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Comparing analytical outliers and the percent of emergency department patients with results above the 99th percentile upper reference limit for 2 conventional and one high sensitivity troponin assay

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ABSTRACT

Objectives: We compared rates of analytical outliers, and percent of emergency department (ED) patients with cardiac troponin (cTn) values above the 99th percentile upper reference limit (URL), for two conventional and one high sensitivity cTn assay.

Methods: We measured 3008 samples from 1931 ED patients by Roche e411 4th generation Troponin T (cTnT); and Abbott STAT Troponin I (cTnI) and high sensitivity troponin I (hscTnI) on an Architect i2000. Within 24 h of initial measurement, samples were aliquoted, re-centrifuged, and repeated in duplicate by all methods. Outliers were defined as one or both replicates exceeding initial value by a critical difference (CD): where $CD = z \times \sqrt{2} \times SD_{analytical}$ ($z = 3.29$ at a probability of 0.0005), *and* at least one replicate on a different side of 99th percentile URL compared to initial value. We also assessed percent of ED patients with values > 99th percentile by all methods (excluding outliers), using both sex-neutral and sex-specific hscTnI URL.

Results: The outlier rate for cTnI (3.66%) was significantly higher than the outlier rate for either cTnT (0.33%) or hscTnI (0.47%) ($p < 0.0001$). More ED patients (33%) had elevated cTnT values compared to either cTnI (25%) or hscTnI (29%). Application of sex-specific URL did not change the percent of ED patients with > 99th percentile hscTnI values.

Conclusion: Abbott STAT cTnI had more analytic outliers than Roche cTnT or Abbott hscTnI. Compared to cTnT, use of hscTnI will significantly decrease the percent of ED patients with elevated cTn values without increasing analytical outliers.

1. Introduction

The introduction of high sensitivity cardiac troponin assays has raised multiple clinical and analytical questions about the ability to smoothly transition to more sensitive assays. These questions include the impact of high sensitivity assays on the rate of analytical outliers (false positive and negative results) and percent of emergency department (ED) patients with cardiac troponin (cTn) values above the 99th percentile upper reference limit (URL) [1].

False positive cTn values, values that are falsely elevated above the 99th percentile URL, are more common than anticipated for immunoassays and may have negative consequences on interpretation of results and clinical management of patients. Analytical false positive cTn results have been attributed to heterophile antibody interference, rheumatoid factor, endogenous alkaline phosphatase activity, and other causes [2–5]. Analytical false positives due to “outliers”, irreproducible analytical errors in cTn analysis, are more common than false positives due to other causes and have been described for most cTn assays [6–10]. One study demonstrated that the outlier rate for a new high sensitivity troponin I assay was lower than that observed for conventional troponin I [9]; while another study found no difference in outlier rates between conventional and high sensitivity assays [8]. False negative troponin results have been described due to anti-troponin antibodies or hemolysis/lipemia interference [11,12].

There are also concerns that use of high sensitivity cTn assays may increase the percent of patients being evaluated for acute coronary

Abbreviations: ED, emergency department; cTn, cardiac troponin; URL, upper reference limit; cTnT, cardiac troponin T; cTnI, cardiac troponin I; hscTnI, high sensitivity cardiac troponin I; CD, critical difference; CV, coefficient of variation

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syndromes that have cTn values > 99th percentile URL, impacting clinical workflow. Several studies suggested that use of high sensitivity cTn assays increases the number of patients with > 99th percentile values; and may even increase the number of patients diagnosed with myocardial injury [13–15]. In contrast another study found that use of high sensitivity troponin I did not impact rate of ED patients with elevated troponin values compared to conventional troponin I [16]. The impact of analytical false positives and false negatives on rates of > 99th percentile URL results has not been systematically studied.

In this study we evaluated the rate of outliers for three troponin assays: Roche 4th generation troponin T (cTnT), Abbott STAT troponin I (cTnI), and Abbott high sensitivity troponin I (hscTnI). Unlike previous studies that relied upon duplicate analysis of samples for outlier identification, we analyzed all samples in triplicate in order to identify outliers. The initial analysis was done on fresh samples collected and processed as a stat clinical sample. After aliquoting and re-centrifugation, samples were analyzed again in duplicate by all methods. This allowed us to classify most outliers as either initial false positive or false negative results. We also compared the number/% of emergency department samples and patients with elevated (> 99th percentile URL) cTn values, after excluding analytical outliers.

2. Materials and methods

2.1. Patient samples and sample processing

3008 samples collected from 1931 Emergency Department (ED) patients who had cTnT ordered clinically in the ED were used for the study. Samples were collected in rapid clot serum separator tubes (BD) and transported by pneumatic tube system to a stat laboratory. Upon arrival in the laboratory, samples were centrifuged at 4000 × *g* for 5 min in a StatSpin 4 centrifuge (Iris Sample Processing). Primary tubes were first put on the Roche Cobas e411 immunoassay analyzer for determination of cTnT using the Roche 4th generation stat TnT assay (Roche Diagnostics). Within 15 min of cTnT analysis, primary tube samples were placed on the Abbott i2000 SR for analysis of cTnI and hscTnI by Abbott STAT troponin I and Abbott high sensitivity troponin I assays (Abbott Diagnostics). Separated serum samples were stored at 2-8C within 2 h of analysis. Within 24 h of initial analysis, samples were warmed to room temperature, aliquoted, and centrifuged at 1500 × *g* for 15 min before analysis in duplicate for cTnT, cTnI, and hscTnI on the same Roche e411 and Abbott i2000 SR analyzers used for initial testing. Enrollment was not consecutive as analysis of samples on the Abbott i2000 SR was available only during certain laboratory shifts. The study was completed between May and October 2015. The study was approved by the Mayo Clinic Institutional Review Board.

2.2. Precision experiments

Quality control was run daily at 2 levels using BioRad Liquichek Cardiac Markers Plus Controls on the Roche e411, and at 2 levels using assay-specific quality control material for the Abbott cTnI and hscTnI assays. Precision for each assay was determined by creating four serum pools with target cTn concentrations of 0.15–0.20 ng/mL cTnT (high pool), 0.05–0.10 ng/mL cTnT (middle pool), 0.01–0.02 ng/mL cTnT (low pool), and 15–30 ng/L hscTnI (very low pool). Serum pools were run 5 times per day over 4 consecutive days (20 times total) to determine mean and standard deviation by each assay (cTnT not detectable in very low pool). Linear regression of SD vs. mean concentration was used to determine $SD_{analytical}$ at manufacturer-defined 99th percentile URL values of 0.010 ng/mL (cTnT), 0.028 ng/mL (cTnI) and 26 ng/L (hscTnI).

2.3. Definition of outliers

Outliers were defined as at least one repeat value (after aliquoting and re-centrifugation) that differed from the initial value by a critical difference (CD): where $CD = z \times \sqrt{2} \times SD_{analytical}$; where the initial and at least one replicate value were on different sides of the 99th percentile URL decision level [8]. In addition to 99th percentile values listed above, we also separately analyzed sex-specific 99th percentile URL values for hscTnI of 15 ng/L (females) and 36 ng/L (males). Previous studies used duplicate analysis in a similar study design of cTN outlier rate and an assumed z-value of 3.48 for a probability of 0.0005 (5 in 10,000) [7–9]. Because we did triplicate analysis (initial and two replicates), we used a z-value of 3.29 which results in a probability of 0.0005 for either one of the two replicates exceeding the CD compared to the initial result. Outlier rates were assessed to see if they significantly differed from the expected rate of 5 per 10,000 using a chi-squared test. Outlier rate among the three cTn assays was evaluated and compared using generalized estimating equations with dependent variable the outlier rate and independent variable cTn assay, assuming a Poisson model with a log link, adjusting the standard errors of the rate estimates per method for multiple observations per person. For comparison of outlier rates to chance and between assays, significance was defined as a $p < 0.05$. Statistical analysis was performed in SAS version 9.4.

Use of a z score to define outliers has limitations, including assumptions about the distribution of replicate results (assumes Gaussian distribution) and dependence upon measured precision to define outliers. To overcome these limitations one previous study used fixed (in addition to measured) SD based upon an assumed 10% CV at the cut-off for troponin assays [7]. We used a similar approach based upon manufacturer claims that assay precision is just above (cTnI) or below (hscTnI) 10% at the URL for these assays. For cTnT, the manufacturer does not make a precision claim for the e411 instrument, but claims < 20% CV near the URL on the e601 platform. We therefore did analysis using a fixed 10% CV assumption for cTnI and hscTnI and 20% CV for cTnT. For this analysis *both* replicates had to exceed the fixed percent difference and be on a different side of URL compared to initial value. The fixed cut-off definitions for outliers were as follows:

TnT: False negative = initial value < 0.01 ng/mL with *both* replicates ≥ 0.012 ng/mL; false positive = initial value ≥ 0.012 with *both* replicates < 0.01 mg/dL.

TnI: False negative = initial value ≤ 0.028 ng/mL with *both* replicates > 0.031 ng/mL; false positive = initial value > 0.031 with *both* replicates ≤ 0.028 mg/dL.

hsTnI sex-neutral: False negative = initial value ≤ 26 ng/L with *both* replicates > 29 ng/L; false positive = initial value > 29 ng/L with *both* replicates ≤ 26 ng/L.

hsTnI sex-specific: False negative = initial value ≤ 15 (female) or ≤ 36 (male) ng/L with *both* replicates > 17 (female) or 40 (male) ng/L; false positive = initial value > 17 (female) or > 40 (male) ng/L with *both* replicates ≤ 15 (female) or ≤ 36 (male) ng/L.

2.4. Percent of samples and patients with > 99th percentile values

We determined the number of ED samples with cTn values above the 99th percentile URL value for all three cTn assays, based upon initial results (and excluding samples with outlier results). For hscTnI, analysis was performed at the sex-neutral 99th percentile URL value of 26 ng/L, and also using sex-specific values of 15 ng/L (females) and 36 ng/L (males). Because most ED patients contributed more than one sample for the study, we also calculated the percent of ED patients with one or more > 99th percentile URL value by each assay. Rates of > 99th percentile URL among the three cTn assays were compared using generalized estimating equations with dependent variable the percent elevated samples or patients, and independent variable cTn assay used (or sex for hscTnI).