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Highly Sensitive Immunoassay for Long Forms of Cardiac Troponin T Using Upconversion Luminescence

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BACKGROUND: Long cardiac troponin T (cTnT) has been proposed to be a promising and more specific biomarker of acute myocardial infarction (AMI). As it represents a subfraction of circulating cTnT, detection of very low concentrations is a requirement. The aim of this study was to develop a novel, highly sensitive immunoassay for long cTnT.

METHODS: A two-step sandwich-type immunoassay for long cTnT was developed, utilizing upconverting nanoparticles (UCNPs) as reporters. The limits of detection and quantitation were determined for the assay. Linearity and matrix effects were evaluated. Performance with clinical samples was assessed with samples from patients with non-ST elevation myocardial infarction (NSTEMI, n = 30) and end-stage renal disease (ESRD, n = 37) and compared to a previously developed time-resolved fluorescence (TRF)-based long cTnT assay and a commercial high-sensitivity cTnT assay.

RESULTS: The novel assay reached a 28-fold lower limit of detection (0.40 ng/L) and 14-fold lower limit of quantitation (1.79 ng/L) than the previously developed TRF long cTnT assay. Li-heparin and EDTA plasma, but not serum, were found to be suitable sample matrixes for the assay. In a receiver operating characteristics curve analysis, the troponin ratio (long/total cTnT) determined with the novel assay showed excellent discrimination between NSTEMI and ESRD with an area under the curve of 0.986 (95% CI, 0.967–1.000).

CONCLUSIONS: By utilizing upconversion luminescence technology, we developed a highly sensitive long cTnT assay. This novel assay can be a valuable tool for investigating the full potential of long cTnT as a biomarker for AMI.

ClinicalTrials.gov Registration Number: NCT04465591

Introduction

Cardiac troponins (cTn) are the most important and widely used biomarkers for the diagnosis of acute myocardial infarction (AMI). As cTn is a biomarker of myocardial injury, testing the patient blood sample for cTn plays a particularly significant role in the diagnosis of non-ST elevation myocardial infarction (NSTEMI) (1). However, commercial high-sensitivity cTn (hs-cTn) assays can also detect cTn elevations in other conditions such as atrial fibrillation, heart failure, chronic kidney disease, sepsis, and strenuous exercise, complicating the interpretation of the test results—especially if the patient presents with AMI-like symptoms (2–6).

Cardiac troponin T (cTnT) is known to be susceptible to proteolytic degradation, and multiple cleavage sites have been identified on the cTnT molecule (7). During AMI, cTnT is released from ischemic cardiomyocytes as a combination of intact molecule (approximately 40 kDa) and fragments of various sizes (8 to 37 kDa). The long forms of cTnT (intact cTnT and long fragments of cTnT, 28 to 40 kDa) are most prominent in early-presenting AMI patients (7–12). Interestingly, only small cTnT fragments (<18 kDa) have been detected in marathon runners and patients with end-stage renal disease (ESRD), indicating different mechanisms of cTnT release (13, 14). However, these studies have been conducted with small numbers of samples and methods with limited sensitivity. Commercial hs-cTnT assays target the stable central part of the cTnT molecule and recognize intact cTnT as well as mildly and heavily truncated cTnT fragments (Fig. 1). Therefore, such assays can be called "total cTnT" assays (11).

Recently, a simple time-resolved fluorescence (TRF) immunoassay based on europium(III) chelate labels was developed for the measurement of long cTnT molecules (Fig. 1). The assay showed increased specificity for AMI by discriminating between cTnT elevations

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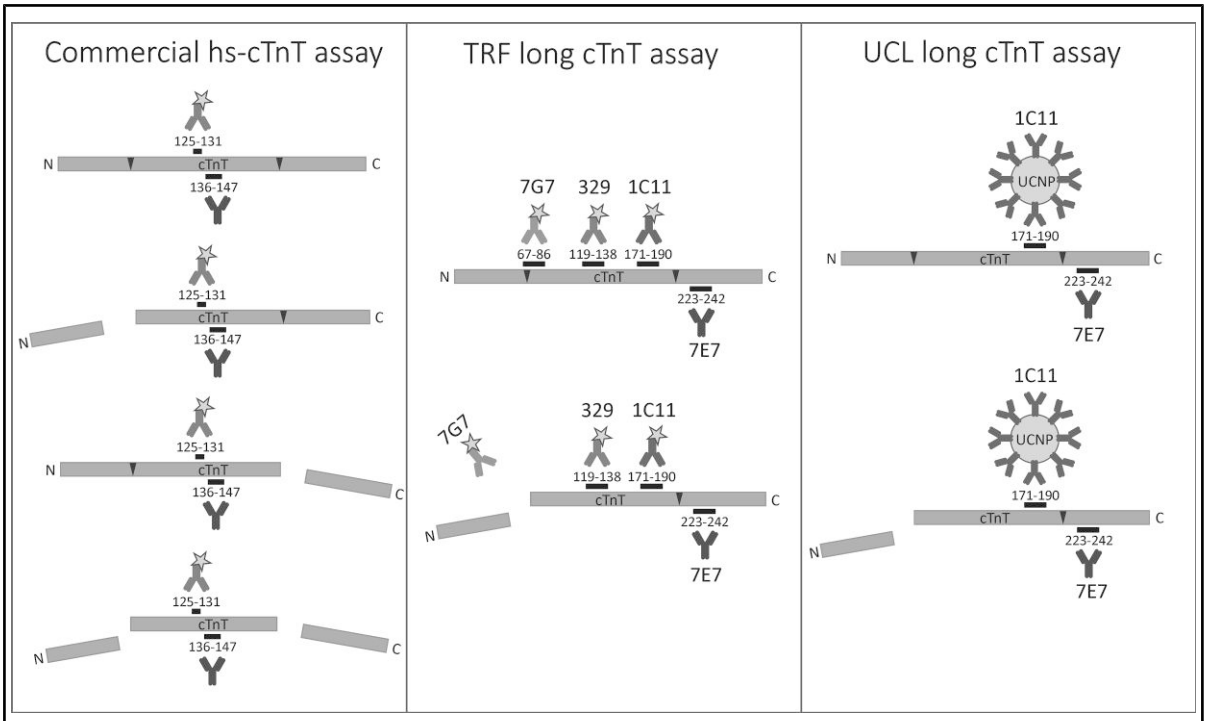

Fig. 1. Molecular forms of cTnT recognized by the commercial hs-cTnT assay ("total cTnT assay"), TRF long cTnT assay, and UCL long cTnT assay. The commercial hs-cTnT assay detects intact as well as mildly and heavily truncated cTnT forms (i.e., long forms and short forms of cTnT), while the TRF and UCL long cTnT assays detect intact and only mildly truncated cTnT forms that are not degraded at aar 189–223. Three tracer antibodies are used in the TRF long cTnT assay to increase signal, but not all of them have to bind to cTnT to produce signal. Epitopes are specified for each antibody and dark grey triangles indicate the major cleavage sites of cTnT, aar 68–69 and 189–223. For simplicity, the figure shows the most prominent forms of cTnT detected in AMI patients.

in AMI and ESRD (15). However, the detection of long cTnT molecules requires particularly low detection limit since only a fraction of the circulating cTnT is present in long forms. The TRF long cTnT assay with a limit of quantitation (LOQ) of 25 ng/L lacks sufficient sensitivity to reliably analyze patient samples with small cTnT elevations (15).

Upconverting nanoparticles (UCNPs) are attractive reporters for the development of highly sensitive bioaffinity assays. The unique feature of UCNPs is that they emit anti-Stokes shifted upconversion luminescence by converting low-energy near-infrared excitation to high-energy emission at visible wavelengths. Consequently, UCNPs can be measured entirely without autofluorescence background (16, 17). UCNPs have been previously utilized in highly sensitive immunoassays for cardiac troponin I (cTnI) (18–20). In this study, we developed a highly sensitive upconversion luminescence (UCL)-based immunoassay for the detection of long cTnT forms to improve the performance of the previously developed TRF long cTnT assay and to enhance further investigations of long cTnT as a promising diagnostic biomarker of AMI.

Materials and Methods

CLINICAL SAMPLES

All clinical blood samples were collected for the troponin fragmentation in myocardial injury (Tropo-Fragm) study (ClinicalTrials.gov Identifier: NCT04465591). The selection of AMI and ESRD patients and the adjudication process for AMI are described in the online Supplemental Data. The study protocol was approved by the Medical Ethics Committee of the Hospital District of Southwest Finland and the study was conducted in accordance with the Declaration of Helsinki as revised in 2013. Written informed consent was

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obtained from all participants. The ethical approval does not allow sharing of participant data to other parties.

Lithium-heparin (LiH) plasma samples from NSTEMI patients (n = 30) were collected within 24 h [mean 17 (5) h] after symptom onset. LiH plasma samples from ESRD patients (n = 37) were collected during a dialysis clinic visit before the hemodialysis. Serial LiH plasma samples were collected from 13 patients with ST elevation myocardial infarction (STEMI) at 3 varying time points (4 to 64 h) after symptom onset. LiH plasma samples matched with ethylenediaminetetraacetic acid (EDTA) plasma samples (n = 9) and serum samples (n = 9) were collected from STEMI patients. LiH plasma samples from 6 STEMI patients were divided into aliquots which were centrifuged (2200*g*, 15 min) either immediately upon arrival at the laboratory [17 (6) min after sample collection] or after 2 h at room temperature. All other samples were centrifuged within 2 h after the samples were drawn. All samples were analyzed from fresh for total cTnT and stored at −70°C. Prior to long cTnT analysis, the samples were thawed at room temperature, mixed, and briefly spun. As the long cTnT assays are investigational batch-based research methods, it was impossible to analyze the samples with these methods from fresh.

UCL LONG CTNT ASSAY DESIGN

The capture antibody (7E7 monoclonal antibody [mAb], HyTest) and the tracer antibody (1C11 mAb, HyTest) of the sandwich-type immunoassay target amino acid residues (aar) 223–242 and 171–190, respectively. The C-terminal region of cTnT between these 2 epitopes (aar 189–223) contains several cleavage sites. Thus, similarly to the previously developed TRF long cTnT assay, this immunoassay detects cTnT molecules that are not degraded at aar 189–223 (Fig. 1).

PREPARATION OF ANTIBODY CONJUGATES

The capture antibody (7E7 mAb) was conjugated with biotin isothiocyanate (Biotechnology unit, University of Turku, Finland) as described previously (21). Oleic acid-capped $NaYF_4$: 17% Yb^{3+}, 3% Er^{3+} UCNPs (online Supplemental Fig. 1) were synthesized, coated with poly(acrylic acid) (PAA), and conjugated to 1C11 mAb using EDC/sulfo-NHS chemistry as described previously (18, 22).

UCL LONG CTNT ASSAY PROTOCOL

The immunoassay for long cTnT forms was performed in C8 Lockwell LUMI White microtiter plates (Thermo Scientific), which were passively coated with 1 μg/well streptavidin (Biospa) as described previously (23). Half an hour prior to initiating the assay, the tracer antibody (1C11 mAb-UCNP) was diluted to a concentration of 8 μg/mL in colorless assay buffer (Uniogen Oy) supplemented with 0.05 wt% PAA (MW 1200, Sigma-Aldrich), 1 mM KF, 0.2 wt% fat-free milk powder (Valio Oy), 0.8 g/L native mouse IgG (Meridian Life Science), and 0.05 g/L denatured mouse IgG (denaturation at 63°C for 30 min). The plate was prewashed with wash buffer (Uniogen) and 200 ng of biotinylated 7E7 capture antibody in 50 μL of colorless assay buffer was added to the wells and incubated at room temperature with slow shaking for 30 min. The wells were washed with wash buffer and 30 μL of sample or calibrator (human cardiac troponin ITC-complex [HyTest] in tris-buffered saline with azide [TSA buffer, 50 mM Tris-HCl, pH 7.75, 150 mM NaCl, 0.5 g/L NaN_3] supplemented with 75 g/L bovine serum albumin [BSA, Probumin, Merck Millipore]) was mixed with 40 μL of sample buffer (13 mM Tris, pH 8, 175 mM NaCl, 0.13 g/L NaN_3, 8.75 g/L BSA [Bioreba AG]), 17.5 g/L D-trehalose (Sigma-Aldrich), 0.21 g/L bovine γ-globulin (Sigma-Aldrich), 0.28 g/L native mouse IgG, 0.0175 g/L denatured mouse IgG, 0.7 g/L casein (Calbiochem, Merck Millipore), and 13.13 U/mL heparin (Sigma-Aldrich) and added to the wells in 3 replicates. Calibrators and samples were incubated at room temperature with slow shaking for 30 min, after which the plate was washed with wash buffer. The tracer antibody dilution was sonicated using a vial tweeter sonicator (3 cycles, 0.5 s with 100% amplitude, Hielscher Ultrasonics) and added to the wells (50 μL volume). The wells were incubated at room temperature with slow shaking for 15 min. The plate was washed 4 times using pH-adjusted wash buffer (NaOH, pH 10.25) and left to dry for 90 min at room temperature. A modified Plate Chameleon microplate reader (Hidex Oy) equipped with a 980 nm laser was used to measure the upconversion luminescence at the dry well bottoms at 540 nm (16).

TOTAL CTNT ASSAY

All LiH plasma samples were analyzed with the Elecsys® hs-cTnT assay (Roche Diagnostics) by the certified laboratory services of Turku University Hospital (Fig. 1). The limit of detection (LOD) and LOQ for this assay are 3 and 13 ng/L, respectively.

TROPONIN RATIO

The troponin ratio, which represents the fraction of long cTnT forms within the detectable total cTnT, was calculated by dividing the long cTnT result by the total cTnT result (the Roche hs-cTnT assay). The ratios are expressed as percentages.

TRF LONG CTNT ASSAY

The previously developed TRF long cTnT assay utilizing 3 tracer antibodies with europium(III) chelate labels was used as a reference method (Fig. 1). The assay was

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calibrated using human cardiac troponin ITC-complex (HyTest). The LOD and LOQ of this assay are 11 and 25 ng/L, respectively (15).

EVALUATION OF ASSAY PERFORMANCE

The limit of blank (LOB) and LOD of the UCL long cTnT assay were determined following the classical approach of the Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2 with minor modifications. One batch of 7.5% BSA-TSA was analyzed as the zero calibrator in 63 replicates over 18 days for nonparametric LOB. Low-level clinical samples with long cTnT concentrations of 1–5×LOB (n = 20) were analyzed in 3 replicates over 9 days for parametric LOD. The LOQ with an accuracy goal of 10% coefficient of variation (CV) was determined using the within-run precision profile of 113 clinical patient samples measured in 3 replicates over 16 days.

LiH plasma samples from STEMI patients were compared to matched EDTA plasma (n = 9) and serum samples (n = 9). Dilution linearity was evaluated by making 1/2 and 1/4 serial dilutions of LiH plasma samples from NSTEMI patients (n = 6, initial long cTnT concentrations 4–411 ng/L) in 7.5% BSA-TSA buffer.

The performance of the UCL long cTnT assay with clinical samples was evaluated by analyzing LiH plasma samples from NSTEMI (n = 30) and ESRD (n = 37) patients and comparing the results to the commercial hs-cTnT assay and the TRF long cTnT assay.

TIME-DEPENDENT CTNT FRAGMENTATION

The progression of cTnT fragmentation over time was evaluated using serial LiH plasma samples collected from STEMI patients (n = 13) at 3 varying time points. An exponential decay function was utilized to estimate the half-life of long cTnT in each patient and to extrapolate for the initial concentration to allow visual comparison using the remaining percentage of long cTnT at different time points between patients.

STATISTICAL ANALYSIS

The normality of continuous variables was assessed by visual examination and the Shapiro–Wilk test. Continuous variables following a normal distribution are reported as mean and standard deviation (SD), whereas median, lower quartile, and upper quartile [25th percentile–75th percentile] are reported for skewed variables.

Linear regression analysis was used to assess dilution linearity. In the comparison of 2 long cTnT assays, Deming regression was used. The comparison between 2 different sample preparation procedures was performed using the Wilcoxon signed rank test. Long cTnT and total cTnT concentrations and their ratios in NSTEMI and ESRD patient groups were compared with the Mann–Whitney *U*-test. Receiver operating characteristic (ROC) curve analyses were performed to evaluate the ability of the investigated assays to distinguish between AMI patients and ESRD patients. ROC curves were compared using paired-sample design. The optimal cutoff point for the troponin ratio was defined using the Youden index. All tests were two-tailed and $P < 0.05$ was considered statistically significant. Linear regression analyses were performed with Origin 8 (OriginLab), Deming regression and ROC analyses with Sigmaplot 15 (Inpixon), and other statistical analyses with IBM SPSS Statistics software version 27 (IBM Corp.).

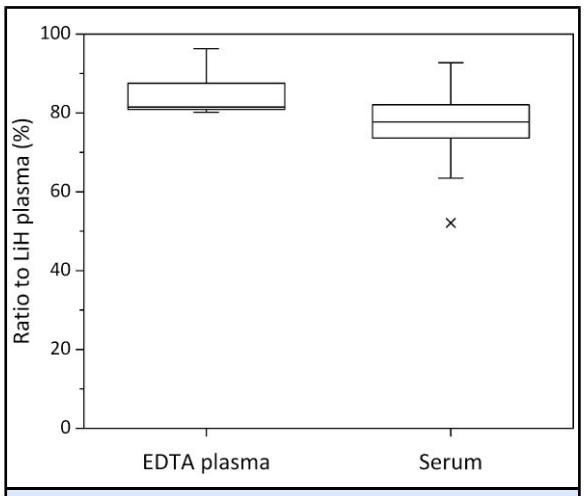

Fig. 2. Matrix effect in EDTA plasma and serum samples. The long cTnT concentrations in EDTA plasma and serum samples were compared to the long cTnT concentrations in matched LiH plasma samples.

Results

ANALYTICAL PERFORMANCE OF THE ASSAY

The UCL long cTnT assay had an LOB of 0.15 ng/L and LOD of 0.40 ng/L. The LOQ determined using the 10% CV accuracy goal and within-run precision profile was 1.79 ng/L (see online Supplemental Fig. 2 for comparison with the within-run precision profile of the TRF long cTnT assay in online Supplemental Fig. 3). The calibration curve was linear between 0.1 and 1000 ng/L ($R^2 = 1.000$) and showed low CV% values above the LOD (online Supplemental Fig. 4). The assay exhibited good linearity in LiH plasma samples from NSTEMI patients (n = 6) diluted in BSA-TSA buffer ($R^2 =$ 0.993–1.000) (online Supplemental Fig. 5).

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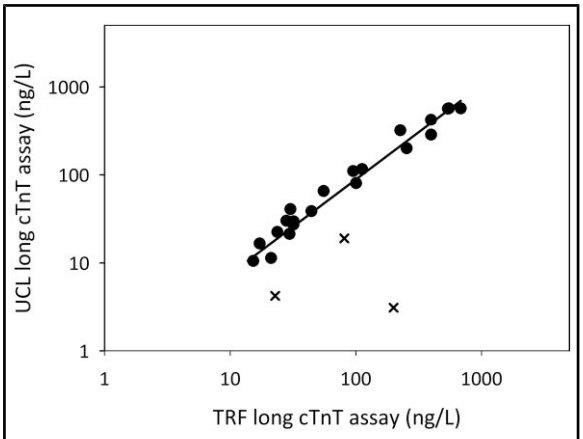

Fig. 3. Comparison of 2 long cTnT assays above the LOD of the TRF assay (11 ng/L). Long cTnT concentrations of the patient samples (n = 21) are presented as the means of 3 replicates (black circles). Deming regression analysis resulted in an equation of $y = 1.03x - 3.56$ and a correlation coefficient of 0.98. The crosses indicate patients with outlier results (n = 3).

The assay was developed for use with LiH plasma samples. The possibility of using EDTA or serum samples was investigated with matched samples from STEMI patients (online Supplemental Table 1). The median EDTA plasma result was 82% [81%–91%] of the results given by LiH plasma (Fig. 2). For serum samples the concentration decrease was more pronounced (serum result median 78% [69%–85%] of LiH plasma results) and highly variable among the studied samples (Fig. 2). A 2-hour delay in centrifugation of LiH plasma did not significantly affect long cTnT [mean change −3% (7%), $P = 0.917$].

When 3 outliers with more than 70% difference in long cTnT concentration were removed from the analysis, the UCL and TRF long cTnT assays showed strong correlation ($r = 0.98$) with 21 patient samples with long cTnT concentrations above the LODs of the assays (Fig. 3). Deming regression resulted in a slope of 1.03 (95% CI, 0.93–1.12) and y-intercept of −3.56 (95% CI, −8.94–1.83). With the 3 outliers excluded, the concentrations measured with the UCL long cTnT assay were on average 5% [−21%–7%] lower than those measured with the TRF long cTnT assay.

PERFORMANCE OF THE ASSAY WITH CLINICAL SAMPLES

The median long cTnT concentrations measured with the UCL long cTnT assay were significantly lower in ESRD patients (2.5 [1.9–3.9] ng/L) than in NSTEMI patients (28.4 [6.4–136.6] ng/L, $P < 0.001$) while total cTnT concentrations did not significantly differ between ESRD patients (76.0 [50.5–124.5] ng/L) and NSTEMI patients (103.0 [47.3–268.3] ng/L, $P = 0.144$). The troponin ratio was consequently lower in ESRD patients (3.3% [2.4%–4.5%]) than in NSTEMI patients (21.5% [12.5%–53.3%], $P < 0.001$) (Fig. 4).

ROC analyses were performed to assess the ability of the assays to discriminate between NSTEMI and ESRD. The area under the curve (AUC) for total cTnT was 0.605 (95% CI, 0.461–0.748). The AUCs for long cTnT [0.905 (95% CI, 0.824–0.985)] and the troponin ratio [0.986 (95% CI, 0.967–1.000)] measured with the UCL long cTnT assay were significantly higher ($P < 0.001$) (Fig. 5). The AUC of the troponin ratio was also numerically higher than the AUC of 0.955 (95% CI, 0.900–1.000) determined for the troponin ratio of the TRF long cTnT assay ($P = 0.293$) (Fig. 5). In the ROC analysis, at the optimal troponin ratio cutoff point 7.5% for the UCL long cTnT assay the sensitivity and specificity for separating NSTEMI and ESRD patients were 93% and 95%, respectively. A specificity of 100% and sensitivity of 83% were achieved with the troponin ratio cutoff point at 11.5%. Comparison to long cTnT and total cTnT and predictive values are presented in online Supplemental Table 2.

TIME-DEPENDENT CTNT FRAGMENTATION

In serial samples of 13 STEMI patients, long cTnT and the troponin ratio followed an exponential decay function [mean R^2 0.98 (0.03) and 0.99 (0.01), respectively] (online Supplemental Fig. 6). The estimated mean half-lives of long cTnT and the troponin ratio were 8.7 (2.7) h and 10.5 (2.9) h, respectively. For total cTnT it was not possible to estimate half-life, as varying trends were seen and for 4/13 patients the values increased in a later sample.

Discussion

The measurement of cTn in blood is widely used as an important parameter for diagnosing AMI. As cTn is a biomarker of myocardial injury, current hs-cTn assays often detect cTn elevations also in patients without AMI. Thus, there is a clinical need for a laboratory test exhibiting higher specificity for AMI. Long forms of cTnT seem to be a promising diagnostic biomarker of AMI, and recently a TRF-based immunoassay was developed for long cTnT (15). However, accurate detection of long cTnT requires particularly high sensitivity as only a fraction of circulating cTnT is measured, and the sensitivity of the TRF-based cTnT immunoassay is not sufficient. In the present study, a novel highly

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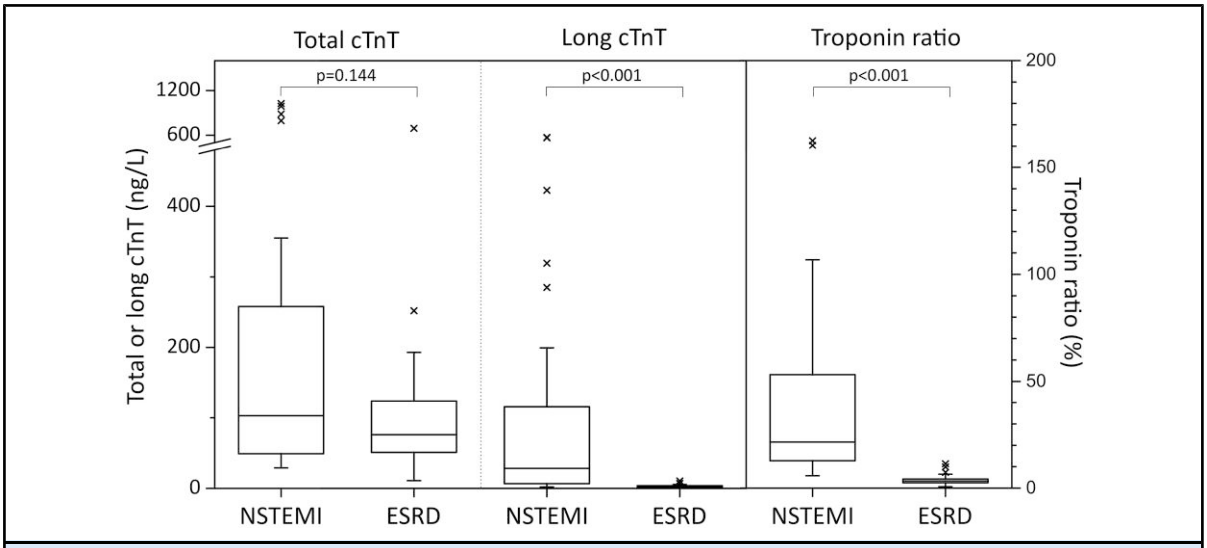

Fig. 4. Total cTnT, long cTnT, and troponin ratio in patients with NSTEMI (n = 30) and ESRD (n = 37). Total cTnT and long cTnT concentrations were measured with the commercial hs-cTnT assay and the novel UCL long cTnT assay, respectively. The troponin ratio was calculated by dividing the long cTnT result by the total cTnT result.

sensitive immunoassay for the detection of long cTnT forms was developed by utilizing UCNP reporters.

The antibody combination was selected, similarly to the previously published TRF long cTnT assay, to target cTnT forms that are not degraded at the major C-terminal cleavage site (aar 189–223) (7, 15). Despite the differing number of tracer antibodies, the 2 long cTnT assays still detect the same cTnT forms (Fig. 1). Compared to the TRF long cTnT assay, the UCL long cTnT assay achieved significantly higher sensitivity, with approximately 28-fold and 14-fold lower LOD and LOQ values at 0.40 ng/L and 1.79 ng/L, respectively (15). Thus, the UCL long cTnT assay allows more sensitive and precise quantification of long cTnT forms in patients with low cTnT elevations and small fractions of long cTnT. This would be particularly important when analyzing early samples of NSTEMI patients with low cTnT elevations.

EDTA plasma samples produced results that were close to 18% lower than the results obtained with matched LiH samples. However, these findings should be confirmed with larger sample panels. Previously, EDTA has been observed to promote dissociation of the ITC complex into subunits (24). As the C-terminal part of cTnT is involved in the ITC complex formation, ITC complex dissociation may change the conformation of the epitope of the capture antibody in the C-terminal region of cTnT and lead to the decreased values for EDTA samples. Serum samples matched with LiH samples showed an even greater reduction in results and there was high variation in the reduction between the samples. Thus, even based on this small number of samples, it can be stated that serum samples are not recommended for use with the assay. Previously, activation of thrombin during the preparation of serum samples has been found to induce degradation of cTnT at a well-characterized cleavage site, aar 68–69 (10, 25). However, other cleavage sites have also been discovered on cTnT related to thrombin or other yet unknown proteases, which are present in serum and inactivated by heparin (7, 26). These enzymes could affect the performance of the developed assay, as some cleavage sites are located between the epitopes of the antibodies used in the assay. Differences in the processing times may have caused significant differences between the serum samples in respect to such fragmentation and explain variation in the results.

The UCL long cTnT assay produced very similar results to the TRF long cTnT assay within the dynamic range of the assays, except for 3 outlier samples for which the TRF assay gave significantly higher results. The outliers may be due to interference caused by the use of multiple tracer antibodies in the TRF assay as the likelihood of interference by cross-linking heterophilic antibodies may increase when the number of antibodies in the assay is higher. For one of these samples the troponin

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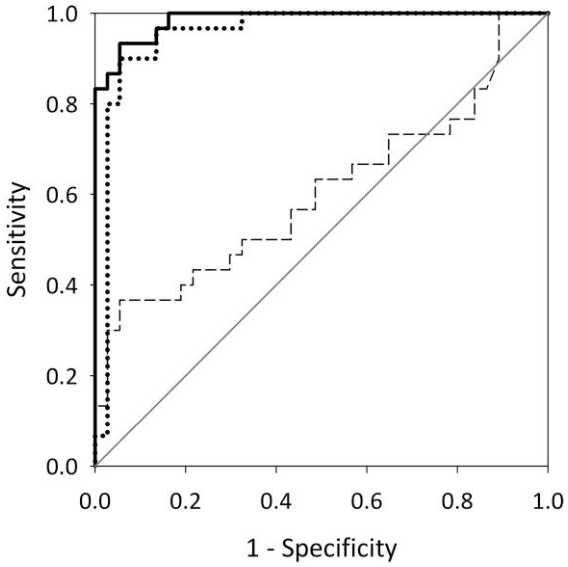

Fig. 5. Receiver operating characteristic curves illustrating diagnostic abilities of the troponin ratios and the commercial hs-cTnT assay (total cTnT) to discriminate between ESRD and NSTEMI patients. The thick solid line and the dotted line indicate the troponin ratios determined with the UCL long cTnT assay (AUC 0.986) and the TRF long cTnT assay (AUC 0.955), respectively. The dashed line indicates total cTnT (AUC 0.605).

ratio was extraordinarily high (200%), also implying that positive interference may have been the reason for higher results with the TRF assay.

Similarly to the TRF long cTnT assay, the developed UCL long cTnT immunoassay was able to discriminate between the patients with NSTEMI and ESRD. However, due to the highly improved sensitivity, the UCL assay was able to discriminate these groups with even higher AUC in the ROC analysis, offering almost perfect separation when the troponin ratio in samples measured within 24 h of symptom onset was used.

The UCL long cTnT assay was also used to evaluate time-dependent degradation of cTnT in serial blood samples of STEMI patients. The half-lives of long cTnT (8.7 h) and the troponin ratio (10.5 h) indicate a relatively rapid decrease in the long cTnT concentration compared to the total cTnT concentration. Our results are in line with existing knowledge, as commercial assay total cTnT levels have been shown to remain elevated for days and often express a biphasic pattern (27, 28). The time from symptom onset to sample collection varied considerably between the patients and many factors may affect the release and decrease of long cTnT, including the progression of myocardial cell damage and blood flow perturbations in the infarcted tissue. Therefore, the reported half-lives should be considered as approximations of long cTnT and troponin ratio kinetics in the circulation, not as exact elimination constants. The apparently short half-life reported here suggests that long cTnT and the troponin ratio could possibly perform well as biomarkers of reinfarction.

A significant limitation for the UCL long cTnT assay is that the assay in its current form is not suitable for clinical practice at emergency clinics, as it requires manual labor and 5 h of total assay time. However, it can still serve as a highly valuable research tool for discovering the full potential of long cTnT and the troponin ratio as diagnostic markers of AMI. As the UCL long cTnT assay and total cTnT assay have been calibrated differently, some calibration-related bias may exist in the concentrations reported by the assays. However, as the assays measure different molecular forms of cTnT, the assay results should be considered to represent different biomarkers for which concentrations do not have to match, although ultimately metrological standardization would be useful.

In the present study the number of samples used in the evaluation of the assay is small. Larger studies should follow to confirm the preliminary findings. Although we present data on long cTnT fragmentation in repeated samples in STEMI patients, further studies should investigate the fragmentation more thoroughly especially in early stages after the onset of symptoms. One limitation of the study is the difficulty to accurately define the timing of the onset of symptoms, as we are relying on the information provided by the patient.

In conclusion, we successfully developed a highly sensitive long cTnT immunoassay utilizing UCL technology that provides much better sensitivity than a previously developed TRF long cTnT assay. The novel assay allows accurate and precise detection of long cTnT forms in patients with low cTnT elevations and exhibits excellent performance in discriminating between patients with NSTEMI and ESRD. In the future, this assay can be used to thoroughly explore long cTnT and the troponin ratio as biomarkers in various patient groups.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: cTnT, cardiac troponin T; AMI, acute myocardial infarction; UCNP, upconverting nanoparticle; NSTEMI, non-ST elevation myocardial infarction; ESRD, end-stage renal disease; TRF, time-resolved fluorescence; cTn, cardiac troponin;

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hs-cTn, high-sensitivity cardiac troponin; LOQ, limit of quantitation; UCL, upconversion luminescence; LiH, lithium-heparin; STEMI, ST elevation myocardial infarction; mAb, monoclonal antibody; aar, amino acid residue; BSA, bovine serum albumin; LOD, limit of detection; LOB, limit of blank; ROC curve, receiver operating characteristic curve; AUC, area under the curve.

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