Improved troponin T ELISA specific for cardiac troponin T isoform: assay development and analytical and clinical validation

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The first generation of troponin T ELISA (TnT 1) can yield false-positive results in patients with severe skeletal muscle injury. Therefore, a cardiac-specific second-generation troponin T ELISA (TnT 2) was developed, in which the cross-reactive antibody 1B10 has been replaced by a high-affinity cardiac-specific antibody M11.7. No cross-reactivity of TnT 2 was observed with purified skeletal muscle troponin T (1000 µg/L) or in test samples from 43 marathon runners and 24 patients with rhabdomyolysis and highly increased creatine kinase. TnT 2 was increased >0.2 µg/L in 5 of 40 patients with renal failure and in 4 of 20 muscular dystrophy patients. The detection limit is 0.012 µg/L. Day-to-day imprecision (CV) within the range 0.19–14.89 µg/L was <5.8%. In 4955 patients without myocardial damage, 99.6% had TnT <0.10 µg/L. Assay comparison (TnT 1 vs TnT 2) over the whole concentration range (i.e., in 323 samples from AMI-suspected patients) showed a slope, intercept, and standard error of estimate (Sey) of 1.18, 0.01 µg/L, and 0.81 µg/L, respectively.

INDEXING TERMS: cardiac markers • monoclonal antibodies • biotin–streptavidin interaction • creatine kinase

Biochemical markers such as creatine kinase (CK) MB isoenzyme mass, troponin I, and troponin T have become indispensable tools for the diagnosis of myocardial injury.5 In patients admitted with massive acute myocardial infarction (AMI), the troponin T ELISA has provided an equivalent [1] or even a better [2, 3] diagnostic sensitivity than the most-sensitive CK-MB mass determination. Because of its prolonged release, the marker cardiac troponin T (cTnT) is also useful in diagnosing subacute myocardial infarction. Thus, determinations of lactate dehydrogenase are no longer needed [2]. In patients with suspected myocardial infarction or minor myocardial damage, cTnT was found in the blood of many patients with anginal pain when CK-MB mass was not or only minimally increased and the electrocardiograms (ECGs) did not indicate AMI [4, 5]. Consequently, cTnT increases in patients who did not fulfill the World Health Organization (WHO) criteria of AMI [6] were rated as false-positive results, and the diagnostic performance of cTnT was considered to be worse than that of CK-MB mass in patients with suspected AMI [5, 7]. This conclusion, however, was definitely premature. Evidence has accumulated showing that the WHO criteria for AMI allow the detection of only more-extensive myocardial cell necrosis but are of little value for detection of minor myocardial cell damage or for risk stratification of chest pain patients [8, 9]. Furthermore, investigators observed that patients with chest pain and cTnT increases who did not fulfill WHO criteria of AMI experienced the same acute [2, 10] and long-term [11–13] outcome as the patients who had clear AMI according to WHO criteria. The more recent

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5 Nonstandard abbreviations: TnT 1, first version of the troponin T ELISA; TnT 2, newly developed assay with two cardiac-specific antibodies; AMI, acute myocardial infarction; cTnT, cardiac troponin T; CK, creatine kinase; Sey, standard error of the estimate (S\text{ey}); SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin; and PBS, phosphate-buffered saline.
findings indicate the clinical need to properly identify this large subgroup of patients with minor myocardial cell damage.

However, the first-generation troponin T ELISA (TnT 1) could also show falsely increased values in patients with severe skeletal muscle damage. These false-positive results may be explained by an unspecific binding of skeletal muscle troponin T to the wall of the test tube, which can then be detected by the cross-reactive enzyme-labeled antibody used in the TnT 1 assay. Obviously, this made TnT 1 results unreliable in patients with severe skeletal muscle injury, and we therefore decided to improve the specificity of the assay by replacing the cross-reactive antibody with a cardiac-specific monoclonal antibody (mAb). We here report on the development of this improved assay (TnT 2), its analytical characteristics, and its diagnostic performance.

**Materials and Methods**

**Materials**

Diaminobenzidine, Tween 20 surfactant, and dithiothreitol were from Serva (Heidelberg, Germany); 8-mercaptoethanol and sodium perborate were from Merck (Darmstadt, Germany); high-grade acrylamide and bisacrylamide were from Bio-Rad Labs. (München, Germany); all other chemicals, immunoreagents, and streptavidin-coated tubes (binding capacity >14 ng of biotin) were from Boehringer Mannheim (Mannheim, Germany).

Reagents included with the newly developed Boehringer Mannheim Troponin T assay kit (TnT 2) are incubation buffer (40 mmol/L phosphate, pH 7.0), biotinylated anti-troponin T antibody M7 (1.5 mg/L), anti-troponin T antibody M11.7 labeled with horseradish peroxidase (>100 U/L), and five human cTnT calibrators (0–18.8 μg/L) in human serum matrix. Substrate buffer (100 mmol/L phosphate–citrate buffer, pH 4.4, containing sodium perborate, 3.2 mmol/L) and ABTS® substrate (2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonate), 1.9 mmol/L, were also from Boehringer Mannheim.

**Preparatory Procedures**

**Preparation of muscle homogenates.** Tissues from human skeletal muscle and myocardium were obtained postmortem from the Department of Pathology no longer than 10 h after the patient’s death. Muscle tissue (100 g) was homogenized in 1 mL of a solution of 0.05 mol/L KCl, 0.05 mol/L Tris, 5 mmol/L EDTA, 1 mmol/L dithiothreitol, and 2 KIU/L aprotinin (TrasyloL), pH 7.0, with three bursts of a homogenizer (Ultraturrax, Germany) at room temperature. This first supernatant, containing the myofibrillar proteins, was also saved. If not used immediately, both supernatants were stored as aliquots at −20 °C.

**Purification of troponin T.** The troponin T isoforms are encoded by different genes and thus are proteins of differing amino acid sequences. In the healthy human adult, the cTnT isoform is expressed exclusively in the heart [14]. Skeletal muscle troponin T was prepared from human psoas muscle, and cTnT from cardiac muscle tissue, by use of the same conventional techniques [14–16] outlined previously [17]. Tissue was obtained from the Department of Pathology no more than 12 h after the death of the patient. The molecular homogeneity of the purified antigens was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [18]. The specific absorption of troponin T at 280 nm was 0.706 L · g⁻¹ · cm⁻¹ [17].

**Antibody selection and characterization.** Selection and characterization of antibodies followed the protocols outlined in detail previously [17]. MAbs against human cTnT were produced by somatic cell fusions of P3 × 63-Ag8–653 myeloma cells and splenocytes from Balb/C mice immunized with four intraperitoneal injections of purified human cTnT in Freund’s adjuvant. The cell supernatants were screened by ELISA and the positive cells were selected by a cell sorter (FACS; Becton Dickinson, Heidelberg, Germany) and cloned. The cell clones selected by ELISA and the FACS were grown in culture and screened again by ELISA for antibody secretion [17]. Cell clones secreting antibodies specific for human cTnT were grown in culture or cryopreserved.

**Characterization of the antibodies by Western blot.** Crude myofibrillar proteins and purified troponin T from human cardiac and skeletal muscles were fractionated on a 9% SDS-PAGE [18]. The gels were stained with AgNO₃ (1 g/L, 30 min) and developed with formaldehyde (15 mmol/L) plus Na₂CO₃ (25 g/L). Immunoblotting was performed as described by Towbin et al. [19]. The proteins were electrotransferred (100 mA, 50 V for 25 min) from the SDS-PAGE to nitrocellulose membranes (Immobilon P membrane; Millipore, Waltham, MA). The membranes were then blocked with 10 g/L bovine serum albumin (BSA) solution. The troponin T antigen was identified by incubating the membranes for 30 min at room temperature in phosphate-buffered saline (PBS), pH 7.4, containing 2 μg/L anti-troponin T antibody and 50 g/L BSA. Unbound antibodies were removed by washing with PBS. The membranes were then incubated for 30 min at room temperature with 4000-fold-diluted goat-anti-mouse antibody labeled with peroxidase in PBS containing 25 g/L BSA. The membranes were stained with 50 g/L diamobenzidine in the presence of 3 mL/L H₂O₂ for 3 min at room temperature (alternatively, with 0.025 mol/L 4-chloro-naphthol in PBS + 50 μL H₂O₂). Purified human...
cTnT and proteins of known molecular mass were used as controls.

ASSAY DEVELOPMENT AND CHARACTERIZATION

Antibodies. To determine the binding characteristics of different pairs of antibodies with cTnT, we performed a biospecific interaction analysis. The system we used for this (BIAcore-System; Pharmacia Biosensor, Uppsala, Sweden) uses surface plasmon resonance to detect real-time binding and dissociation of interacting molecules to provide information on the affinity, specificity, kinetics, and cooperativity of an interaction. The binding epitopes on the cTnT molecule for the different antibodies chosen for assay development were determined by testing the antibody binding to peptide fragments of cTnT (as outlined in detail by Geysen et al. [20]) by using 12-amino acid peptides with a 1-amino-acid frameshift, starting from the amino-terminus.

The biotinylation of the capture antibody (M11.7) was performed according to the method of Peters and Baumgarten [21] with the use of biotin cross-linked with N-hydroxysuccinimide. Horseradish peroxidase was coupled to the second mAb (M7) as outlined in detail before [17].

These two antibodies are applied in a one-step sandwich assay. The biotinylated capture antibody M11.7 binds with high affinity to the streptavidin-coated test tube. The signal antibody M7 is labeled with horseradish peroxidase. Addition of ABTS substrate produces a 405 nm absorbance signal that is proportional to the cTnT concentration, and the concentration is calculated by using Rodbard functions and dedicated software. As adapted to the ES 300 and 600 analyzers (Boehringer Mannheim), the turnaround time of this newly developed assay is 45 min.

Calibrators. Five calibrators (purified bovine cTnT in human serum) are used to generate the calibration curve. The new calibrators have been measured by TnT 1. With the resulting preliminary calibrator values a method comparison between TnT 1 and TnT 2 had been performed. The difference between both methods expressed as percent of deviation of the slope of the correlation curve had been calculated. The preliminary calibrator values had been corrected by this percentage.

Performance evaluation. The specificity of the assay was tested by serially diluting extracts of cytosolic proteins from skeletal muscle and the troponin T purified from diaphragm, psoas, and quadriceps muscle. The cross-reactivity was calculated as the relative concentrations of skeletal troponin T and cTnT at 50% maximal binding on the calibration curve. The lower detection limit was determined by analyzing in single determination 21 blood samples from healthy volunteers. The mean concentration + 3SD for these samples was calculated.

Intraassay precision was assessed by measuring 10 times five serum samples with cTnT concentrations of 0.19, 5.14, 5.38, 9.39, and 13.74 µg/L. Day-to-day imprecision was determined by analyzing five serum samples of different cTnT concentrations (0.19, 0.30, 0.54, 5.28, and 14.89 µg/L) once each on 10 subsequent days. For each sample we calculated the mean ± SD concentration and CV.

OTHER CARDIAC MARKERS

Total CK activity was determined in the clinical chemistry laboratory with a Clin Chem Analyzer (Miles, Tarrytown, NY) and the reagents provided by the manufacturer. The upper reference limit for total serum CK is 80 U/L in men and 75 U/L for women (25 °C). CK-MB activity was determined by the immunoinhibition method (CK-MB-NAC; Boehringer Mannheim). The upper reference limit for CK-MB activity at 25 °C is 10 U/L, or <5% of the total CK activity.

The TnT 1 assay was performed according to the manufacturer’s instructions manual. Turnaround time for the TnT 1 is 90 min at room temperature. The discriminator value of cTnT used to indicate myocardial damage was >0.1 µg/L.

BLOOD SAMPLING

Blood (10 mL) was drawn into tubes and centrifuged, and the serum samples obtained were stored at 4 °C. If analysis was not done within 8 h, serum samples were stored at −20 °C. For all AMI-suspected patients, the time from onset of pain to assay was recorded. Serial blood samples were taken from each patient, on admission and afterwards at 4–6-h intervals for the first 24 h, at 8-h intervals on day 2, and one per day until day 14 or until discharge. In patients without ischemic heart disease and patients with skeletal muscle injury, one admission sample was drawn. We drew two samples from 43 marathon runners, one before and one after the event.

SUBJECTS

We assayed samples from five groups of subjects. All gave written informed consent to participate in the study after thorough explanation of the study protocol. This investigation was approved by the Ethics Committee of the University of Heidelberg.

1. We compared TnT 1 and TnT 2 results in 323 samples from 47 AMI-suspected patients, an average number of 6.8 samples per patient having been collected during the hospital stay; slope, intercept, and Sey were calculated for the scatter plot.

2. To determine the reference range for cTnT measured with TnT 2, we assayed samples from 4955 patients suspected of endocrine disorders but without clinically apparent myocardial damage, who had been to ambulatory care units and offices of general practitioners.
3. The in vivo specificity of TnT 2 was tested in 43 healthy individuals after a 3-day marathon race and in 24 patients with rhabdomyolysis but without clinical evidence of myocardial damage.

4. Serum samples from AMI-suspected patients seen in the emergency room of the University Hospital in Heidelberg were used to assess the diagnostic performance of TnT 2. The final diagnoses in these patients were:
   a) Chest pain but no evidence for acute cardiac disorders as assessed by history, clinical examination, ECG, echocardiography, and cardiac enzymes (47 serum samples, 47 patients). Additionally, the control group included 132 serum samples from 132 healthy volunteers.
   b) Definite AMI (67 serum samples, 21 patients). The diagnostic criteria for definite AMI (WHO) were: monophasic ST-segment elevations of at least 0.2 mV in at least two adjacent leads of the 12-lead ECG with appearance of new Q-waves and reduction of R-waves and (or) a time-dependent change of serum activities of CK and CK-MB more than twice the upper limit of normal or an increased CK activity with a CK-MB fraction exceeding 5% of the total CK activity.
   c) Possible (minor) myocardial cell damage (74 serum samples, 52 patients). These patients complained of either accelerating angina (Braunwald class I) or angina at rest (Braunwald class III) [22]. AMI was excluded by ECG and results of cardiac enzyme measurements.

5. Patients with Duchenne muscular dystrophy (n = 20) and patients with end-stage renal disease undergoing chronic maintenance hemodialysis (n = 40).

STATISTICAL CALCULATIONS
Data analyzed are given as either mean ± SD or as median with 25th and 75th percentiles (quartiles). Method comparison was performed as a scatter plot, showing slope, intercept, and Sey.
Figure 2 shows how much circulating cTnT was recovered by three cTnT assays with the antibody combinations M7–M11.7, M7–M1.16, and 1B10–M7 (the combination used in TnT 1). Recovery of circulating cTnT by the antibody combination M7–M1.16 was markedly less than that by the 1B10–M7 and the M7–M11.7 combinations.

ASSAY CHARACTERIZATION

The in vitro specificity (Fig. 3) of the antibody combination M7–M11.7 was tested with use of purified troponin T and homogenates from psoas muscle and cardiac muscle as antigens. Using troponin T purified from human skeletal muscle at concentrations as great as 1000 μg/L gave a cross-reactivity <0.1%. When extracts of freshly prepared skeletal muscle were tested in serial dilutions, no binding of the M7–M11.7 antibody combination was observed even at a 1:10 dilution. The same data were obtained when homogenates from quadriceps muscle and diaphragm or purified TnT from these tissues were tested.

Detection limit. The lower detection limit of TnT 2, as assessed by 21 determinations of serum samples from healthy individuals, was 0.011 or 0.012 μg/L (mean ± 2SD or mean ± 3SD, respectively).

Calibration curves for TnT 1 and TnT 2 (Fig. 4). Fig. 4 shows the calibration curves for TnT 1 and TnT 2. In TnT 2 the number of calibrators is reduced from 6 to 5. The slope and the measuring range of both tests are comparable, because both assays use a common cTnT calibrator.

Intraassay precision. The analysis of 10 determinations of the same serum sample showed a CV ≤4.1% at all cTnT concentrations tested. The mean ± SD concentrations measured, in μg/L (and CV), were: 0.19 ± 0.01 (4.1%), 5.14 ± 0.06 (1.3%), 5.38 ± 0.12 (2.2%), 9.39 ± 0.11 (1.2%), and 13.74 ± 0.1 (0.7%).

Day-to-day imprecision. Between-day imprecision over the whole range of the controls, 0.19–13.74 μg/L, was <5.8%. The mean ± SD concentrations, in μg/L (and CV), were: 0.19 ± 0.01 (5.8%), 0.3 ± 0.01 (3.8%), 0.54 ± 0.02 (4.5%), 5.28 ± 0.17 (3.2%), and 14.89 ± 0.29 (2.0%).
Heparin plasma. TnT results for heparin plasma correlated well with those for serum (n = 95, r = 0.97, P < 0.005; data not shown).

Specificity in individuals with skeletal muscle injury. In the 43 marathon runners, the median values (and 25th and 75th percentiles) for troponin T determined by the new TnT 2 method and by TnT 1 were 0.0 µg/L (0.0, 0.025) and 0.08 µg/L (0.03, 0.33), respectively. The total CK activity in these subjects was 818 U/L (546.5, 1107). The respective values for the 24 patients with rhabdomyolysis were: TnT 2, 0.0 µg/L (0.0, 0.0); TnT 1, 3.07 µg/L (2.04, 3.74); and CK activity, 14 820 U/L (3445, 32 200). All runners and all patients with rhabdomyolysis had markedly increased activities of total CK. As tested with the TnT 1, troponin T was >0.2 µg/L in 15 of 43 volunteers after their marathons and in 23 of the 24 patients with rhabdomyolysis. No increase in troponin T (>0.1 µg/L) was observed when the samples were tested by the newly developed TnT 2.

TnT 2 reference range. The distribution of cTnT concentrations in patients with nonacute cardiac diseases was analyzed in serum samples from 4955 patients tested for possible endocrine disorders in general practitioners' offices (Fig. 5). cTnT was =0.02 µg/L in 80% of the patients, =0.04 µg/L in 95% of the patients, =0.06 µg/L in 98.7% of the patients, and =0.1 µg/L in 99.6% of the patients. cTnT was =0.1 µg/L in 21 of the 4955 patients, exceeding 0.2 µg/L in 15; the highest concentration measured was 1.02 µg/L.

Method comparison: correlation between TnT 1 and TnT 2 in AMI-suspected patients. We compared TnT 1 with TnT 2 in 323 samples from 47 AMI-suspected patients (Fig. 6). The slope, intercept, and Sey were 1.18, 0.01 µg/L, and 0.81 µg/L, respectively. For the lower range of values, 0–0.5 µg/L (n = 117 serum samples), the values were 1.18, 0.004 µg/L, and 0.088 µg/L, respectively.

CLINICAL EVALUATION

Control group. cTnT measurements by TnT 2 and total CK activity of blood samples from healthy volunteers and patients without any clinical evidence for cardiac ischemia are shown in Fig. 7A. The median (and quartiles) concentration values measured in 132 healthy volunteers with the cardiac-specific TnT 2 were 0.0 µg/L (0.0, 0.0), with the TnT 1 were 0.0 (0.0, 0.01), and total CK activity was 37 U/L (26, 51). For the 47 patients without myocardial ischemia, the respective values were: 0.0 µg/L (0.0, 0.03), 0.0 µg/L (0.0, 0.0), and 38 U/L (24.2, 50.5). CK activity was increased in 16 of these latter patients (maximum 1235 U/L).

Patients with AMI. In the 67 samples from patients with AMI (Fig. 7B), the median (and quartiles) cTnT concentration measured with the specific TnT 2 was 1.6 µg/L (0.245, 8.87) and with the TnT 1 was 1.57 µg/L (0.145, 9.3); CK was 118.5 U/L (47, 586.5). In 10 of 14 cases with increased cTnT but normal-range CK activity, the samples
had been obtained in the subacute phase of AMI, the other 4 samples having been drawn early in AMI development. In 41 samples both CK and cTnT were increased, rising to 20- and 100-fold the upper limit of normal, respectively.

Patients with unstable angina. In the 74 samples from the patients with possible myocardial cell damage clinically classified as unstable angina (Fig. 7C), median (and quartiles) cTnT concentration values measured with TnT 1 and TnT 2 were 0.035 µg/L (0.0, 0.2) and 0.07 µg/L (0.01, 0.25), respectively. CK activity in these samples was 42.5 (25, 91.7) U/L.

In 35 of the 74 samples, cTnT was >0.1 µg/L, and CK activity exceeded the upper limit of normal in 14 of these 35 samples. In 5 of the 7 patients with an increased CK but normal cTnT, the coronary angiography was normal, suggesting a noncardiac origin of CK in these patients.

Renal failure and muscular dystrophy patients. We tested 40 serum samples from patients with renal failure undergoing chronic hemodialysis. Median cTnT concentration values (and quartiles) were 0.07 µg/L (0.027, 0.25) by TnT 1, 0.09 µg/L (0.06, 0.13) by TnT 2. The concentrations were above normal (>0.2 µg/L) by TnT 1 in 11 of the 40 patients and in 5 by TnT 2.

Serum samples from 20 patients with muscular dystrophy gave median (and quartiles) cTnT concentration values of 0.54 µg/L (0.13, 1.8) by TnT 1 and 0.1 µg/L (0.02, 0.18) by TnT 2. Concentrations were >0.2 µg/L by TnT 1 in 14 of the 20 patients and in 4 by TnT 2. CK values were 1986 U/L (632, 3214), being above normal in all but 1 patient.

Discussion

The first-generation troponin T ELISA was originally developed to provide a more sensitive and specific method than CK-MB mass for detecting myocardial cell damage in patients with suspected myocardial infarction. To achieve this goal, we initially selected two high-affinity anti-troponin T antibodies and combined them in a sandwich-type assay. The specificity of this assay was obtained with the capture antibody, which did not react with the skeletal muscle isoforms of troponin T, whereas the labeled antibody was cross-reactive. Clinical trials with patients with suspected AMI substantiated that TnT 1 specificity was equivalent to [1], or even greater than, the specificity of CK-MB mass assays [2]. Furthermore, as has
been shown in several prospective multicenter trials, cardiac cell necroses can indeed be detected by the troponin T ELISA method that were missed by CK-MB mass determinations; the patients detected were classified as having minor myocardial damage. In several blinded and prospective trials, it has also been shown that these minor increases of troponin T in chest pain patients do not denote a “troponin T disease,” but rather indicate a risk of troponin T-positive patients with chest pain not different from that of patients with definite AMI [4, 10, 23, 24].

When we and others analyzed patients with more extensive skeletal muscle injuries and CK activities in blood exceeding 25-fold the upper limit of normal, cTnT increases were observed in some individuals without clinical findings of acute myocardial cell necrosis [7, 17]. Our hypothesis regarding these troponin T increases is that they may have been false-positive results attributable to an unspecific binding of skeletal muscle troponin T to the test tube and detection by the cross-reactive mAb 1B10 used as label.

To avoid these false-positive results, we developed a new, more-specific, version of the Troponin T ELISA utilizing a new antibody combination (two cardiospecific mAbs). Great care was taken in the selection of a suitable new pair of mAbs, to find antibodies that would be more specific than but at least as sensitive as the former pair. Selection for specificity was carried out by Western blotting and ELISA of purified cardiac and skeletal troponin T as well as homogenates of human myocardium and of different human skeletal muscles such as diaphragm, psoas, and quadriceps muscle.

The binding capacity of different pairs of antibodies was determined with a biospecific interaction analysis (BIAcore). The mAb pairs with the highest relative response were selected for further investigation.

Various pairs of high-affinity antibodies were checked for their recovery of troponin T in patients’ blood. Antibody combination M7–M11.7 showed a recovery similar to that of antibody combination M7–1B10, used in TnT 1 assay; M7–M1.16 detected only 50% of circulating cTnT.

The epitope-mapping showed that the binding epitopes of antibody combination M7–M11.7 are only 6 amino acid residues apart, whereas combination M7–M1.16 binds epitopes ~45 amino acid residues apart. Possible cleavage by proteases between the binding epitopes of mAbs M7 and M1.16 might explain the decreased detection of circulating troponin T by this antibody combination. The number of “sandwiches” formed and the resulting signal would accordingly be diminished. We therefore used the antibody combination M7–M11.7 for further assay development. In TnT 2, mAb M11.7 is coupled to biotin and mAb M7 is labeled with horseradish peroxidase.

The in vitro specificity analysis showed no signal for troponin T purified from skeletal muscle, even at concentrations as great as 1000 μg/L. No false-positive results were obtained when we tested 43 healthy marathon runners and 24 patients with severe skeletal muscle damage but no evidence for acute cardiac disorders.

The highly specific TnT 2 assay detected above-normal TnT concentrations in some patients with chronic renal failure (5 of 40 patients tested) or muscular dystrophy (4 of 20 patients tested). That is, the proportion of cTnT-positive patients is less when measured with the TnT 2 assay than with the TnT 1 assay, indicating that some of the previously reported positive cTnT results may result from an unspecific binding. The reason for cTnT increases in the patients with muscular dystrophy and the patients on maintenance hemodialysis remains to be determined and is the subject of ongoing clinical investigations. Some have suggested that cTnT may be reexpressed in regenerating rat or human skeletal muscle [25, 26]. Alternatively, the increased troponin T may reflect true myocardial damage because dilative cardiomyopathy may result from muscular dystrophy [27] and a substantial proportion of patients with end-stage renal failure suffer from severe coronary artery disease [29, 30]. In a separate investigation on 105 patients on maintenance hemodialysis, we observed a significant positive correlation between the presence of coronary artery disease and increased cTnT concentrations (Hall et al., in preparation for publication).

The time-dependent concentration changes of troponin T in patients with AMI and the number of positive troponin T results in patients with unstable angina or AMI as measured in patients’ blood by the new TnT 2 show good agreement with the results by the TnT 1 over a wide concentration range. Thus, the diagnostic sensitivities of the first- and second-generation troponin T assays appear to be equivalent. Moreover, the improved specificity of the new assay provides sufficient reproducibility to be useful in clinical practice (intra- and interassay CVs of ≤4.1% and ≤5.8%, respectively).

The detection limit of the new assay, defined as 3SD above the mean absorbance at 405 nm for 21 determinations of blood samples from healthy volunteers, is 0.0123 μg/L—another improvement over TnT 1 (detection limit 0.04 μg/L). In addition, the turnaround time could be reduced from 90 min for TnT 1 to 45 min for TnT 2.

Testing troponin T concentrations in a large number of patients with endocrine disorders allowed us to set the clinical discriminator value for troponin T at 0.1 μg/L. This discrimination value correctly classified 99.6% of the patients with endocrine disorders as having normal heart function. However, if the few patients with troponin T >0.2 μg/L are considered to be true-positive patients with unrecognized acute cardiac disorders, the proportion of patients classified correctly at a discriminator value of 0.06 and 0.1 μg/L would be 99% and 99.8%, respectively. Prospective study of an unsel ected group of chest pain patients may thus be necessary to clarify the clinical significance of troponin T values between 0.06 and 0.1 μg/L. Preliminary data from the Fragmin in Acute Ischemic Syndrome [12] trial indicate an increasing risk
for cardiac events in patients with unstable angina and non-Q-wave AMI, beginning at troponin T concentrations of 0.06 μg/L. The GUSTO II investigators also noted a linear increase of cardiac risk with increasing troponin T concentrations [10]. The GUSTO II and other investigators, however, used a 0.1 μg/L discriminator value in their clinical trials on patients with acute ischemic syndromes and efficiently stratified their patients according to risk by using this discriminator value.

In summary, this second-generation ELISA for troponin T is substantially improved in specificity, allowing the differentiation of cardiac and skeletal muscle damage even in patients with severe skeletal muscle injury. At the same time, the turnaround time of the assay could be reduced to 45 min without loss in analytical precision and clinical sensitivity. Therefore, we expect that TnT 2 will replace TnT 1 in clinical practice.

References


