The Effect of Freezing and Long-Term Storage on the Stability of Cardiac Troponin T

Majid Basit, MD,1 Nasir Bakshi, MD,2 Mustafa Hashem, MD,1 Zuhair Allebban, PhD,1 Noel Lawson, MD,3 Howard S. Rosman, MD,1 and James J. Maciejko, MS, PhD1

Key Words: Cardiovascular disease; Troponin T; Sample storage; Sample stability; End-stage renal disease; Hemodialysis

DOI: 10.1309/LR7FC0LUGLHT8X6J

Abstract

Cardiac troponin T (cTnT) levels are widely used to assess for evidence of myocardial infarction. We studied the effect of freezing and long-term storage on the stability of cTnT in blood samples from 178 patients with end-stage renal failure. The serum was separated and divided into multiple aliquots. Baseline cTnT levels were measured in the unfrozen aliquots. The remaining aliquots were frozen using standard techniques. The aliquots were thawed after 3, 6, 12, or 24 months and cTnT levels measured.

There were no significant changes in the mean ± SEM cTnT levels up to 12 months (0.111 ± 0.098 µg/L) compared with baseline (0.114 ± 0.098 µg/L); after 24 months, cTnT levels were significantly lower (0.107 ± 0.095 µg/L) than baseline (P = .004). The cTnT assay is a reliable method of measuring the cTnT level in human serum up to 12 months of frozen storage. However, after 24 months, the cTnT level was 0.007 µg/L lower than baseline, potentially causing erroneous interpretations. The clinical significance of the change in the cTnT level after long-term frozen storage is unclear. Further studies, including prospective analysis of patient outcomes, should be helpful.

Materials and Methods

Blood samples were obtained from 178 patients with ESRD undergoing routine hemodialysis treatment. The patients were participants in a clinical study designed to evaluate whether the level of troponins (I and T) and C-reactive protein prognosticated morbidity and mortality.7 The patients underwent hemodialysis for at least 6 months and less than 3 years and had no evidence of active or ongoing myocardial ischemia.
Blood samples were drawn in two 10-mL tubes before the start of hemodialysis. The samples were collected in phlebotomy tubes containing no anticoagulant or preservative and centrifuged at 1,000g for 10 minutes. The serum was divided into 5 aliquots, 1.5 mL each, and collected in plastic tubes. Nitrogen gas was passed over the individual aliquots before sealing them in airtight tubes. One aliquot was stored at 4°C and shipped on water ice within 2 weeks to Bi-County Hospital Clinical Laboratory, Warren, MI, for measurement of cTnT. The protocol for testing the unfrozen samples is based on a previous study in which the concentration of unfrozen cTnT remained unchanged for up to 14 days. The other 4 aliquots were labeled as 3, 6, 12, or 24 months and stored at −70°C until they were thawed. The delay between venipuncture, serum separation, and storage of the aliquots was less than 48 hours. The frozen samples were thawed by placing them at room temperature. They were mixed thoroughly and then assayed in batches within 8 hours after thawing.

The Elecsys 2010 Immunoassay Analyzer and reagent (Roche Diagnostics, Indianapolis, IN) were used to measure the cTnT concentration. The total duration of the assay is approximately 9 minutes. The assay uses 2 monoclonal murine antibodies specific for cTnT and electrochemiluminescence as the detection technique. A biotinylated monoclonal cTnT antibody and a monoclonal cTnT antibody labeled with an electrochemiluminescent compound (ruthenium complex) react with the test sample to form a sandwich complex. After removing unbound substances and applying voltage, chemiluminescent emission is induced, which is measured by photomultipliers. This value is converted to micrograms per liter. The assay has been standardized with human recombinant cTnT and is a third-generation cTnT detection test. The sensitivity of the assay is 0.01 µg/L with a detection range of 0.01 to 25 µg/L.

We divided subjects into 3 categories, based on baseline cTnT concentrations, to determine the effect of the freezing and thawing of the samples on clinical relevance relative to prognostication. The categories were low risk (<0.010 µg/L), moderate risk (0.010-0.099 µg/L), and high risk (≥0.100 µg/L) as demonstrated by James et al. The St John Hospital and Medical Center Institutional Review Board (Detroit, MI) approved the study. Written informed consent was obtained from all participants. The investigators had full access to the data and take full responsibility for its integrity.

All statistical analyses were performed using SPSS for Windows (version 12.0, SPSS, Chicago, IL).

Results

The characteristics of our study population are summarized in Table I. The mean age of the cohort was 62.1 years, with more than 50% older than 70 years. Somewhat more than half were white, and about half were women. A large proportion of the patients had a history of hypertension or diabetes mellitus. The mean serum creatinine level was 9.1 mg/dL (804 µmol/L; range, 3.1-24.1 mg/dL [274-2,130 µmol/L]). The mean ± SEM cTnT concentrations were as follows: baseline, 0.114 ± 0.098 µg/L; 3 months, 0.113 ± 0.097 µg/L; 6 months, 0.120 ± 0.100 µg/L; 12 months, 0.111 ± 0.098 µg/L; and 24 months, 0.107 ± 0.095 µg/L. The cTnT concentrations at 3, 6, and 12 months were not significantly different from the baseline concentrations (P = .9, P = .1, and P = .5, respectively). The cTnT concentrations obtained from the frozen specimens stored for 24 months were significantly lower than baseline (P = .004). Although the concentrations were significantly lower, the correlation between the baseline cTnT concentrations and those obtained from the frozen specimens stored for 24 months was high (r = 0.91; P < .0001).

![Table I](https://example.com/table1)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>47.8</td>
</tr>
<tr>
<td>White</td>
<td>52.8</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>91.0</td>
</tr>
<tr>
<td>Diabetic</td>
<td>52.2</td>
</tr>
<tr>
<td>Current smokers</td>
<td>19.1</td>
</tr>
<tr>
<td>Previous smokers</td>
<td>51.1</td>
</tr>
<tr>
<td>Mean ± SD age (y)</td>
<td>62.1 ± 15.7</td>
</tr>
<tr>
<td>Mean ± SD body mass index (kg/m²)</td>
<td>28.6 ± 8.3</td>
</tr>
<tr>
<td>Mean creatinine level (mg/dL)</td>
<td>9.1 (3.1-24.1)</td>
</tr>
</tbody>
</table>

Data are given as percentages unless otherwise indicated.

![Figure 1](https://example.com/figure1)

Comparison of the mean ± SEM level of cardiac troponin T (cTnT) in fresh samples with the level of cTnT of the same samples frozen for 3, 6, 12, and 24 months, measured using the Elecsys cTnT assay (Roche Diagnostics, Indianapolis, IN). A Kruskal-Wallis analysis of variance was used to test for changes over time. * Significant difference between fresh samples and samples frozen for 24 months (P = .004).
The interassay coefficient of variation (CV) of this assay was between 4.1% and 7.2%. The interassay CV was obtained for the whole measuring range, and the highest CVs were used in the range of the cutoff.

Analysis of variance for repeated measures and post hoc analysis were used to account for storage time effect on the stability of the TnT molecule from baseline and months 3, 6, 12, and 24. There was no difference between the baseline mean difference and the 3-, 6-, and 12-month mean difference (−0.001, SE = 0.001, P < 1.0; −0.006, SE = 0.003, P < 0.62; and 0.002, SE = 0.001, P < 0.52, respectively). At 24 months, the mean difference was significantly lower than baseline (0.006, SE = 0.001, P < .0001). In all cases, a P value of less than .05 was considered to indicate statistical significance.

By dividing subjects into low-, moderate-, and high-risk categories, we demonstrated that greater than 95.5% of patients remained within their baseline cardiovascular risk categories throughout the study, including at the 24-month point. The percentages of deviation for each time point compared with baseline for the 3 decision cutoff points is given in Table 2.

### Discussion

Many clinical trials and research studies rely on off-site or delayed batch analysis of patient blood samples. To accomplish this analysis, blood samples are separated by centrifugation and the resulting serum or plasma samples are quickly frozen. At specific follow-up times, the samples are thawed and analyzed as designated by a study protocol. This analysis can take place days, months, or years after initial sample collection.9 The freezing and thawing process and the resulting serum or plasma samples are quickly accomplished by centrifugation, or delayed batch analysis of patient blood samples. To accomplish this analysis, blood samples are separated by centrifugation and the resulting serum or plasma samples are quickly frozen. At specific follow-up times, the samples are thawed and analyzed as designated by a study protocol. This analysis can take place days, months, or years after initial sample collection.9 The freezing and thawing process and the resulting serum or plasma samples are quickly frozen.

Several studies have demonstrated that serum troponin concentrations are sensitive and specific markers of cardiac injury, such as in the 15% to 20% of patients who have an acute coronary syndrome with normal creatine kinase MB levels.10 Also reported, yet somewhat controversial, is the elevation in troponin levels observed in patients with ESRD without coronary heart disease. Ooi et al4 hypothesized that in renal failure, there may be circulating substances that modify troponins differently, affecting their clearance rate or reactivity in the analytic assay. However, the elevated troponin levels may have major clinical implications for patients at high risk of coronary heart disease.1,4

Despite the numerous studies evaluating the diagnostic performance of troponins T and I, only a few studies have addressed the issue of long-term (ie, >12 months) on the in vitro stability of these cardiac markers.5,11 In fact, published and manufacturers’ data on the long-term in vitro stability of troponins are limited. This issue becomes important when long-term clinical trials are undertaken, requiring the study of stored patient samples. This is analogous to the Cholesterol and Recurrent Events study, which attempted to establish the association of increased risk of recurrent coronary events with inflammation.9 In that study, C-reactive protein and serum amyloid A, both markers of inflammation, were measured from serum samples frozen for 5 years.9,12 In addition, retrospective analysis of cardiac markers is occasionally requested clinically if the diagnosis of myocardial infarction is not initially apparent or if clinical manifestations change. Because there is now evidence to suggest that elevated cTnT levels can be used to influence therapy for acute coronary syndromes, the need for knowing the long-term stability of cTnT in vitro cannot be overemphasized.11,13,14

The stability of cardiac troponins in serum was assessed in a smaller study in which the sample storage was restricted to short periods (1-6 months).8 The present study was conducted as part of ongoing research to assess the ability of cardiac troponins T and I and C-reactive protein to predict cardiac events in asymptomatic patients with ESRD.7

Our data demonstrate that serum cTnT results are reliable for up to 12 months of frozen storage. Our findings suggest that investigators can use stored specimens for up to 12 months to measure cTnT. The results of our study may help substantiate the validity of results using the cTnT assay on previously frozen samples and may be applicable to other assays as well. However, after 24 months of frozen storage, the measurement of cTnT in serum samples may not be accurate, potentially causing erroneous interpretations. The clinical significance of the change in the cTnT level after long-term frozen storage is unclear, and further studies, including prospective analysis of patient outcomes, may be helpful.

### Table 2

<table>
<thead>
<tr>
<th>Cutoff Point (µg/L)</th>
<th>Percentage of Deviation of Cardiac Troponin T Levels in Samples Frozen for 3 to 24 Months Compared With Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline vs 3 mo</td>
</tr>
<tr>
<td>≥0.100</td>
<td>0.006</td>
</tr>
<tr>
<td>0.010-0.099</td>
<td>0.006</td>
</tr>
<tr>
<td>&lt;0.010</td>
<td>0.006</td>
</tr>
</tbody>
</table>

From the 1Division of Cardiology, Department of Medicine, St John Hospital and Medical Center, Wayne State University School of Medicine, Detroit, MI; 2Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City; and 3Department of Pathology, St John Hospital and Medical Center, Detroit.

Address reprint requests to Dr Maciejko: Division of Cardiology, St John Hospital and Medical Center, 22201 Moross Rd, Suite 470, Detroit, MI 48236.
Roche Diagnostics provided funding for the laboratory assays performed in this study.

References