Troponin-T as a Serum Marker for Myocardial Infarction

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We report here our experience with serum troponin T (TnT), measured with the sandwich immunoassay introduced by Boehringer Mannheim as a marker for myocardial infarction. We assayed TnT in serial serum samples from 30 patients with time courses of serum CK, CK-MB, AST, and LD that we consider typical of acute myocardial infarction (MI). In every patient but one, TnT rose

in parallel with both CK-MB and AST, but remained elevated significantly longer. The ratios of the elevations of the different markers varied from patient to patient with marked variation in the ratio of TnT to CK-MB. There appeared to be a significant association between the magnitude of that ratio with the level of ALT. *J. Clin. Lab. Anal.* 11:125–128, 1997. © 1997 Wiley-Liss, Inc.

Key words: enzymometric immunoassay; cardiac troponin-T; CK electrophoresis; creatine kinase-MB isoenzyme; serum cardiac enzyme markers

INTRODUCTION

Cardiac troponin-T (TnT) is a polypeptide subunit of the tropomyosin complex of cardiac myofibrils (1). Katus and others (2–4) have suggested that the recently introduced immunoassay for TnT in serum (Boehringer Mannheim, Indianapolis, IN) is a better diagnostic marker for early diagnosis of acute myocardial infarction (AMI) than creatine kinase-MB (CK-MB). The aim of this study was to evaluate this hypothesis.

MATERIALS AND METHODS

TnT Assay

Serum TnT was measured using the Cardiac T Elisa Troponin T immunoassay reagents and the ES 300 analyzer from Boehringer Mannheim. This immunoassay employs streptavidin-coated reaction tubes, a biotin-labeled capture antibody specific for cardiac TnT, and a second antibody directed against TnT that is labeled with horseradish peroxidase. Both antibodies react during the initial 1 hr incubation period with TnT in the serum sample to form an antibody sandwich immobilized on the inner wall of the streptavidin-coated tube. At the end of the incubation, nonadherent components are removed by aspiration, and the tube is washed with saline.

The remaining, immobilized horseradish peroxidase activity is measured with di-ammonium 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonate) (ABTS) as substrate in a conventional end-point assay format: the absorbance at 405 nm of the product formed after 30 min incubation is directly proportional to TnT concentration in the sample. A calibration curve constructed from duplicate assays of six calibrators

supplied by the manufacturer, which have TnT concentrations ranging between 0 and 13 µg/L is used to compute the serum TnT concentration in the sample. The samples assayed were selected from those sent to the laboratory for cardiac enzyme assays, which had been assayed on the day they were received and then stored at –70°C until TnT was assayed. Serial samples that showed the typical rise and fall of creatine kinase (CK), CK-MB, aspartate aminotransferase (AST), and lactate dehydrogenase (LD) enzyme activities we considered consistent with AMI.

All enzyme assays (including alanine amino transferase [ALT]) were performed with the Technicon DAX analyzer and reagents supplied by the Miles-Technicon Division (Tarrytown, NY). CK-MB was assayed by the Helena REP electrophoresis procedure (5) with gels and reagents from Helena Labs (Beaumont, TX). The CK-MB enzyme activity was calculated from the percent CK-MB of the total shown by electrophoresis and the total CK activity measured by the Technicon DAX. CK-MB mass in 30 serial samples from six patients was also assayed using the Stratus II CK-MB mass assay (Baxter Healthcare, Miami, FL). Previous studies in our laboratory indicated that the CK-MB mass assays gave quantitative results for µg/L that were approximately numerically equal to the enzyme activity in U/L, except that the mass assay measured low but significant levels of CK-MB in sera in which no CK-MB activity could be detected by the equally sensitive electrophoresis method.

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Patient Samples

A total of 235 serial serum samples from 50 patients were assayed. These included 65 serum samples taken at intervals of 6–8 hr after the initial samples (at the time of admission) from 20 patients with no elevations of the enzyme markers, which we considered to be non-MI, and 170 samples from 30 patients with elevated cardiac enzyme activities, which we considered as MI. These latter included samples from 20 patients that had been taken over a period of as long as 1 week following admission. All the procedures followed were in accordance with the ethical standards established by the standing Committee for Clinical Investigations of Jacobi Hospital and the Albert Einstein College of Medicine.

Statistical Analysis

SAS statistical software package was used for the repeated measures data analysis by the mixed model approach (6).

RESULTS

TnT Assay Performance

Duplicate assays of random serum samples agreed closely: 0.18 vs. 0.17; 1.52 vs. 1.44; 5.28 vs. 5.30 $\mu\text{g/L}$. Within-run imprecision of the assay, estimated from assays of 20 replicates of the two control solutions supplied with the assay kit, showed mean $\pm$ SD to be $0.21 \pm 0.01 \mu\text{g/L}$ (5% CV) for TnT-1, and $5.06 \pm 0.14 \mu\text{g/L}$ (2.8% CV) for TnT-2. Between-run precision was estimated by assaying the 5 TnT calibrators once each on 10 different days. Results ranged from 0.96 ± 0.07 to $10.3 \pm 0.32 \mu\text{g/L}$ with CVs from 7.0% for the samples with low concentrations to 1% for those with higher concentrations.

Assay Linearity

The proportionality of the observed result to TnT concentration was studied by assaying five mixtures containing different proportions of two serum pools, according to NCCLS protocol. The results were directly proportional to concentration over the range between 0 and 9.5 $\mu\text{g/L}$, the highest serum TnT concentration used.

Reference Range

The mean TnT $\pm$ S.D. in sera from 20 patients that had been sent for assay of theophylline concentration was $0.02 \pm 0.01 \mu\text{g/L}$, suggesting an upper limit of "normal" of 0.04 $\mu\text{g/L}$. The reference range for TnT reported by the manufacturer of the assay kit is between 0 and 0.1 $\mu\text{g/L}$.

TnT Concentrations in Sera From Hospitalized Patients

TnT concentrations in 65 serum samples from 20 patients with no cardiac enzyme results (CK, AST, LD) suggesting

myocardial infarction were less than the upper limit of the reference range. A profile of one such patient that we thought typical is shown in Figure 1. In all patients with cardiac enzyme patterns and time courses thought to be consistent with a recent myocardial infarction, serum TnT was elevated. In those early in the course of their illness in whom the serum CK-MB was increasing, the TnT rose in parallel with CK-MB. It remained elevated for at least a week, long after the CK-MB activity had disappeared below the detection limit (2 U/L) of our electrophoresis assay (Fig. 2). In the patients with AMI, the first sample of all series had CK-MB levels higher than those of TnT, compared to their respective reference ranges (Figs. 2 and 3). Achievement of a diagnostic rise in CK-MB thus seemed to precede that of TnT.

Because of how the samples were selected, all 30 patients classified as having had AMI exhibited abnormal MB. They all had elevated TnT result as well.

Serum samples from 9 of the 30 patients with CK-MB undetectable by our routine CK-MB electrophoresis assay, exhibited above normal TnT, indicating that the samples available may have been drawn from recovering MI patients several hours after the typical rise and fall of the cardiac enzyme markers. All nine patients showed above normal CK and LD and three of the nine patients also exhibited higher than normal ALT. TnT in at least one or more samples from each patient was above 0.04 $\mu\text{g/L}$, the upper limit of reference range. In one patient, a 25–50-fold TnT concentration

Fig. 1. TnT concentrations in serial serum samples from a hospitalized patient. The reference ranges of cardiac enzymes are: AST (OT), ALT (PT), 10–40 U/L; LD, 100–250 U/L; CK, 10–100 U/L; CK-MB, 0–1 U/L, and TnT, 0–0.04 $\mu\text{g/L}$. The logarithmic concentrations of each marker are depicted as a function of time (hr) after collection of first sample (zero time). Note that values of 0.1 for CK-MB and 0.01 for TnT on the log scale of Y axis actually represent 0, to enable plotting. CK-MB; —■— TnT; —□— AST (SGOT); —◆— ALT (SGPT); —◇— CK; —△— LD; —▲— Upper limit of reference value -----.

Fig. 2. Typical pattern of enzyme markers and TnT concentrations in serial serum samples from a suspected AMI patient. See legend to Figure 1 for other details.

above the reference range persisted in the first four samples without any CK-MB activity present (Fig. 4).

When we classified AMI patients with abnormal CK-MB results, based on whether the ALT became elevated along with the AST, the peak TnT concentrations were in the range of 2 $\mu\text{g/L}$ in those with ALT in the reference range, ~ 50 times the upper limit of the reference range. However, when the ALT was elevated (72 ± 26 U/L), peak

Fig. 3. Typical pattern of cardiac markers in a second AMI patient. CK-MB mass concentrations in $\mu\text{g/L}$ instead of isoenzyme activity units are given. All other details same as in Figure 2.

Fig. 4. Profile of cardiac markers in serial serum samples obtained from a patient with no CK-MB present. Note the high TnT concentrations in the first four samples. All other details same as in Figure 1.

TnT concentration was ~ 100 times the upper normal reference concentration (Table 1).

Although the peak TnT results in the two groups appear to overlap, statistical comparison of the data from serial samples from all the patients in the two groups, by subjecting them to repeated measures analyses, and comparing all the results for CK-MB, TnT, AST, and ALT, showed that the differences in the two groups of patients were significant ($P = 0.0001$). Higher levels of TnT correlated well with elevated ALT. The differences in AST in the two groups, although not as pronounced as ALT, were also significant ($P = 0.0047$). All other enzyme markers studied were not significantly different.

DISCUSSION

TnT proved to be a very sensitive marker for AMI. The assay described could detect very low TnT concentrations in serum with good precision and accuracy. Wu et al. (7) suggest that TnT may be more useful as a marker of myocardial ischemia rather than infarction.

Although it is contended that TnT is a more sensitive AMI marker than CK-MB, that comparison is apparently based on use of the immunochemical assay for CK-MB, which measures CK-MB, albeit at low concentration, when none is detectable by electrophoresis. This tends to diminish the sensitivity of CK-MB for detection of early infarction.

The upper reference value of TnT concentration of 0.04 $\mu\text{g/L}$, in our hospitalized non-AMI patient population is much lower than the value reported by this group. We also have found that rise in CK-MB often precedes the appearance in serum of abnormal concentration of TnT by 6–8 hr (data not shown).

TABLE 1. Troponin-T, AST, CK, and ASLT in the Serum of AMI Patients at the Time of Appearance of Peak CK-MB Concentration in Serum

No. of Patients	TnT, $\mu\text{g/L}$			CK-MB, U/L			Total CK, U/L			AST, U/L			ALT, U/L		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
10	2.1	2.1	0.1–7.4	63	92	5–314	294	229	91–733	62	52	16–186	22	27	9–36
10	4.4	4.4	0.1–12.7	57	63	5–201	645	388	220–1410	107	74	65–235	72	26	46–121

In our studies, we chose to plot the logarithms of the concentrations of the different enzyme markers and TnT as functions of time of admission after infarction, rather than “normalizing” the enzyme activities by dividing the values by the upper reference value of each marker. In logarithmic plots, parallel rises indicate proportionate changes, which are what would be expected if the markers were released from the same source, such as myocardial muscle, where the markers are in constant proportions to each other. As reported, the parallel rises of CK-MB and TnT and AST in several patients suggest a common source and a common mechanism. In other patients, CK-MB rise precedes that of AST and that of TnT. This suggests a different mechanism of release. The lack of parallelism in the return of the different markers to the reference range has, of course, been noted before. LD was noted to persist elevated longer than AST in the earliest studies reported. Here we note that TnT persists elevated appreciably longer than LD. The mechanism for this long persistence is not clear, as is the mechanism of clearance of all the markers. The graphs suggest that the return of TnT to the reference range after infarction is a function of slower clearance. This, however, is not what was reported by others, who note a very short half-life of TnT. In either event, the long persistence of elevated TnT may prove useful in detecting recent, but not very recent, infarction. The peak TnT concentration in serum of patients with abnormally elevated ALT was nearly twice that seen in patients whose ALT was within the reference range. The mean TnT results in the two groups of patients were 2.1 (range: 0.13–7.44) $\mu\text{g/L}$ and 4.4 (range: 0.1–12.7) $\mu\text{g/L}$, respectively, whereas the peak CK-MB concentrations between the same two groups were not significantly different.

Persistent elevations in TnT concentrations in serum of patients without any evidence of AMI were unexpected. A report by Hafner et al. (8) indicated that patients with chronic renal failure tend to exhibit abnormal elevated serum TnT concentration.

Minor drawbacks of this assay is the requirement of 300 μL sample, which precluded our attempts to assay all samples in duplicate to assess the accuracy of the test. The unusually long time of 1 hr and 40 min needed to complete the assay was also a drawback.

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