Analytical performance of the i-STAT cardiac troponin I assay

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Abstract

Background: This study determines the analytical characteristics of the i-STAT cardiac troponin I assay (cTnI; i-STAT, Princeton, NJ), a 10-min POC assay, designed to be performed at the bedside. Methods: Three different hospitals participated in a patient specimen and analytical validation study (n = 186) for the i-STAT cTnI assay carried out in real time. A total of 186 whole blood specimens (lithium heparin) were collected from patients presenting with symptoms suggestive of acute coronary syndromes (ACS) for correlation studies as well as from 162 healthy subjects for reference interval determination. Factors studied included antibody specificity, detection limit, imprecision, linearity, assay specificity, sample type stability, interferences, reference limit determination and comparison vs. the Dade Stratus CS cTnI assay. Results: Total imprecision (CV) of 10% and 20% were seen at 0.09 and 0.07 μg/l, respectively. The detection limit was 0.02 μg/l. The 99th percentile reference limit was 0.08 μg/l. The assay was not affected by common interferents. An equimolar response within 5% was found for reduced and phosphorylated forms of TIC and IC complexes. Regression analysis for the i-STAT cTnI between whole blood and plasma specimens and for whole blood between the i-STAT and Stratus CS cTnI assays demonstrated slopes of 1.06 and 0.89, respectively. Conclusions: The i-STAT cTnI assay is a sensitive and precise monitor of cTnI, poised for point-of-care/near bedside clinical utilization for triage, diagnostics and risk management of acute coronary syndrome patients.

Keywords: Myocardial infarction; Cardiac troponin; Point-of-care testing; Emergency medicine

1. Introduction

Cardiac troponin (cTn) has been designated as the preferred biomarker for the diagnosis of myocardial injury [1–3]. In the clinical setting of ischemia, the designation of myocardial infarction (MI) is predicated on an increased cTn above the 99th percentile reference cutoff. In addition, prognosis and risk of death and cardiac events are related in part to the extent of increases of cTn in patients with an ischemic mechanism of injury [4]. Differentiating patients with acute coronary syndromes is predicated on an increased cTn (non-ST-elevation MI) and normal cTn (unstable angina) [3]. When the central laboratory is
used to monitor biomarkers the American College of Cardiology, American Heart Association, and the National Academy of Clinical Biochemistry have designated that cTn results be available in < 60 min from the time a patient’s blood is drawn to reporting of results to the clinician [2,5,6]. Point-of-care (POC)/ near bedside testing systems have been developed to reduce delays in specimen transportation and processing that often occur when cardiac markers are measured in a central laboratory. In addition Emergency Medicine has endorsed the need for rapid turn around of cardiac biomarker testing, specifically cardiac troponin. In chest pain centers, patient triage and therapy management are often reliant upon cardiac troponin findings over the initial 6 to 9 h after presentation in the emergency department [7,8]. Quality specifications that apply to cardiac troponin monitoring using central laboratory instrumentation, including both analytical (antibody selection, calibration to appropriate standards, calculation of limits of detection, interferent studies) and pre-analytical (sample storage effects, specimen types) factors, must also be applied in the assessment of POC cTn assays [9]. The goal of the current study was to determine the analytical characteristics of the i-STAT cTnI assay (i-STAT, Princeton, NJ), a 10-min POC assay, designed to be performed at the bedside.

2. Methods

Three different hospital sites as well as i-STAT scientists participated in the study. Patient specimen comparisons were performed against the Dade-Behring Stratus CS (Dade-Behring, Newark, DE). Each hospital site carried out the cTnI analyses in real time on both analyzers. A total of 186 whole blood specimens (lithium heparin) were collected from patients presenting in the emergency department and cardiology units with symptoms suggestive of acute coronary syndromes (ACS). The determination of the final diagnosis of each patient was not part of this study. Normal healthy volunteers (n = 162) without a known history of heart disease or injury were recruited to donate blood for the normal, reference limit determination. All patients gave informed consent following approval of each site’s institutional review board.

All whole blood samples (heparinized) were analyzed by non-laboratory personnel (nurses, research assistants) at all three sites within 60 min of collection on the i-STAT analyzer. Immediately following whole blood analysis, specimens were centrifuged to separate plasma, and the plasma was then immediately reanalyzed by the same non-laboratory personnel on the i-STAT analyzer within 30 min. A separate whole blood tube was also analyzed within 1 h on the Stratus CS analyzer, following manufacturer’s guidelines [10]. As the comparative device, the Stratus CS cTnI assay has been shown to demonstrate the following analytical characteristics: limit of detection 0.03 μg/l (mean + 2SD of 20 determinations of the zero calibrator), upper limit of linearity 50 μg/l, and the concentration at which 10% total imprecision (10% CV) was 0.1 μg/l.

3. Results

The i-STAT cTnI assay demonstrated a limit of detection (mean + 2SD of 20 replicates of the zero calibrator) at 0.02 μg/l (mean + 3SD was 0.03 μg/l) and an upper limit of linearity, determined by serial dilutions of a high cTnI plasma specimen, of 50 μg/l (data not shown). Dilution of whole blood samples is not recommended due to matrix effects. Recovery experiments at approximately 3 μg/l were 101% to 104%.

The monoclonal (capture) and polyclonal (conjugate) antibody paired assay (both directed within the central 30 to 100 amino acids of cTnI) demonstrated equimolar response (within 95%) to the following cardiac troponin I forms (obtained from HyTest, Turku Finland): ternary cTnT–cTnl–cTnC (TIC), binary cTnl–cTnC (IC), and the reduced and phosphorylated forms of TIC and IC. The oxidized form, which was not commercially available, was not tested. In contrast, the relative responses of the Stratus CS to the same cTnl isoforms tested ranged from 83% to 121%. Neither citrated nor EDTA whole blood samples were acceptable for analysis due to interference of chelators. Serum was not tested. Based on spiked whole blood specimens, no interferences were found for cTnT, skeletal troponin I isoforms, heterophile antibodies, rheumatoid factors, bilirubin, lipemia, or numerous drugs. Only heparin, at 90 U/ml, was determined to cause substantial interference.
Total, within-lot and lot-to-lot imprecision over 20 days at concentrations ranging from 0.58 to 33.6 µg/l were < 6.1%. cTnI cartridges, tested for three different lots, and three concentrations of whole blood control specimens (cTnI concentrations of 0.58, 2.38, 4.21 µg/l) demonstrated <4% change stored refrigerated or at room temperature over 14 days.

Fig. 1 shows the low-end precision profile in whole blood spiked with the TIC ternary complex, run on 18 i-STAT analyzers across three lots of cartridges, performed over 1 day. Each point represents the mean statistics for 18 replicates. The lowest concentration demonstrating a 10% coefficient of variation (CV) was approximately 0.09 µg/l and a 20% CV was found at approximately 0.07 µg/l. Regression analysis demonstrated the following equation following removal of 13 specimens with concentrations <0.02 µg/l: [i-STAT cTnI]= 0.89 [Stratus CS cTnI] – 0.035; \( r = 0.97 \); range 0.02 to 36.2 µg/l (n = 173 whole blood specimens). The 95% confidence intervals for slope and intercept were 0.8 to 0.92 and −0.31 to 0.24, respectively. Regression analysis for the same samples for plasma i-STAT vs. whole blood Stratus CS was not significantly different (slope 0.86; intercept −0.032). Regression analysis of the whole blood specimens with cTnI concentration <3 µg/l demonstrated the following equation: [i-STAT cTnI] = 0.88 [Stratus CS cTnI] − 0.036; \( r = 0.97 \) (n = 112). An acceptable correlation was also found between the whole blood and plasma paired specimens (n = 173) for the i-STAT cTnI assay compared across three lots of i-STAT cTnI cartridges: (whole blood cTnI) = 1.06 (plasma cTnI) − 0.07; \( r = 0.99 \). The 95% confidence intervals for slope and intercept were 1.04 to 1.07 and −0.19 to 0.05, respectively. Fig. 2A and B shows the Bland Altman bias plots for cTnI concentrations <10 µg/l (n = 148) for whole blood i-STAT vs. whole blood Stratus CS samples, and i-STAT whole blood and i-STAT plasma samples, respectively. No clinical information was available on any subjects due to IRB restrictions.

The 99th percentile reference limit determined (non-parametric statistic) for 162 normal (healthy) volunteers (55% male, 87% Caucasian, age range 23 to 65 years) demonstrated a whole blood cutoff of 0.08 µg/l and a plasma cutoff of 0.04 µg/l. One hundred fifty one (93%) normal subjects had concentrations less than the limit of detection (<0.02 µg/l). No specific mechanism can be given to explain the whole blood/plasma difference in 99th percentile limits. However, the assay was insensitive to variations in hematocrit in the range of 0% to 65%; with higher hematocrits exhibiting poor imprecision.

4. Discussion

These findings demonstrate acceptable analytical and reference limit characteristics regarding the i-STAT whole blood and plasma POC cTnI assay. The
i-STAT POC cTnI assay was quite close to meeting the ESC/ACC consensus criteria for 10% imprecision at the 99th percentile; demonstrating a ratio of 1.20 [0.09 μg/l, the lowest concentration to give 10% CV/0.08 μg/l, the 99th percentile concentration]. These findings complement the total imprecision findings of Panteghini et al. [11], which addresses imprecision profiles of 17 different cardiac troponin assays involving both central laboratory and POC platforms. The 0.07 μg/l concentration, which demonstrated a 20% CV (functional sensitivity), was <99th percentile limit. In addition, the 99th percentile whole blood reference limit (0.08 μg/l) was fourfold above the lowest detection limit (0.02 μg/l). The few measurable (>limit of detection) cTnI concentrations found in normal volunteers cannot be explained as either false positives or true positives, because the current study was not designed to gather clinical information on these subjects. However, these findings are similar to those observed for other cardiac troponin assays in a larger reference range study [12]. These findings will increase the evidence-based literature for implementing the i-STAT cTnI system along ESC/ACC guidelines [6].

The i-STAT cTnI assay system was described by users at all three hospital sites as easy to use and provided results 10 min after placing a specimen on a cTnI cartridge and initiating analysis on the analyzer; permitting flexibility in use in multiple locations, including cardiac units, emergency departments, chest pain centers, ambulances, and helicopters, as well as in the central laboratory. While a minor negative bias was observed at higher cTnI concentrations (Fig. 2A) between the i-STAT and Stratus CS, these biases would likely not affect the clinical interpretation for either assay. However, as limited data is available around the 99th percentile limits, and no clinical information was gathered in this study, additional larger studies will be necessary to address this finding. The i-STAT system (which measured cTnI directly in whole blood without plasma separation) now provides an alternative to quantitative POC troponin assays (such as the Dade-Behring CS, Biosite Triage, Roche Cardiac Reader, all of which separate plasma from cells prior to cTn analysis) as well as qualitative cardiac troponin assays (Spectral Diagnostics, Roche Cardiac). These claims are supported by the fact that the non-laboratory healthcare workers (nurses, assistants) in cardiac units and emergency departments performed the i-STAT whole blood analyses for patients and controls in the current study. While serum was not tested in the present study, decreased concentrations have been observed in heparinized plasma compared with serum due to direct molecular interaction between cTnI and heparin [13,14]. Therefore, for any cTnI or cTnT assay system, whether point-of-care or central laboratory, direct comparison should be carried out between serum and heparinized plasma to validate potential biases.

In conclusion, the i-STAT assay system (FDA cleared) is now poised for patient care use in emergency departments, chest pain centers, cardiology
units as well as in central laboratories. Patient-based clinical studies, including diagnostics for detection of MI, risk outcomes assessment in ACS patients, and outcome studies in non-ischemic myocardial injury pathologies, are needed for this point-of-care, rapid cTnI assay, to provide the evidence-based support for management of acute coronary syndrome patients in emergency department practice utilizing POC testing systems [7,8].

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References


