG findings of massive AMI.

Attempts to recanalize an occluded coronary artery by thrombolytic treatment fail in 10-20% of all AMI patients treated, who may benefit from rescue percutaneous transluminal coronary angioplasty, provided they are properly identified beforehand. Cardiac enzyme markers may be useful for the non-invasive assessment of reperfusion due to the fact that their elevation depends on the time delay from onset of ischaemia to success of reperfusion therapy. Analyses of
cardiac enzyme markers showed that they predicted successful reperfusion therapy with an accuracy of 80–90%[8,9]. Thus it was hoped that laboratory-based assays would facilitate selection of patients for rescue percutaneous transluminal coronary angioplasty. However, due to the time delay from venipuncture to report of a test result and the high risk associated with failed rescue percutaneous transluminal coronary angioplasty the non-invasive monitoring of successful reperfusion therapy by biochemical assays has not gained widespread application[10].

Non-Q wave infarction and unstable angina

The improved survival of patients with massive AMI and early success of reperfusion therapy results from more effective salvage of jeopardized myocardium. In patients with less extensive areas of myocardial ischaemia, such as in patients with non-Q wave AMI, immediate thrombolytic treatment did not improve survival. In patients with unstable angina, thrombolytic treatment was either not beneficial or resulted in an increased rate of death or a more frequent progression to AMI[11–14]. For these reasons immediate thrombolytic therapy is not required in patients with acute ischaemic syndromes other than AMI characterized by ST-segment elevations.

Cardiac enzyme markers in patients with acute ischaemic syndromes, however, may serve to identify a high risk subgroup of patients with small AMI or minor myocardial damage. Several trials have shown that the survival rate of patients with small non-Q wave AMI or more massive Q-wave AMI is essentially the same at 6, 12, and 24 months of follow-up[15–18]. The recent Thrombolysis in Myocardial Infarction (Phase IIIIB) analysis reports a similar mortality rate for patients with smaller AMIs based on cardiac enzymes (non-Q wave) and more extensive AMIs based on ECG (Q wave) at 3 and 6 weeks of follow-up[19].

Patients with unstable angina also represent a high risk subgroup. There is progression to AMI within 6 months in 45% of all patients with crescendo type angina, in 62% of patients with acute rest angina, and in 25% of patients with subacute angina at rest[20]. In a recent review of patients with unstable angina, the rate of progression to AMI and cardiac death varied from 4%–25% and 2%–18%, respectively[21].

Characteristics of the marker molecule troponin T

Cardiac troponin T is a myofibrillar protein, only expressed in myocardial cells, for which highly specific assays have been developed that allow precise differentiation of cardiac and skeletal muscle damage. However, the high specificity of troponin T measurements was not convincingly demonstrated using the first generation of the troponin T ELISA, due to the use of a cross-reactive monoclonal antibody as label in this test kit[22]. In the second generation troponin T enzyme immunoassay, two cardiospecific monoclonal antibodies are combined, yielding less than 0.5% cross-reactivity with skeletal muscle troponin T[23]. With this assay, cardiac troponin T was not elevated in blood of patients with rhabdomyolysis or healthy persons after a 3 day marathon event, despite creatine kinase elevations in the blood of more than 300 fold the upper limit of normal. Figure 1 shows an example of a patient with severe skeletal muscle injury following ethanol intoxication, in whom creatine kinase activities in blood were 35 000 IU·l−1 (upper limit of normal creatine kinase activity 200 IU·l−1). In this patient, the absence of troponin T in the circulation indicates that creatine kinase originate exclusively from skeletal muscle injury.

The measurement of cardiac troponin T also allows a more sensitive diagnosis for myocardial cell necrosis than cardiac enzyme measurements. This is, among other factors, due to the specific intracellular compartmentation and release kinetics of troponin T. Troponin T is present in the myocyte in high concentrations both in a cytosolic and structurally bound protein pool. The cytosolic pool, which may serve as a precursor pool for myofibrillar assembly, amounts to 6% of the total troponin T mass in the cardiomyocyte[24]. This mass corresponds to the total mass of cardiac creatine kinase MB. Most cardiac troponin T (94%), however, is bound in the myofibrils. The release of this pool depends on disintegration of the contractile apparatus during irreversible cell damage, which is the consequence of intracellular acidosis and activation of proteolytic enzymes, a process which continues during infarct evolution. As a consequence, troponin T may be liberated from the infarcting myocardium, for more than 2 weeks[25].
Thus, in spite of a short serum half life of only 2-3 h, troponin T levels are elevated for at least 140 h after onset of symptoms in all patients with AMI.

The sensitivity of a diagnostic assay depends not only on the diagnostic window (duration of elevation) but also on the relative increase of the marker in circulation above its upper limit of normal (signal to noise ratio). In contrast to cardiac enzymes, normal levels of cardiac troponin T have not been detectable in healthy individuals. Thus, the inter-individual variability of normal serum levels which limits the sensitivity of cardiac enzymes does not affect the sensitivity of troponin T measurements.

For these reasons, cardiac troponin T in the circulation is a highly sensitive marker for myocardial cell damage. Figure 2 shows the relative increase of troponin T in comparison to creatine kinase activity in a patient with reperfused myocardial infarction. The increase of troponin T in the circulation is seven times higher than that of creatine kinase MB mass and remains elevated five times longer than creatine kinase MB. The serum concentrations of troponin T are biphasic, revealing a rapid wash out of the cytosolic troponin T pool on day 1 and persistent elevation in the circulation resulting from ongoing degradation of the myofibrils. In patients with persistent occlusion of the infarct-related artery there is no evidence of a troponin T peak on day 1 resulting from wash out of cytosolic troponin T. The differences in kinetics of troponin T release on day 1 can thus be analysed to non-invasively assess the success of reperfusion therapy.

The role of troponin T in patients with acute ischaemic syndromes

Since the first publication in 1991, more than 4000 patients with acute ischaemic syndromes have been analysed for release of troponin T in 12 different trials (Fig. 3). In these trials the rate of troponin T elevations in patients finally classified as having unstable angina varied from 19-64% (mean 33%). Thus, despite non-significant changes of cardiac enzymes in blood, release of cardiac troponin T could be shown in one third of all unstable angina patients.

The increased risk associated with the appearance of cardiac troponin T in the circulation, but no other evidence for AMI according to WHO criteria (minor myocardial damage), was first shown in 1991. In 388 patients with chest pain and suspected AMI, 79 patients were classified as having angina at rest based on WHO criteria. In five of the six patients who progressed to AMI during their stay in hospital, five had elevated troponin T levels at least 12 h before the diagnosis of AMI could be established by ECG or cardiac enzymes. A negative troponin T result was highly predictive of an
uneventful course (negative predictive value = 0.98). These findings were confirmed in the European Multicenter Trial on 112 patients with unstable angina.\(^{28}\) The cardiac event rate (AMI, death) was 30% in the 39% of patients with an elevated troponin T result, but only 2% in the 61% of patients without elevated troponin T. In this trial, creatine kinase MB mass was not predictive for cardiac events. Similar results were reported in many smaller trials.

The clinical significance of an elevated troponin T value on admission was prospectively studied in the multicentre Global Utilization of Streptokinase for Occluded Coronary Arteries (GUSTO II) troponin T substudy on 865 patients.\(^{30}\) The patients were classified on admission according to ST segment changes (ST elevation, n = 531; no ST elevation, n = 334). Creatine kinase MB mass was elevated (>7 µg·L\(^{-1}\)) in 33% of the patients and troponin T was elevated in 36% of patients without ST elevation. In the patients without ST segment elevation and a normal troponin T value, the rates of death, shock, and congestive heart failure were 1%, 2%, and 7%, respectively, but were 6%, 9%, and 16% in the troponin T-positive patients. In patients with ST elevations, the rates of death, shock, and congestive heart failure were 5%, 3%, and 10%, in the troponin-T negative patients, but were 12%, 9%, and 15%, in patients with an elevated troponin T value on admission. Troponin T elevations were strongly associated with mortality (relative risk = 17; \(P<0.0001\)). In a stepwise regression model, creatine kinase MB mass added no information after troponin T levels were included.

The Scandinavian Multicenter Trial\(^{39}\) and the Fragmin for Risk in Acute Ischemic Syndromes Trial\(^{29}\) tested the prognostic implications of an elevated troponin T result on outcome in patients with suspected AMI at 5 and 6 months follow-up. In the Scandinavian Multicenter Trial, 298 patients with suspected AMI were enrolled. These were classified on WHO criteria as having definite AMI (n = 155), ischemic heart disease but no evidence for AMI (n = 127), and no ischemic heart disease in 16 patients. The rates of death or AMI after 6 months were 14% in patients with definite AMI, 7% in patients with ischemic heart disease, and 0% in patients without ischemic heart disease. When the patients with ischemic heart disease but no AMI were reclassified according to their troponin T result, the troponin T-positive subgroup had a cardiac event rate of 13% after 6 months follow-up which was similar to the 14% found in patients with definite AMI. In contrast, patients with ischemic heart disease but a negative troponin T result had a cardiac event rate of 3-6%.

The FRISC\(^{40}\) trial enrolled 968 patients. The patients were classified in quintiles of their troponin T levels. The 5 months mortality rate increased from 0.8 to 2.6%, 3.9%, and 8.4% with increasing troponin T levels, as did the combined event rate (4.4%, 11.4%, 14.1%, 17.7%). There was no specific discriminator value of troponin T associated with an increased risk but a parallel increase of risk with increasing troponin T levels.

In 384 patients with chest pain followed for a median time of 3-7 years, the mortality rate of patients with elevated troponin T at index admission was significantly higher at 3 years follow-up, as compared to patients with a normal troponin T result.\(^{33}\)

These studies provide firm evidence that the elevation of cardiac troponin T indicates the presence of minor myocardial cell damage in patients with chest pain, and that minor myocardial damage is associated with a high risk for subsequent progression to AMI and death. Various cardiac markers, including creatine kinase MB mass, do not add any further prognostic significance to troponin T measurements. As the WHO criteria are not suitable to detect these patients, a new diagnostic classification is needed, to take into account the clinical importance of minor myocardial damage in patients with chest pain.

The ideal treatment strategy for patients with chest pain and elevated troponin T but no evidence of AMI according to WHO criteria remains to be defined. As it has now become impossible to identify these patients by troponin T measurements, randomized trials of different treatment modalities, for example emergency percutaneous transluminal coronary angioplasty, new thrombin antagonists or antiplatelet agents, can be designed. It is mandatory that in such trials the physicians have rapid and immediate access to troponin T measurements, which are facilitated by the availability of a rapid troponin T test strip assay, which allows the determinations of troponin T levels in whole blood at the patient's bedside.

The bedside assay for troponin T

The rapid, whole blood assay T utilizes two anti-troponin T antibodies (Fig. 4).\(^{40}\) One cardiospecific antibody is labelled with gold particles. The second, high-affinity capture antibody, is labelled with biotin. Both antibodies, the buffer substances and detergents are absorbed onto a paper fleece, which is then mounted below the application well of the assay device. Heparinized blood (160 µl) applied to the well of the test device solubilizes the reagents. Troponin T in blood then reacts with the gold and biotin-labelled antibodies. The immune complexes formed are then concentrated in a line by the interaction of biotin-labelled capture antibodies with streptavidine chemically linked to the nitrocellulose membrane and become visible as a violet line due to the accumulation of the gold-labelled antibodies. Unreacted gold-labelled antibodies may bind to purified cardiac troponin T linked in a second line to the cellulose acetate strip. The appearance of this second line indicates proper function of the reagents and unimpeded flow of plasma in the test device. Blood cells are separated from plasma during the diffusion process by a glass fibre fleece.

The detection limits of this device is 0.2 µg·L\(^{-1}\). The higher the troponin T concentrations are, the more intense and rapid is the development of the violet line.
Diagnostic utility of troponin T

Blood 150 μl

Retention of blood cells
Immunoreaction
Immobilization
cTnT positive control

Figure 4 Principle of rapid bedside assay of cardiac troponin T.

giving a positive result within 5 min if troponin T concentrations exceed 2 μg·L⁻¹. The cross-reactivity of this device is 1%. However, with an improved version using two cardiospecific antibodies the cross-reactivity is less than 0.1% giving no false-positive results even in patients with severe skeletal muscle injury.

In a recent multicentre evaluation the diagnostic performance of the whole blood troponin T assay, the troponin T ELISA (discriminator value 0.1 μg·L⁻¹), and the creatine kinase MB mass assay (discriminator value 10 μg·L⁻¹) were compared in patients with suspected acute and subacute myocardial infarction (485 patients, 773 samples). The sensitivities of the three methods were 85%, 89%, and 67% and the specificities were 92%, 87%, and 96%, respectively. In patients with unstable angina and minor myocardial damage according to troponin T ELISA result >0.1 μg (158 patients, 266 samples) the whole blood assay was also positive in 62% whereas creatine kinase MB was elevated in only 27%.

These data indicate that the rapid assay is a useful tool to confirm acute or subacute AMI. It is at least as useful as creatine kinase MB mass in patients with suspected AMI but is superior to creatine kinase MB mass in patients with unstable angina. The rapid assay can be performed conveniently in the emergency room or in laboratories with less sophisticated equipment and holds the potential to aid in the triage of chest pain patients and the selection of therapeutic strategies.

References


