Troponin T and I Assays Show Decreased Concentrations in Heparin Plasma Compared with Serum: Lower Recoveries in Early than in Late Phases of Myocardial Injury

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Background: Heparinized plasma samples allow more rapid analysis than serum samples, but preliminary studies showed lower cardiac troponin T (cTnT) results in plasma. We undertook a multicenter study to characterize this effect for cTnT and cardiac troponin I (cTnI).

Methods: Blood samples were collected with and without heparin at five hospitals. cTnT was measured by a “third generation” assay (Elecsys®), and cTnI was measured by a commercial immunoassay (IMMULITE®).

Results: Mean cTnT was 15% lower in heparin sampling tubes than in serum. Measured concentrations of cardiac troponins also decreased with increasing heparin concentrations added to sera. Heparin-induced losses were greater in early than in late phases after onset of chest pain. Addition of heparin (∼100 IU/mL) to serial samples from nine acute myocardial infarction patients produced mean cTnT losses of 33% at 1–12 h after onset of chest pain, 17% at 13–48 h, and 7% after 48 h. The changing heparin effects were seen for both cTnT and cTnI during time courses of individual patients with myocardial infarction.

Conclusion: We suggest that binding of heparin to troponins decreases immunoreactivity, especially in early phases of myocardial injury. The resulting losses may depend on the antibodies used in each troponin assay.

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The usefulness of cardiac troponins T and I (cTnT and cTnI)6 in the diagnosis and prognosis of myocardial damage is well documented. Assay methodologies have steadily improved over the years. In Helsingborg, we routinely determine cTnT (“third generation” assay), creatine kinase MB isoenzyme (CKMB), and myoglobulin in serum on the Elecsys 2010 (Roche, Mannheim, Germany) (1, 2). The package insert for “Troponin T STAT” (August 1999) allows for use of serum with or without separator gel, lithium, ammonium-sodium-heparin, tripotassium EDTA, and sodium citrate plasma.

To shorten needle-to-report time for cardiac markers, we wished to change from serum to plasma samples. When we obtained parallel samples in heparin and serum sampling tubes, we found values as much as 30% lower for cTnT (Elecsys 2010) in plasma from various heparin sampling tubes. Aware of the possible clinical implications, we asked for independent confirmation on cTnT from other Swedish laboratories and from the Katus group in Germany.

Noting that the manufacturers’ directions for several
current cTnI assays describe 19–30% losses with heparin plasma compared with serum, we also collected data on cTnI with one of the methods (3).

Materials and Methods

A total of 231 paired samples were drawn in parallel from acute myocardial infarction (AMI) patients at five hospitals into tubes without anticoagulant [Becton Dickinson (BD) serum separator tube (SST; cat. no. 367788) and, for laboratory 2 (Karolinska Sjukhuset), Terumo Venoject SST (cat. no. VP-054SAS)] and into various BD heparin glass and plastic tubes. Preliminary studies (not shown) indicated no differences in measured cTnT concentrations between BD and Terumo serum tubes, serum tubes with or without serum separator gel, or serum tubes with or without thrombin. Laboratories 1 and 5 drew serial samples on admission and at 6-h intervals (see Table 2 and Fig. 3) after estimating the time of onset of chest pain as accurately as possible. We also performed a closer study of the variability of heparin-induced cTnT and cTnI losses during the time course of AMI. From each of 10 patients, 14–15 serial samples were drawn from a separate intravenous line, initially at 30-min intervals. Patients gave informed consent.

cTnT (third generation), myoglobin, and CKMB mass were determined on the Elecsys® 2010 according to each laboratory’s routine. Nearly all serum and heparin plasma samples were assayed within a few hours. All three markers are stable in both serum and heparin plasma over 24 h at 4 °C. In a few cases, in which heparin-plasma cTnT was determined after storage for 20 h, serum cTnT was redetermined at the same time. cTnI and CKMB mass were also determined on the IMMULITE® system (Diagnostic Products Corporation).

For reasons of imprecision, only samples with values >0.04 μg/L in both plasma and serum were used for calculation of the ratios of plasma cTnT to serum cTnT (P-TnT/S-TnT).

Initial heparin titration experiments were carried out on 10 sera by adding 5, 10, and 50 μL of heparin (5000 IU/mL) to 500-μL serum aliquots, giving final concentrations of 50, 98, and 450 IU/mL. Samples were gently mixed on a vortex-type mixer, and cTnT or cTnI was determined after 30 min. All results were corrected for dilution. If heparin experiments were carried out later than ~4 h after the initial cTnT determination, cTnT or cTnI was redetermined immediately before the heparin addition. In a small number of samples, low-molecular heparin (Fragmin) was added to serum aliquots to a final concentration of 98 IU/mL and P-TnI/S-TnI was determined.

Data were analyzed with the statistical software Statistics for Windows (StatSoft). Results were expressed as the mean ± 95% confidence interval. To compare mean values for different tubes, ANOVA with the Shefﬁ test was used. P < 0.05 was considered statistically significant.

Results

The results obtained with heparin and serum sampling tubes are compared in Table 1 and given as the P-TnT/S-TnT ratio as a percentage. We recalculated the manufacturer’s stated heparin content per tube to the heparin concentration per milliliter of plasma (in filled tubes; range, 40–70 IU/mL of plasma). Table 1 shows the lower cTnT results obtained for various heparin sampling tubes compared with serum tubes. The mean P-cTnT/S-cTnT value for all 231 data pairs (eight subsets) from six heparin tubes was 84% ± 2% with no statistically signifi- cant differences among types of tubes (ANOVA). A probable reason for the slightly lower mean value with the BD 367993 plasma separator tube (PST) is discussed below.

The lower cTnI (IMMULITE) results obtained with the BD 367993 PST (lower part of Table 1) were similar to those for cTnT. The loss of ~15% was smaller than the 30% given in the IMMULITE assay insert (n = 73). CKMB mass and myoglobin (Elecsys) and CKMB mass (IMMULITE) showed no significant losses with heparin tubes. Myoglobin was not evaluated on the IMMULITE system.

The data from all 231 sample pairs in Table 1 are shown in Fig. 1. P-TnT/S-TnT was not correlated with cTnT concentrations.

HEPARIN TITRATION EXPERIMENTS

The addition of increasing heparin concentrations to serum aliquots from nine AMI patients consistently produced decreasing cTnT values. The measured troponin concentrations did not decrease further after standing with heparin for several hours. Preliminary experiments showed identical results with two different heparin preparations. Unexpectedly large variability was seen at each heparin concentration: 50 IU/mL gave P/S values of 86–109%, 98 IU/mL gave values of 67–99%, and 450 IU/mL gave values of 51–78%.

On closer scrutiny, we discovered that the highest cTnT losses of up to ~30% occurred in samples with the combination of increased cTnT, myoglobin, and CKMB mass. This pattern is typical for the early phase of myocardial damage with an initial peak of (“free”) cyto- solic cTnT (4–6). In contrast, the smallest cTnT losses, 5–10%, occurred in samples with only increased cTnT. This pattern is found in the late stages of myocardial damage, during which cTnT is released from degenerating cellular structures (4–6).

Heparin titration experiments with cTnT on 10 samples showed similar P-TnI/S-TnI values of 77% and 69%, respectively, at 98 and 450 IU/mL.

DATA FROM AMI PATIENTS

Data from the 144 samples from Table 1 with parallel cTnT and CKMB mass values were used to calculate the mean P-TnT/S-TnT values for different CKMB concentra-
tions. Fig. 2 shows that P-TnT/S-TnT values decreased with increasing CKMB mass, which is increased only during the first 2–3 days after AMI.

For additional studies, we added heparin to a final concentration of 98 IU/mL, 40% higher than the concentration in BD tube no. 367793, to serial serum samples from AMI patients. Two examples of such time curves are shown in Fig. 3. In these cases, both P-TnT/S-TnT and P-TnI/S-TnI (IMMULITE) increased continuously with time after onset of chest pain. For P-cTnI, low-molecular heparin (Fragmin) caused losses similar to those seen with heparin (Fig. 3B). Table 2 summarizes the differences

<table>
<thead>
<tr>
<th>Type (cat. no.) of tube</th>
<th>Laba</th>
<th>No. of TnT determinations</th>
<th>Mean ± 95% confidence interval, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys 2010</td>
<td></td>
<td></td>
<td>P-TnT/S-TnT</td>
</tr>
<tr>
<td>Nonsiliconated soda glass tube with separator gel; Li-heparinate ~70 IU/mL plasma (BD 367793 PST)</td>
<td>1</td>
<td>31</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Nonsiliconated PET tube; Li-heparinate, 45 IU/mL plasma (BD 367994 PST)</td>
<td>3</td>
<td>28</td>
<td>88 ± 6</td>
</tr>
<tr>
<td>Nonsiliconated plastic tube, separator gel; Li-heparinate, 60 IU/mL plasma (Greiner Vacuette 456087)</td>
<td>1</td>
<td>4</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>Nonsiliconated soda glass tube; Li-heparinate ~65 IU/mL plasma (BD 367684 PST)</td>
<td>3</td>
<td>28</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>Nonsiliconated soda glass tube; no separator gel; Na-heparinate ~40 IU/mL plasma (BD 367679)</td>
<td>2</td>
<td>34</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>Nonsiliconated PET tube with separator gel; Li-heparinate ~50 IU/mL plasma (BD 367993 PST)</td>
<td>1</td>
<td>14</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>Total no. of data pairs</td>
<td>231</td>
<td>144</td>
<td>132</td>
</tr>
<tr>
<td>Immulite (BD 367793 PST)</td>
<td>5</td>
<td>20</td>
<td>84 ± 5</td>
</tr>
</tbody>
</table>

a Reference serum tube was BD 367788 SST siliconated glass tube (except for laboratory 2, where the Terumo VP-054SAS Venoject tube with separator gel was used).

b Laboratories: 1, Helsingborg; 2, Karolinska Sjukhuset; 3, Lübeck Medical University; 4, Lund Medical University; 5, Ryhov Sjukhus.

c MYO, myoglobin.

Fig. 1. P-TnT/S-TnT ratio vs natural logarithm of mean concentrations (μg/L) from plasma and serum. All 231 sample pairs from Table 1 are shown. The heparin-induced losses do not correlate to the cTnT concentration.

Fig. 2. Mean values (± 95% confidence intervals) for P-cTnT/S-cTnT grouped by increasing concentrations of the early marker, serum CKMB mass (S-CKMB; μg/L), measured in the same sample.
among P-TnT/S-TnT values at 98 IU/mL heparin in 43 samples from “early”, “middle”, and “late” phases in nine AMI patients. These classifications were based on the estimated time of onset of chest pain.

**Discussion**

To the best of our knowledge, loss of troponin T in heparin sampling tubes has not previously been published. For cTnI, a recent study reported that low concentrations of heparin (15 IU/mL) had no effect on two cTnI assays (3). However, in their respective assay inserts, several cTnI manufacturers (including IMMULITE) describe 19–30% lower values in heparin than in serum tubes.

In this study, five laboratories independently confirmed a mean decrease of ~15% of both cTnT (third generation, Elecsys) and cTnI (IMMULITE) using heparin plasma from various sampling tubes. Among possible causes, we could exclude both separator gel and adherence of troponins to nonsiliconated glass tube walls. The highest values were always found in the serum reference tube with separator gel (Table 1). We found no significant differences among heparin tubes. The lower mean for BD tube no. 367793 (74% ± 68%; Table 1) was probably not attributable to the tube itself. This small series (n = 14) contained an unusually high percentage (79%) of initial-phase samples with increased CKMB mass (range, 11–500 µg/L), giving a P-TnT/S-TnT range of 48–89%. The remaining samples with CKMB mass <10 µg/L had P-TnT/S-TnT values of 80–100%.

Titration of sera with heparin concentrations below and above tube concentrations caused increasing losses of both troponins with increasing heparin concentrations. However, for a given heparin concentration, we found an unexpectedly large variability in the P-TnT/S-TnT ratio among individual samples from AMI patients. We found that these differences in heparin sensitivity were related to early and late samples from AMI. One hundred forty-four samples showed decreasing P-TnT/S-TnT values with increasing values of the “early marker” CKMB mass (Fig. 2).

We further studied this relationship by determining P-TnT/S-TnT in serial serum samples with heparin added to a constant concentration of 98 IU/mL. This concentration is ~40% higher than that calculated for the BD 367793 PST. Therapeutic concentrations of heparin at AMI and at cardiac surgery have been estimated to 1 IU/mL and 5 IU/mL, respectively (7). These correspond to 1.5% and 7% of the concentrations in heparin tubes and do probably not cause significant in vivo losses of cardiac troponins.

Fig. 3 confirmed that P-TnT/S-TnT values generally are lowest in the early phase and increase toward the late phases of myocardial damage. Individual variability occurs during AMI. cTnI (IMMULITE) showed similar patterns.

Table 2. P-TnT/S-TnT ratio at 98 IU/mL heparin in serial samples from nine AMI patients.

<table>
<thead>
<tr>
<th>Estimated time interval from onset of chest pain</th>
<th>n</th>
<th>1–12 h</th>
<th>13–48 h</th>
<th>&gt;48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-TnT/S-TnT, mean ± 95% confidence interval</td>
<td></td>
<td>67.2 ± 2.3</td>
<td>82.5 ± 5.2</td>
<td>92.5 ± 1.5</td>
</tr>
</tbody>
</table>

Thus, the decreased immunoreactivity of both troponins apparently depends on the different distributions of troponin forms found in plasma during evolution of myocardial damage. Dolci (8) reported in an abstract that the problem is common to both troponins (cTnT third generation and cTnT Stratus II) with both lithium heparinate and tripotassium EDTA sampling tubes. EDTA is known to release free cTnI from a calcium-dependent cTnI-troponin C complex (3, 9). This causes decreased
We find it probable that negatively charged polyanions on heparin bind to positively charged troponins (10). This may reduce immunoreactivity either by causing conformational changes of the troponin molecule or by directly covering analytical epitopes (10). In the early phase of myocardial damage, troponin T occurs mainly as a “free cytosolic” form; in the later phase, it occurs in the “structurally bound” form and fragments (4, 5). Troponin I is primarily released into plasma as a binary complex with troponin C and later occurs as a distribution of a variety of forms (3, 9, 10). Heparin binding with different affinities to different troponin forms would explain our findings of varying P-TnT/S-TnT ratios at 98 IU/mL heparin in serial serum samples from AMI patients. For cTnI, the different losses in heparin plasma described in the respective assay inserts would be explained by different analytical antibodies binding to differently located epitopes on the cTnI molecule. WHO (11) recommends calcium-titrated heparin, 40–60 IU/mL of blood (dry heparinization) and 8–12 IU/mL of blood (liquid heparinization). The IFCC (12) recommends 40–60 kIU/L of blood, corresponding to ~80–120 IU/mL of plasma in tubes. Sample tubes for troponin determinations should be validated and specified in the respective assay inserts as recommended by the IFCC (13). The recent National Academy of Clinical Biochemistry Recommendations (14) suggest that manufacturers target their assays for use in plasma and recommend: “plasma or anticoagulated whole blood are the specimens of choice for the stat analysis of cardiac markers”.

We conclude that heparin decreases the measured concentrations of cardiac troponins, probably by binding to troponins and reducing their immunoreactivities. The magnitude of the decrease depends on the distribution of different troponin forms in circulation during and after myocardial damage and on analytical antibodies used in different troponin assays. Future cardiac troponin assays should be resistant to interference by both heparin (10) and EDTA (9). Until such methods are available, the sample of choice for cardiac troponin determinations is serum collected in tubes with or without gel, or in thrombin tubes with and without gel.

We thank Stig and Ragna Gorthons Stiftelse, Helsingborg, for a research grant for reagents that made it possible for the Helsingborg to initiate this study. Dr. Katus is a consultant for Roche Diagnostics and holds a patent for troponin T jointly with Roche Diagnostics. Additional graphs may be found at the Clinical Chemistry Online Web site (http://www.clinchem.org/content/vol46/issue6).

Addendum: Since November 1999, a separate product information sheet for Elecsys Troponin T STAT (cat. no. 2017423) and for Troponin T (cat no. 2017644; Roche Diagnostics) informs about reduced recovery in heparin samples as described here.

References
8. Dolci A. Biochemical markers of myocardial damage: what sample can we use? [Abstract]. Preanalytical Phase in Patient Care and Hospital Management, June 5–6, 1999, Pratolino-Florence, Italy.