Cardiac markers used in the detection of myocardial injury

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ABSTRACT

Objective: Activities of total creatine kinase and its isoform creatine kinase are usually significantly elevated in patients with myocardial or skeletal muscle injury as well as in those with renal failure. The purpose of this study was to compare findings for creatine kinase mass, cardiac troponin T and cardiac troponin I with those of creatine kinase and creatine kinase MB activity.

Methods: Blood samples from 118 patients were studied. Fifty eight patients had significantly elevated creatine kinase activity (39 with and 19 without clinically proven myocardial injury or infarction) and 60 were normal controls. The sensitivity, specificity, positive and negative predictive values were calculated for all markers.

Results: Cardiac troponins had 100% sensitivity and negative predictive value, for myocardial injury, as compared with 92% and 96% for creatine kinase activity and 96% and 97% for creatine kinase-mass. Cardiac TnI had the highest specificty and positive predictive value (99% and 98%) as compared with cardiac troponin T (96% and 93%), creatine kinase-mass (92% and 86%) and creatine kinase activity (89% and 80%).

Conclusion: Cardiac troponins, especially cardiac troponin T, have very high sensitivity, specificity and predictive value for myocardial injury.

Keywords: Cardiac markers, cardiac troponins, creatine kinase, myocardial injury.

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Accurate detection of myocardial injury is crucial for the proper management of individuals in diverse clinical situations. Determination of the total activity of creatine kinase (CK) and its isoform CKMB have proven useful in most situations in the accurate diagnosis of cardiac injury. However, these activities can be increased in patients with skeletal muscle injury or renal failure.[1,2]

CKMB is produced in healthy skeletal muscles to a variable degree, up to 3% of total CK.[3] The proportion of B-subunit formed in skeletal muscle cells, and therefore the amount of CKMB present, increases after skeletal muscle insult. Therefore, after any skeletal muscle insult, the serum may show raised activities of CK and CKMB, complicating the accurate diagnosis of myocardial injury by routine means. CK and CKMB activities also rise significantly in 5% of patients with chronic renal failure, probably as a consequence of skeletal myopathy.[1,4]

Several different technical methods are available for CKMB measurement. Its activity can be quantified by electrophoresis, immunoinhibition or kinetic enzymatic assays with results presented as percentage or units per litres.[5] On the other hand, CKMB mass is measured by a 2- site

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Table 1 - Patients distribution.

Patients distribution	CKMB activity	CKMB activity	cTnT	CKMB activity
Pts with clinical evidence of myocardial injury and (+) test	36	37	39	39
Pts with clinical evidence of myocardial injury and (-) test	3	2	0	0
Pts without clinical evidence of myocardial injury and (+) test	9	6	3	1
Pts without clinical evidence of myocardial injury and (-) test	70	73	76	78
CKMB-creatine kinase, cTnT-cardiac troponin T, cTnI-cardiac torpnin I				

immunoenzymometric assay or by electron chemiluminiscence immunoassay, with results given in nanograms per millilitre.[6] The CKMB isoenzyme concentration itself is measured by one monoclonal antibody binding to its M-subunit and the other binding to its B-subunit. Compared to CKMB activity, CKMB mass show a better performance.[7,8]

Measurement of cardiac troponins has been useful in diverse clinical situations. Troponins I, C and T form a complex that regulates the calcium–modulated interaction of actin and myocin in striated muscle.[9,10] Troponin I (cTnI) and troponin T (cTnT) have cardiac isoforms that are the products of unique gene sequences with corresponding unique protein structures.[11-16]

This has allowed the development of monoclonal antibodies used in assays that differentiate the cardiac isoforms from those produced in skeletal muscles.[17,18] Both cardiac troponins I and T have proven to be highly sensitive markers for cardiac injury, yielding powerful prognostic information in patients with acute coronary syndromes.[19-21] The purpose of this study was to evaluate cTnI, cTnT, and CKMB mass for the detection of myocardial injury and to compare these results with assays of CKMB activity.

Methods. Fifty eight blood samples with significantly high total CK activity (sent urgently to the lab from different departments in the hospital) were used for the evaluation. The plasma or serum of these samples were frozen at -20°C until the time of analysis, within two weeks from the time of collection. Thirty nine samples (Group A) were collected from patients with clinically proven myocardial injury or infarction, 12-24 hours from the event, and the other 19 samples (Group B) were from patients with muscular disease and renal failure or both without any clinical evidence of myocardial injury. In addition, we studied 60 blood samples with normal CK activity from normal age and sex matched controls who had no clinical evidence of myocardial injury or infarction

Clinical evidence of myocardial injury or infarction was diagnosed or ruled out by history, physical examination, echocardiograms and ECG review.

Blood samples (plasma or serum) with significantly raised CK activity were evaluated for CKMB activity and for CKMB-mass, cTnT and cTnI. Also, blood from controls was assayed for all markers.

Total CK activity (normal $\leq$ 195 IU/L) and CKMB activity (normal $\leq$ 24 IU/L) were measured on a Hitachi 917 (Boehringer Mannheim) analyzer with a kinetic enzymatic method. CKMB-mass (normal $\leq$ 5 ng/ml), as well as cTnT were assayed by an electron chemiluminiscence immunoassay on a 2010

Table 2 - Calculated parameters for the tested markers.

Calculated parameters	CKMB activity	CKMB mass	cTnT	CtnI
Sensitivity	92%	95%	100%	100%
Specificity	89%	92%	96%	99%
(+) Predictive value	89%	86%	93%	98%
(-) Predictive value	96%	97%	100%	100%
CKMB-creatine kinase, cTnT-cardiac troponin T, cTnI-cardiac torpnin I				

Elecsys System (Roche Diagnostic, formerly Boehringer, Mannheim). The cut-off value of cTnT utilizing this method to assess for myocardial injury was 0.1 ng/ml with a lower detection limit of 0.01 ng/ml as recommended by the manufacturer.

Cardiac TnI assays were performed on an AxSYM random access immunoanalyzer (Abbott) using microparticle enzyme immunoassay (MEIA) technology with a cut-off value of 2 ng/ml and a lower detection limit of 0.3 ng/ml as recommended by the manufacturer.

Results. The high CK group included 39 patients with (Group A) and 19 without (Group B) clinical evidence of myocardial injury. Group B included 3 patients with acute skeletal muscle disease, 12 with chronic muscle disease and 4 with chronic renal failure. None of the 60 samples from the control group had elevated CK activities.

CKMB activity and CK MB-mass were elevated significantly in 36/39 patients as they were in 37/39 patients in Group A and 9/19 and 6/19 in Group B. Both cTnT and cTnI were significantly elevated in all (39/39) blood samples from Group A. On the other hand, Group B had 3/19 patients with significantly elevated cTnT and only 1/19 with significantly elevated cTnI. All markers were normal in the control group. The detailed patients distribution is shown in Table 1 and calculated sensitivity, specificity, and positive and negative predictive values for all markers are shown in Table 2.

Discussion. These results demonstrate clearly that cardiac troponins were highly sensitive and specific markers for cardiac injury in our highly selected group with significantly elevated CK activity.

The high sensitivity and specificity of cTnT and cTnI are due to the fact that these proteins are the products of unique gene sequences with corresponding unique protein structures.[11-16] This allowed for the development of monoclonal antibodies that are used in the assay that differentiate these cardiac isoforms of troponins from those produced in skeletal muscles.[17,18]

When compared with each other, cTnT and cTnI were 100% sensitive in this selected groups but cTnI was more specific for myocardial injury than cTnT (98% vs 93%). Two of the three samples found to have falsely elevated cTnT came from patients with polymyositis and their CK activities were 608 and 565 IU/L. One of these samples also had significantly elevated cTnI. The third sample with falsely elevated cTnT was from a patient with chronic renal failure.

The difference in clinical specificity of both troponins supports previous reports.[22-25] Cardiac TnI occurs only in myocardial cells.[22] Neither the protein nor its messenger RNA have ever been detected outside of myocardial tissue at any point in ontogeny or in any pathologic state.[22] In multiple clinical evaluations, elevation of cTnI have been found only in patients with cardiac injury.[22,26-28] It is possible that, in our study, the patient with polymyositis who showed both cTnT and cTnI elevated, had a degree of myocardial inflammation not significant enough to cause detectable impairment in the left ventricular function by echocardiogram.

Although cTnT does demonstrate relative cardiac specificity compared with previously available biomarkers, elevations of this protein in the absence of cardiac injury have been described.[28] Multiple studies have found elevated levels of cardiac troponin T in the absence of discernible cardiac injury, most frequently in individuals with renal failure.[19-24]

Initial assays for cardiac troponin T had 1% to 2% cross-reactivity with the skeletal muscle form of troponin T.[28] This was initially believed to be due to an "analytic" false-positive, but recent data[29] support the contention that many of the observed elevations are due to release of cardiac troponin T from skeletal muscle, rather than merely problems with antibody cross-reactivity. With the development of a second-generation assay for cTnT, the frequency of positive results in these patients is lower than that in the first-generation assay, although still higher than for cTnI.[30,31]

Subsequent studies have shown that the antibodies in the second-generation assays are specific for cTnT isoforms, do not detect the cTnT isoforms expressed in diseased skeletal muscle, and therefore do not produce false-positive cTnT results in skeletal muscle disease and renal patients.[32,33] Preliminary reports have shown that raised levels of cTnT in patients with chronic renal failure are associated with a higher incidence of cardiac death.[34] The importance of these findings is not completely known and the prognostic significance of the low cardiac troponin level in chronic renal failure remains unclear, pending completion of ongoing outcome studies.[35]

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