Specimen Recentrifugation and Elevated Troponin I Levels

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ABSTRACT

Objective: To compare troponin I levels in plasma before and after specimen recentrifugation.

Methods: Over a period of 24 consecutive days, we recentrifuged 189 plasma specimens in which the troponin I level was higher than the positivity cut off (60 ng/L) and then remeasured the troponin I.

Results: Two-tailed Wilcoxon matched-pairs test results identified a statistically significant difference between troponin I concentrations before and after recentrifugation (P <.01). For 94 specimens close to the cut-off value (below the 50th percentile among the overall sample of 189 specimens), Passing-Bablok regression analysis showed a median reduction of 10 ng/L (95% confidence interval, -37.5 ng/L – -10.0 ng/L) in the concentrations of troponin I after recentrifugation.

Conclusion: Specimen recentrifugation is followed by a reduction in troponin I concentrations in specimens with elevated cardiac troponin I, posing a risk for misclassification of patients. Moreover, this practice gives rise to unnecessary prolongation of turnaround time.

Keywords: recentrifugation, troponin I, preanalysis, cardiac biomarkers, cardiovascular disease, clinical chemistry

Since their introduction, cardiac troponin (cTn) assays have been accompanied by observations of falsely elevated results.1 This phenomenon, initially thought to be specific to serum, was eventually also observed in plasma.2 Many authors3-5 speculated that particulate matter (eg, fibrin strands) may be responsible for unexpectedly high troponin results, and proposed recentrifugation of specimens as a solution to this problem.

The available literature on this topic does not clearly support this hypothesis.6 In addition, the common method of identification of putative false-positive results does not take into account the existence of outlier results in reanalyzed specimens (a change in measured cTn not explained by analytical variability after reanalysis of unmodified specimens [ie, not recentrifuged or otherwise manipulated]), which has been demonstrated with many troponin assays,7,8 or the pathophysiology of troponins (which are cardiосpecific but not disease-specific).9 Moreover, to our knowledge, no systematic effort has been made to investigate the effects that recentrifugation might have on the troponin levels in blood specimens.6 The Clinical and Laboratory Standard Institute advised that caution should be taken in recentrifuging specimens because potential effects on the concentrations of analytes could be induced.10 These recommendations are supported by the few reports published on the subject11-13 that demonstrate the influence of recentrifugation on common clinical chemistry analytes, although data regarding troponin have not been published. Nevertheless, some clinical laboratories still recentrifuge specimens that test positive for troponin. For example, in a recent article, Li et al14 proposed a method for identification of falsely elevated troponin I based on the assumption that suspended particulates were the cause. To improve our knowledge of preanalytical variables affecting troponin measurement, we compared troponin levels in plasma before and after specimen recentrifugation.

Abbreviations

cTn, cardiac troponin; CI, confidence interval

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Materials and Methods

We collected data from patient specimens that had undergone troponin determination during a period of 24 consecutive days. Blood was collected in lithium heparin evacuated tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) to obtain plasma after an initial centrifugation (1300 g, 10’; centrifuge not refrigerated). Troponin I was measured via the immunometric assay AccuTnI on an Access 2 platform (Beckman Coulter Inc., Brea, CA). During the study, we monitored the performance of the assay using 2 levels of quality control; calibration was periodically reviewed with the materials provided by the manufacturer. The correct functioning of centrifuges was monitored by medical engineering personnel at the hospital. Laboratory technicians reran patient specimens for which the cTn I level was found to be higher than the positivity cut-off value (60 ng/L); all these specimens were manually aliquotated in plastic tubes (plasma only) and recentrifuged (1300 g, 10’; unrefrigerated) before troponin was remeasured. Specimens were stored at room temperature. We decided to recentrifuge only specimens with initial positive results for 2 reasons. First, since we did not use a high-sensitivity assay, a large fraction of the negative results had unmeasurable troponin I concentrations; second, our approach addresses current recommendations for investigating falsely elevated results. All reanalyzed specimens were assayed within a maximum time of 90 minutes after initial analysis. Statistical analysis was performed with the MethComp package for R statistical software.

Results

We collected data on 189 specimens. The 2 sets of data were not Gaussian-distributed according to the Shapiro-Wilk test. Most of the troponin values were near to the positive cut-off; the median value was 190 ng/L before recentrifugation and 170 ng/L after recentrifugation, whereas the 2.5th- to 97.5th-percentile interval was 60 ng/L to 19,660 ng/L and 10 ng/L to 19,490 ng/L, respectively. Two-tailed Wilcoxon analysis of matched pairs confirmed the difference between troponin determinations before and after recentrifugation, and the difference was statistically significant (P < .01). To evaluate the effects of recentrifugation on positive results near the positive cut-off concentration, we focused our analysis only on concentrations lower than the 50th percentile within our study sample of 189 specimens (first determination, <190 ng/L; 94 specimens). Nonparametric Passing-Bablok regression analysis showed that after recentrifugation, a median reduction of 10 ng/L (95% confidence interval [-37.5 – -10.0 ng/L]) in troponin I concentrations was observed (Figure 1). A difference plot for the paired data appear in Figure 2.

Discussion

Our data revealed that specimen recentrifugation results in reduction of measured cardiac troponin I concentrations. Since outliers are infrequent using this assay, we are confident that our conclusions are not affected by the presence of these anomalous results. A previous article reported a similar negative bias in comparing single centrifuged vs recentrifuged specimens; however, the effect was attributed to different types of collection tubes. The cause of this phenomenon remains unknown. One possible cause is analyte degradation, instead of interference from suspended particulates. Recentrifugation poses a risk of misclassification.
of patients since troponin results near the cut-off concentration after initial centrifugation may be below the positive threshold after recentrifugation. Additionally, as a routine practice recentrifugation prolongs turnaround time, which can potentially affect patient care.

From these observations, and other known causes of error that affect troponin measurement, recentrifugation does not appear to be a beneficial practice and we believe that it should be avoided. Even the outliers observed in troponin assays, the causes of which remain unknown, can be detected by reanalyzing the specimen without recentrifugation.  

In conclusion, our data reiterate the importance of following guidelines and instructions from the manufacturer to prevent erroneous results; moreover, they stress that extreme caution should be taken in implementing any unproven laboratory procedure in clinical practice. In the near future, it will be interesting to subject high-sensitivity troponin assays to similar analysis. However, the cause of these observed effects of recentrifugation on analyte measurement is known, we believe that no evidence supports recentrifugation of specimens for troponin I measurement in clinical laboratories.  

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References
