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Recommended Citation

Christenson RH, Peacock WF, Apple FS, Limkakeng AT, Nowak RM, McCord J, deFilippi CR. Trial design for assessing analytical and clinical performance of high-sensitivity cardiac troponin I assays in the United States: The HIGH-US study.. *Contemp Clin Trials Commun* 2019; 14:100337.

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Trial design for assessing analytical and clinical performance of high-sensitivity cardiac troponin I assays in the United States: The HIGH-US study

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ARTICLE INFO

Keywords:

High-sensitivity cardiac troponin

Immunoassay

Analytical characteristics

Clinical performance

99th percentile

Sex-specific 99th percentile cutoffs

ABSTRACT

Background: High-sensitivity cardiac troponin I (hs-cTnI) assays have been developed that quantify lower cTnI concentrations with better precision versus earlier generation assays. hs-cTnI assays allow improved clinical utility for diagnosis and risk stratification in patients presenting to the emergency department with suspected acute myocardial infarction. We describe the High-Sensitivity Cardiac Troponin I Assays in the United States (HIGH-US) study design used to conduct studies for characterizing the analytical and clinical performance of hs-cTnI assays, as required by the US Food and Drug Administration for a 510(k) clearance application. This study was non-interventional and therefore it was not registered at clinicaltrials.gov.

Methods: We conducted analytic studies utilizing Clinical and Laboratory Standards Institute guidance that included limit of blank, limit of detection, limit of quantitation, linearity, within-run and between run imprecision and reproducibility as well as potential interferences and high dose hook effect. A sample set collected from healthy females and males was used to determine the overall and sex-specific cTnI 99th percentile upper reference limits (URL). The total coefficient of variation at the female 99th percentile URL and a universally available American Association for Clinical Chemistry sample set (AACC Universal Sample Bank) from healthy females and males was used to examine high-sensitivity (hs) performance of the cTnI assays. Clinical diagnosis of enrolled subjects was adjudicated by expert cardiologists and emergency medicine physicians. Assessment of temporal diagnostic accuracy including sensitivity, specificity, positive predictive value, and negative predictive value were determined at presentation and collection times thereafter. The prognostic performance at one-year after presentation to the emergency department was also performed. This design is appropriate to describe analytical characterization and clinical performance, and allows for acute myocardial infarction diagnosis and risk assessment.

1. Introduction

Cardiovascular disease is the leading cause of mortality of both men and women in the United States (US) and worldwide [1]. Biomarkers are the keystone for making the diagnosis of acute myocardial

infarction (AMI), and cardiac troponin (cTn) is the preferred biomarker for aiding in the AMI diagnosis in patients presenting to the emergency department (ED) with signs and symptoms of AMI [2]. Since their entry into US practice in 1995 [3–6], cTn assays have evolved in analytical sensitivity, which has translated clinically into earlier diagnosis and

Abbreviations: IM, immunoassay; hs-cTnI, High-Sensitivity Troponin I; PPV, positive predictive value; NPV, negative predictive value; ACS, acute coronary syndrome; AMI, acute myocardial infarction; MDP, Medical Decision Pools; CLSI, Clinical and Laboratory Standards Institute; Li-Hep, lithium heparin; cTn, cardiac troponin; cTnI, cardiac troponin I; hs-cTn, high-sensitivity cardiac troponin; LoB, Limit of Blank; LoD, Limit of Detection; LoQ, Limit of Quantitation; URL, upper reference limit

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<https://doi.org/10.1016/j.conctc.2019.100337>

Received 19 December 2018; Received in revised form 22 January 2019; Accepted 13 February 2019

Available online 15 February 2019

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earlier rule-out of AMI. The most analytical sensitive assays are termed 'high-sensitivity cardiac troponin' (hs-cTn), and have been defined per recommendations of the Academy of the American Association for Clinical Chemistry (AACC) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)'s Committee for Clinical Application of Cardiac Bio-markers (C-CACB) [2,7–11]. These recommendations address 1) total imprecision at the sex specific 99th percentile upper reference limit (URL) of healthy men and women must be less than or equal to a total coefficient of variation (CV) of 10%; and 2) at least 50% of samples from cohorts of healthy men and healthy women must each have cTn values equal to or exceeding the assay's limit of detection (LoD) [7]. Although the definition of hs-cTn assays is analytical, the benefits of these hs-cTn assays are intended to be clinical, and include earlier time to AMI diagnosis after acute cardiac events [7].

Appropriate study design and conduct of studies intended for characterizing hs-cTn assays are essential for assuring that any device is safe and effective for use in patient care. Our purpose is to present an approach for designing and conducting US Food and Drug Administration (FDA) 510(k) investigations that characterize hs-cTn assays, both analytically and clinically. This methodology was used to determine the nature of multiple Siemens Healthcare Diagnostics Inc. ("Siemens Healthineers") hs-cTn assay systems intended as an aid for diagnosis and risk assessment of patients presenting to EDs with signs and symptoms suggestive of AMI.

2. Materials and methods

2.1. Clinical performance studies

2.1.1. Ethics and governance

Documented approval was obtained from the local Institutional Review Board from all participating centers before the study initiation at a clinical site, according to Good Clinical Practice and local laws and regulations. Written informed consent was obtained from all participants as part of enrollment.

2.1.2. 99th percentile upper reference limits (URLs)

Studies were conducted in a healthy population recruited in accordance with Clinical and Laboratory Standards Institute (CLSI) document EP28-A3c [12] using both lithium heparin plasma (Li-Hep) and serum samples. Twelve sites across the US enrolled subjects 22 years or older, who completed a comprehensive medical history questionnaire and self-reported that they were in generally good health with no symptoms of heart disease. Subjects with the following findings were excluded from enrollment in the study: history of vascular or cardiovascular disease, hypertension, diabetes mellitus, chronic renal disease, rheumatoid arthritis, muscle/skeletal disease or injury currently under the care of a physician, cancer, history of heavy alcohol intake (defined as consuming on average more than two drinks per day or more than 14 drinks per week for men, and on average more than one drink per day or more than seven drinks per week for women; one drink is approximately 14 gm of pure alcohol), morbid obesity defined as body mass index (BMI) above 40, and pregnancy. Subjects were also excluded from enrollment if taking cardio-active drugs with the exceptions of aspirin and statins taken in prophylaxis or for dyslipidemia without a confirmed diagnosis of atherosclerosis. Enrollment was stratified per age group and sex in order to include a population representative of the US.

For analysis of the 99th percentile URL from healthy subject study, the SAS System for Windows (ver. 9.3) was used to compute the 99th percentile URL non-parametrically in accordance with CLSI guideline EP28-A3c [12], with PROC Univariate using the type 4 definition. The 90% confidence interval was also computed.

2.1.3. Clinical performance in the emergency department

Twenty-nine sites across the US enrolled subjects, ≥ 22 years of age,

Table 1
Blood sample collection timetable.

Group	Targeted Blood Collection Times
1	T ₀ : baseline (within ± 90 min of the first local standard of care blood draw for troponin)
2	T ₁ : 45–75 min after T ₀
3	T ₃ : 2–3 h after T ₀
4	T ₆₋₉ : 6–9 h after T ₀
5	T ₁₂₋₂₄ : 12–24 h after T ₀

presenting to the ED with symptoms suspicious for AMI as determined by the treating ED physician and able to be enrolled within 1.5 h of their first clinical blood draw. Possible symptoms of ischemia include one or more of the following: chest, upper extremity, mandibular, or epigastric discomfort during exertion or at rest, or dyspnea or fatigue [2,10]. The study had no enrollment exclusion criteria, except that patients had to be willing to participate and provide informed consent in accordance with Institutional Review Board requirements. Patients were enrolled over the time period from April 2015 to April 2016. Paired Li-Hep and serum baseline samples were collected as soon as possible after presentation and obtaining informed consent (T₀), and at the following four additional time points displayed in Table 1 whenever possible. Samples collected outside of these time windows were retained and tested. All samples were centrifuged within four hours of collection, held at 4 °C until separation into six to seven aliquots to create six sets of ED samples that were then frozen and stored locally at -20 °C or colder within six hours of collection. Samples were all shipped to a Core Laboratory repository on dry ice where they remained frozen during organization and were thereafter maintained at -70 °C or colder. Hospitalized subjects known to have elevated cTn levels were enrolled in a stability study and matched Li-Hep and serum samples were collected from each subject. Samples were centrifuged within two hours of collection. Fresh aliquots were immediately tested by the ADVIA Centaur TNIH assay. The remainder of the plasma and serum was aliquoted into cryovials and frozen at -70 °C up to 690 days. At regular time intervals, aliquots were thawed and cTnI measured using the TNIH assay. Data were plotted with % bias on the y-axis and number of days on the x-axis. A linear regression was performed. The sample stability duration is estimated as one day before the one-sided 95% confidence interval of the regression line intersects with the acceptance criterion of $\pm 10\%$ bias. (Data not included here; however, stability data will be included in a relevant publication utilizing the HIGH- US samples.)

2.1.4. Sample testing

Characterization of analytical parameters was determined for the Atellica[®] IM TnI, ADVIA Centaur[®] TNIH, Dimension Vista[®] 1500 TNIH, and Dimension EXL[®] 200 TNIH[™] Systems in respective clinical trials. By design, the characterization testing for each system was examined at three sites, each of which performed approximately one third of the required testing. Samples were shipped on dry ice from the Core Laboratory in batches to the three qualified clinical testing sites. The study was designed to collect samples within the protocol targeted time windows (Table 1); however samples collected outside of these windows were eligible for analysis.

2.1.5. Subject clinical classification

Patients were classified for AMI status by adjudication, based on the Third Universal Definition of Myocardial Infarction consensus guideline endorsed by the European Society of Cardiology (ESC), the American College of Cardiology Foundation (ACCF), the American Heart Association (AHA), and the World Heart Federation (WHF) [10]. Subject demographics, height, weight, time of onset of last symptoms to blood draw, time for first blood draw from ED presentation, ED admission information, cardiac therapies, cardiac risk factors, medical

history, concurrent therapies, ED discharge summary or transfer note, hospital discharge summary, and treating ED physician initial suspicion of ACS prior to knowing the first cTn measurement result and local standard of care cTn results were provided to the adjudicators. The 29 enrolling sites used any FDA cleared cTn assays (see Supplemental Table 1 for listing) to perform the local standard of care cTnI measurements used for the adjudication. A total of 25 adjudicators were involved. To avoid possible adjudicator panel bias, data for each subject was reviewed by a unique combination of five adjudicators. Approximately half the panels included two cardiologists and three emergency physicians, and half included three cardiologists and two emergency physicians. The majority rule was applied to determine the final AMI classification. Adjudicators were blinded to cTnI results from the new Siemens Healthineers assays being examined and to the patient diagnosis established by the treating hospital. The adjudicators had access to both the manufacturers' package insert cutoffs and to the locally established cTn cutoffs (where applicable) for the diagnosis of AMI. Each adjudicator independently used their expert opinion to assess if the requirements of an AMI adjudicated diagnosis were met, including the presence of a clinically significant rise and/or a fall of cTn levels, as determined by the experienced adjudicators. For this study no relative or absolute threshold was pre-specified for a significant rise and/or fall of cTn levels.

2.2. Analytical performance studies

Characterization of the analytical parameters was determined for the Atellica IM TnIH, ADVIA Centaur TnIH, Dimension Vista 1500 TnIH, and Dimension EXL 200 TnIH Systems in respective clinical trials. The Atellica IM TnIH and ADVIA Centaur TnIH assays are three-site

sandwich immunoassays sharing the same assay design, antibodies (Abs) and direct chemiluminometric technology (Fig. 1 Panel A). Capture epitopes are amino acids 41–50 and 171–190; and the detection epitopes are amino acids 29–34 [13]. Solid Phase Reagent is made of magnetic latex particles conjugated with streptavidin and two bound biotinylated capture monoclonal Abs (one sheep and one mouse) each recognizing a unique cTnI epitope. (The two Abs are pre-bound to the solid phase reagent via streptavidin which eliminates biotin interference.) The solid phase-cTnI complex is detected through binding to Lite Reagent, which consists of recombinant anti-human cTnI sheep Fab and acridinium ester tag for chemiluminescent signaling [14], each bound to bovine serum albumin. A direct relationship exists between the amount of cTnI present in the sample and the amount of chemiluminescent generated relative light units detected by the system [15,16].

The Dimension EXL and Dimension Vista TnIH assays share the same assay design and Abs (Fig. 1 Panel B). The capture epitopes recognized by Abs are amino acids 29–34; the detection epitopes recognized by Abs are amino acids 41–50 and 171–190 [13]. As indicated in Fig. 1 Panel B, the assays employ Luminescent Oxygen Channeling Immunoassay (LOCI) technology that utilizes two sheep monoclonal Abs, one is an intact antibody that coats chemibeads (capture) and the other is an F(ab')₂ fragment (detection), plus one mouse monoclonal Fab fragment (detection). cTnI in the patient sample is 'sandwiched' between the chemibead-sheep monoclonal Ab conjugate and the biotinylated sheep F(ab')₂ and mouse Fab fragments that then bind to streptavidin-coated sensibeads. Sensibeads bind tightly to available Ab-biotin sites on the complex, bringing the acridinium ester chemiluminescent dye particle in close proximity. Exposure of sensibeads to light at 680 nm leads to their release of short-lived singlet oxygen (¹O₂) molecules that react with the acridinium ester in the capture-Ab bead. A

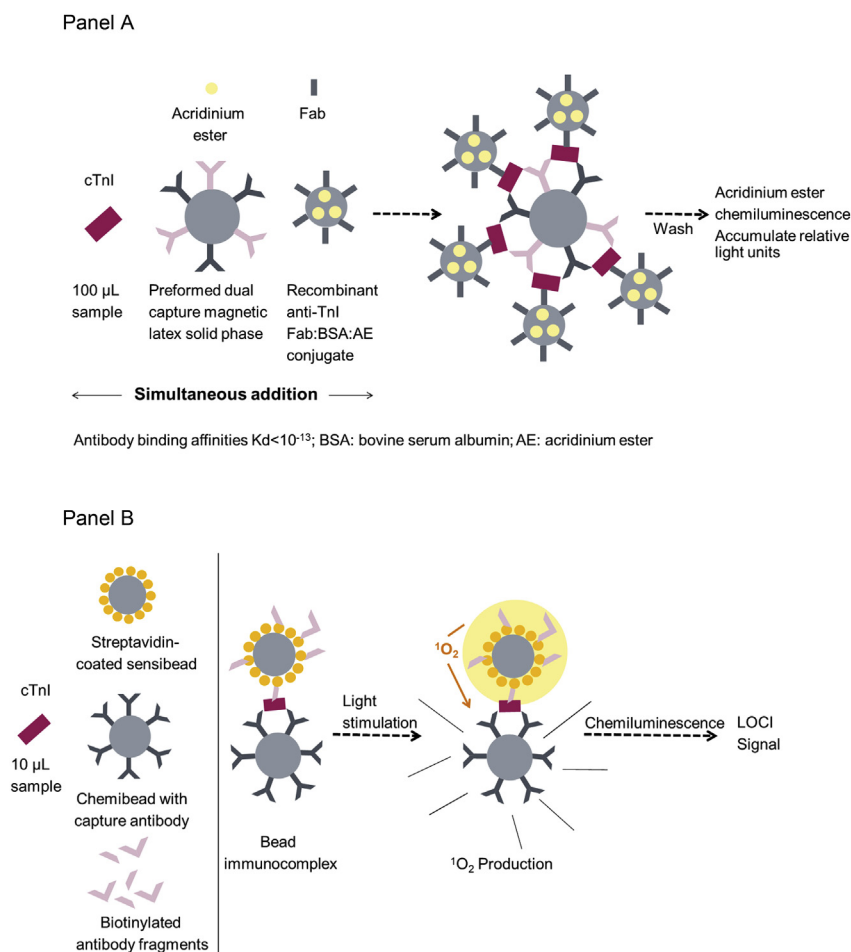


Fig. 1. Panel A. Siemens Healthineers' hs-cTnI assay architecture for the Atellica IM TnIH Assay and the ADVIA Centaur XP/XPT TnIH assays. Patient sample containing cTn complexes and fragments is mixed with the solid phase magnetic latex beads coated with sheep and mouse monoclonal Abs for capture of cTnI. Lite reagent is also added and contains anti-human recombinant sheep Fab fragment linked to bovine serum albumin (BSA) labeled with acridinium ester (chemiluminescent signal producing substance). The complex is washed and addition of acid/base reagent results in a chemiluminescent signal read by the detection system. The time to first result with the Atellica IM TnIH Assay TTF is 9.8 min; the analytical time for the ADVIA Centaur XP/XPT TnIH assay is 18 min.

Panel B. Components of Luminescent Oxygen Channeling Immunoassay (LOCI) technology used for hs-cTnI measurement on the Dimension Vista and EXL Systems. Dye containing chemibeads coated with sheep monoclonal anti-cTnI Abs (capture) and two soluble, biotin labeled monoclonal Ab fragments (one sheep and one mouse) are added to samples containing cTnI to form [Capture-Ab:cTnI:Ab-Biotin] complexes. A streptavidin-coated particle reagent capable of generating singlet oxygen (Sensibead) is added to the mixture. Sensibeads bind avidly to available Ab-biotin sites on the complex, thereby tethering the acridinium ester chemiluminescent dye particle in close proximity. Upon exposure to light at 680 nm, the Sensibeads release short-lived singlet oxygen (¹O₂) molecules that react with the chemiluminescent dye in the capture-Ab bead, causing emission of light at 612 nm, which is read by the detection system. The time to first result on both systems is 10 min.

chemiluminescent signal is triggered and measured at 612 nm, and is a direct function of the cTnI concentration in the sample [17,18].

Detection Capability of the systems was performed in accordance with CLSI document EP17-A2 [19].

Limit of blank (LoB), defined as the 95th percentile value of assay blank samples (e.g., Li-Hep or serum samples in which cTn epitopes were masked) was performed using a nonparametric statistical approach. The details for each system varied; however, each study included a minimum of two reagent lots, measured on three non-consecutive days for each instrument system, and included at least four blank samples. LoB analysis was performed on both Li-Hep plasma and serum sample matrices. A representative study, performed using an Atellica IM Analyzer, employed four Li-Hep plasma blanks and four serum sample blanks, with each of three unique hs-cTnI reagent lots. A total of 1440 measurements were obtained with 480 measurements per reagent lot or 240 measurements for each reagent lot/sample matrix combination. The claimed LoB for the Siemens Healthineers hs-cTnI assay represents the highest LoB observed for all combinations of reagent lots/sample.

Limit of detection (LoD), defined as the lowest analyte concentration detected above the LoB with a probability of 95%, was determined based on LoB and standard deviation (SD) of low concentration samples. Testing included a minimum of two reagent lots, measured on three days, on two systems for each type of instrument system. LoD analysis was performed on both Li-Hep and serum sample matrices. For example, the Atellica IM Analyzer study included 13 samples (six low cTnI Li-Hep plasma pools and seven low cTnI serum pools) in a 20 day, two run per day, three reagent lots, on a minimum of two analyzers, collecting a total of 1040 measurements. The LoD represents the highest calculated LoD across all combinations of instruments, sample matrices and reagent lots.

Limit of quantitation (LoQ), or functional sensitivity, was estimated in accordance with CLSI document EP17-A2 and CLSI document EP05-A3 [19,20]. The LoQ represents the highest observed concentration at 20% CV and the highest concentration determined from the individual reagent lot and instrument combination. As an example, the Atellica IM Analyzer study included three reagent lots tested, and the sample types included six low cTnI Li-Hep plasma pools and seven low cTnI serum pools. For each reagent lot and instrument combination, the within-lab precision over 20 days for each sample, expressed as percent %CV, was plotted against the mean concentration of each sample. Data were fitted using a power function to yield a precision profile.

Analytical measurement range (AMR), or linearity was determined by a series of Li-Hep and serum samples containing cTnI measured in triplicate spanning from the LoQ to the upper measurable limit without dilution. Patient samples exceeding the upper range of reportable results were auto-diluted. Onboard auto-dilutions were performed for all systems using Siemens Healthineers specified diluent.

Precision studies were performed in accordance with CLSI document EP05-A3 to assess repeatability (within-run) and total (within-lab) imprecision [20]. The studies used multiple reagent lots, one calibrator lot per reagent lot, and multiple systems per study. The systems were calibrated every 14 days, and the testing included 20 test days, two runs per test day with a minimum of two hours in between, and two replicate measurements per sample. Samples included control serum pools; contrived high and low spiked Li-Hep plasma and serum samples. Fresh frozen aliquots were thawed daily.

Reproducibility, i.e., between site imprecision, was determined at multiple cTnI concentrations at three sites, over five days, with two runs per day and three replicates per run using one lot of reagent and one lot of calibrator in accordance with CLSI document EP05-A3 [20]. These data were utilized to assess reproducibility, repeatability and within-laboratory precision for the three sites. Reproducibility was estimated by incorporating laboratory-to-laboratory, instrument-to-instrument and within-laboratory imprecision in accordance with CLSI document EP05-A3 [20].

Method comparison studies were conducted in accordance with CLSI document EP09-A3 for the various assays [21]. A minimum of 950 samples was used across the assay range. In this way, harmonization between the systems could be determined with one cTnI reagent lot.

Analytic specificity was tested using human samples containing cTnI concentrations near the overall 99th percentile URL of healthy individuals for each platform. Substances including cardiac troponin T, skeletal troponin I, tropomyosin, actin, troponin C, myosin light chain, myoglobin or CK-MB were added, each at a concentrations of 1,000,000 ng/L. Results from the tested hs-cTnI assay system for the spiked samples were compared with those of unspiked control samples. Cross-reactivity, in percent, was determined in accordance with CLSI Document EP07-A2 [22] and was calculated as:

$$\% \text{ cross - reactivity} = \frac{(\text{hs - cTnI concentration of spiked sample} - \text{hs - cTnI concentration of unspiked sample})}{\text{Concentration of substance spiked into sample}} \times 100\%$$

Interference testing was performed in Li-Hep plasma and serum samples having cTnI concentrations near the 99th percentile URLs of 20–60 ng/L and at a more elevated value of 1000–2000 ng/L in accordance with CLSI document EP07-A2 [22]. Compounds for interference testing included hemoglobin, triglycerides, conjugated bilirubin, unconjugated bilirubin, biotin, cholesterol, and over 50 cardiovascular-associated drugs. A cTnI value $\leq 10\%$ of the initial target cTnI value was considered as no interference.

High Dose Hook Effect was examined with Li-Hep samples with cTnI values up to 500,000 ng/L, i.e., 20-fold higher than the assay's AMR of 25,000 ng/L. Reporting of a value other than ' $> 25,000 \text{ ng/L}$ ' was considered positive for the high dose hook effect.

High-Sensitivity Performance Assessment. This was determined as recommended by the AACC Academy and IFCC Committee for Clinical Application of Cardiac Bio-markers (C-CACB) [7]. Total imprecision at lowest sex-specific 99th percentile URL was determined according to CLSI document EP12-A2 [23]. Assays were used to measure samples from healthy cohorts of ≥ 300 men and ≥ 300 women to determine if $\geq 50\%$ of each sex had cTnI values equal to or exceeding the assay's defined LoD. For this determination, a commonly available cohort, the AACC Universal Sample Bank (USB), was utilized [24]. The characteristics of this repository have been described previously [24], but briefly all samples were collected from self-selected volunteers that participated in The AACC USB activity as part of the 2015 AACC Annual Meeting and Exposition in Atlanta, GA (84% of samples) or were individuals from the Baltimore, MD community (16% of samples), who volunteered to participate within the same time frame at the University of Maryland School of Medicine (Baltimore, MD). The protocol and conduct of the AACC USB was approved by Emory University's Institutional Review Board. Specimens were processed and frozen on dry ice within 60 min of collection, and then stored at -70°C in the Repository at the Center for Disease Control and Prevention in Atlanta, GA. To examine for possible underlying health conditions, aliquots of samples from all subjects were tested for the surrogate biomarkers Amino-terminal proB-type natriuretic peptide (NT-proBNP); hemoglobin A_{1c}; and creatinine, which was used with each subject's age, sex and race to estimate glomerular filtration rate with the equation from the Chronic Kidney Disease (CKD) Epidemiology Collaboration as described earlier [25].

2.3. Statistical methods: clinical performance analyses

2.3.1. Hypotheses

The study tested the following hypotheses. H0: The assay has sensitivity for AMI greater than or equal to 90%; H1: The assay has less than 90% sensitivity.

2.3.2. Sample size

This protocol describes the enrollment of up to 3000 ED subjects to support the validation of multiple Siemens Healthineers TnI assays; however, we tested approximately 2000 of these subjects on each individual assay. For the assessment of diagnostic accuracy and prognostic performance in subjects with AMI, we included a minimum of approximately 2000 adults who presented for emergency care with signs and symptoms of ACS. We anticipated approximately 8%–12% would be diagnosed with AMI, representing 160 to 240 subjects. Given a sensitivity of 90% and an alpha of 0.05 (95% confidence), a sample of 179 subjects would have 80% power to detect a difference of 6% or greater between the hypotheses.

2.3.3. Diagnostic accuracy

This was defined as the medical concordance between the 99th percentile URL and the presence or absence of AMI diagnosis at each of the time points. In addition to the pooled assessment for all subjects, separate assessments were conducted for each sex-specific subgroup using sex-specific specific 99th percentile URLs previously established using the population of apparently healthy subjects. Four measures of diagnostic performance were calculated: sensitivity, specificity, positive predictive value, and negative predictive value. Sensitivity was defined as the proportion of subjects with an assay result above the cutoff among all subjects diagnosed with AMI. Specificity was defined as the proportion of subjects with an assay result equal to or below the cutoff among all subjects not diagnosed with AMI. Positive predictive value was defined as the proportion of subjects diagnosed with AMI among all subjects with assay results above the cutoff. Negative predictive value was defined as the proportion of subjects not diagnosed with AMI among all subjects with assay results equal to or below the cutoff. The 95% Wilson score confidence intervals for sensitivity, specificity, positive predictive value and negative predictive value were estimated [26].

Prognostic performance was assessed at 30-day, 90-day, 182-day and 365-day after baseline T_0 . Endpoints were mortality from all causes and major cardiac events including cardiovascular death, non-fatal MI, heart failure admission, stroke, or revascularization after the index admission. The product-limit method of Kaplan-Meier was used to estimate survivorship, i.e., the proportion of subjects with an initial T_0 reading above the cut-off who reach endpoints within 30, 90, 182 and 365 days, for all subjects and for each sex-matrix subgroup. Cox regression was used to calculate the hazard ratios for each sex-matrix subgroup. A hazard ratio was defined as the proportion of subjects with an initial T_0 reading above the cutoff who reach endpoints divided by the proportion of subjects with an initial T_0 reading equal to or below the cutoff who reach endpoints. Adjustment for demographics, traditional cardiovascular risk factors, presence of known cardiovascular disease, and renal function was also assessed.

3. Discussion

3.1. Importance of study design for safety and effectiveness

Each year, approximately 10 million people in the US present to the ED with signs and symptoms suggestive of AMI; of these, 625,000 patients (~6.25%) are diagnosed with ACS. Of the 625,000 ACS patients, 70% (437,500) are ultimately diagnosed with non-ST-elevation (NSTEMI)-ACS (NSTEMI plus unstable angina) that are often not identified by the ECG [27,28]. Accurate and rapid identification of AMI patients is critical to saving lives, and avoiding unnecessary procedures, lengthy stays and overcrowding in EDs, patient anxiety, and associated healthcare costs [2,10,29–31]. Analytically, hs-cTnI assays are superior for detecting very low cTn concentrations with far better precision than contemporary assays used in the U.S currently.

hs-cTnI assays are not harmonized or standardized; therefore, values from one assay cannot be directly compared with those from

another assay. This report describes the design and rationale for the analytical and clinical performance validation of hs-cTnI assays in ED patients presenting with signs and symptoms of AMI across the US. Here we describe the study design and conduct for characterizing hs-cTnI and for determining the optimal clinical cutoff points for safe and effective rule-out and rule-in of AMI in ED populations, based on FDA 510 (k) requirements. For rule-out, guideline recommended optimal cTnI cutoff points are detection capability parameters (e.g., LoD) and low cutoff values [32], and for rule-in, the guideline recommended optimal cut point is the 99th percentile URL with a temporal rise and/or fall in cTn levels. Superior precision is imperative for both rule-out strategies based on the LoD or low cutoff values, for determining significant changing patterns, and for rule-in strategies based on the 99th percentile. Additionally, this study collected samples and data to allow evaluation of the prognostic utility of hs-cTnI assays up to one year after the index presentation.

hs-cTnI assays and accelerated protocols (0/1-h, 0/2-h, and 0/3-h), often termed Accelerated Diagnostic Protocols (ADPs), have been in use for several years outside the US, (e.g., Europe, Australia, and New Zealand) [32,33]. As hs-cTn assays become available in the US, some of these accelerated protocols may be safely adopted, due to the improved sensitivity and precision of the hs-cTn assays [34].

3.2. Central role of cardiac troponin for management of coronary heart disease and other diseases

As with the Third Universal Definition of MI used for adjudication in the current study design, the Global Task Force for the Fourth Universal Definition of MI defines myocardial injury (cell necrosis/death) as detection of cTn values above the 99th percentile. A 2018 expert consensus document by the AACC Academy and IFCC Committee for Clinical Application of Cardiac Bio-markers (C-CACB) has provided an update to the definition and analytical performance requirements of cTn assays [7,9,10,35].

hs-cTnI measurements will be used in diseases other than suspected AMI including perioperative myocardial injury associated with non-cardiac procedures [2], such as heart failure and Takotsubo syndrome [2], and different study designs will need to be developed for those applications. Also, diseases other than ACS myocardial injury may be associated with non-ischemic, non-cardiac (systemic) conditions such as chronic kidney disease. Elevations above the 99th percentile URL have been observed in most end-stage renal disease patients when using hs-cTn assays, and documenting a changing temporal pattern for diagnosing AMI is important for the CKD and dialysis populations, just as it is for patients with normal renal function [36].

3.3. Strength of study design

Several experienced clinical trial experts along with the US FDA reviewed early protocols and made recommendations to the study design. The clinical experts have participated in several similar trials on patients presenting to the ED with suspected AMI for other cTn assays, and have published their findings in peer-reviewed journals [37–44]. In addition, these experts have contributed to setting international guidelines for use of hs-cTn assays [2,7,9,35]. By design, all subjects presenting to the ED with signs and symptoms of AMI were eligible for inclusion in this study. Not excluding any patients presenting with suspected AMI translates into the findings being more applicable to an unbiased population, as seen in real-life clinical scenarios. In addition, clinicians will have more confidence to use these hs-cTnI assays. Other strengths include that the study was prospective and samples were collected at multiple sites throughout the US; samples were designed to be collected from a large population of diverse subjects; cTnI values were measured in real-world laboratories as well as at Siemens Healthineers (customer simulated environment laboratories). Furthermore, endpoint adjudication used a large pool of experts with diverse

backgrounds. Compared to earlier studies, our intention was to draw the study's first sample (T_0) as close as possible to specimen collected for clinical use.

3.4. Limitations

This study design has several limitations. First, results generated from this US study may not apply to situations outside of the US. Furthermore, the use of multiple local cTn assays to determine the adjudicated diagnosis of AMI may have provided a reference standard that was less than perfect because some of the standard of care assays were clearly inferior to the Siemens Healthineers comparator. Second, a possible limitation was the use of a healthy control population that may have resulted in 99th percentile URL values that were distinctly lower than the usual population of patients presenting to the ED with suspected ACS. Although no healthy individuals had evidence of cardiovascular disease, some subjects taking statins were enrolled which may have lowered cTn values [45]. Using this population likely increased the sensitivity compared to a population with a baseline rate of co-existent pathology, e.g., patients with diabetes, who smoked, and had a prior AMI, etc. Third, although there were no enrollment exclusions, we did not collect samples from all patients presenting to the ED, seven days a week, 24 h a day, so there may have been a selection bias. Further, some eligible patients chose not to participate. Fourth, the investigative teams were unable to obtain samples at all time points from every patient. Furthermore, the volume available for some samples limited testing of all samples on all platforms at all time points, which could have affected diagnostic and/or prognostic performance. Although substantial efforts were made to collect the initial study sample as close as possible to the first clinical specimen, the unavoidable requirement for an appropriate informed consent process necessarily delayed the collection of the first study sample (T_0) compared to timing of the earliest clinical sample.

3.5. Conclusion

This study will be used to define the analytical and clinical performance of the Atellica TnIH, ADVIA Centaur TNIH, Dimension EXL TNIH, and Dimension Vista TNIH assays, based on FDA requirements for patient enrollment. Analytical parameters (LoB, LoD, LoQ) at the lower end of the assay range, imprecision, linearity, 99th percentile URL diagnostic cutoff values, and high-sensitivity performance were determined and used to assess safety and clinical efficacy of the assay for AMI diagnosis and one-year prognosis. Improving triage of myocardial injury patients will lead to better management of patients, thereby improving outcomes and decreasing medical costs.

3.6. Clinical perspectives

More accurate diagnosis of AMI and myocardial injury conditions using hs-cTnI assays can lead to more timely and efficient triage, more accurate therapeutic and management approaches, reduced time in the ED, reduced healthcare costs, reduced disease complications, and improved clinical outcomes.

Acknowledgements

Funding

The HIGH-US study was supported/funded by Siemens Healthineers, 511 Benedict Avenue, Tarrytown, NY 10591, USA. This research did not receive any specific grant from funding agencies in the public, or not-for-profit sectors.

OR, if open access the following would be used

This work is funded/supported by Siemens Healthineers Laboratory Diagnostics Inc.

Author contributions

All authors made substantial contributions to this manuscript. All authors were involved in drafting the manuscript and critically revising it for intellectual content and accuracy. All authors have approved the final version of the manuscript for publication.

Disclosures

Dr. R.H. Christenson has received fees from Siemens Healthineers for consultancy work on design and conduct of high-sensitivity cardiac troponin I clinical trials, and is a consultant for Siemens Healthineers, Roche Diagnostics, Quidel Diagnostics, and Beckman Coulter.

Dr. F. Peacock has received research Grants from Abbott, Braincheck, Immunarray, Janssen, Ortho Clinical Diagnostics, Relypsa, Roche. He has served as a consultant to Abbott, Astra-Zeneca, Bayer, Beckman, Boehringer-Ingelheim, Ischemia Care, Dx, Immunarray, Instrument Labs, Janssen, Nabriva, Ortho Clinical Diagnostics, Relypsa, Roche, Quidel, and Siemens Healthineers. He has provided expert testimony for Johnson and Johnson, and has stock/ownership interests in AseptiScope Inc, Brainbox Inc, Comprehensive Research Associates LLC, Emergencies in Medicine LLC, and Ischemia DX LLC.

Dr. F. Apple's disclosures are as follows. Board of Directors, HyTest; Advisory Board, Instrumentation Laboratory, Siemens Healthineers; Consultant, LumiraDx. Non-salaried PI through the Hennepin Healthcare Research Institute for cardiac troponin studies from Abbott Diagnostics, Abbott POC, Ortho-Clinical Diagnostics; Siemens Healthineers, and Beckman Coulter.

Dr. A.T. Limkakeng has received grant funding from Roche Diagnostics, Inc., Abbott Laboratories, Bristol Meyers Squibb, Ischemia Care, LTD., GE, and Astrazeneca. He has served as a consultant to Biomerieux and ZS Pharma.

Dr. R.M. Nowak has received fees from Siemens Healthineers as a consultant for the design and conduct of this high-sensitivity cardiac troponin I trial. Additionally, he has been or is a consultant for Siemens Healthineers, Roche Diagnostics, Beckman Coulter, Ortho Diagnostics, and Abbott.

Dr. J. McCord has received research support from Roche, Siemens Healthineers, Abbott, and Beckman, and has served as a consultant for Roche and Siemens Healthineers.

Dr. C. deFilippi has received research support through his institution Inova. In addition, he consults for Abbott Diagnostics, FujiRebio, Metabolomics, Ortho Diagnostics, Roche Diagnostics, and Siemens Healthineers. He has received honorarium from WebMD and Royalties from UpToDate.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.conctc.2019.100337>.

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