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Prognostic significance of chronic myocardial injury diagnosed by three different cardiac troponin assays in patients admitted with suspected acute coronary syndrome

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Abstract

Objectives: Chronic myocardial injury (CMI) is defined as stable concentrations of cardiac troponin T or I (cTnT or cTnI) above the assay-specific 99th percentile upper reference limit (URL) and signals poor outcome. The clinical implications of diagnosing CMI are unclear. We aimed to assess prevalence and association of CMI with long-term prognosis using three different high-sensitivity cTn (hs-cTn) assays.

Methods: A total of 1,292 hospitalized patients without acute myocardial injury had cTn concentrations quantified by hs-cTn assays by Roche Diagnostics, Abbott Diagnostics and Siemens Healthineers. The median follow-up time was 4.1 years. The prevalence of CMI and hazard ratios for mortality and cardiovascular (CV) events were calculated based on the URL provided by the manufacturers and compared to the prognostic accuracy when lower percentiles of cTn (97.5, 95 or 90), limit of detection or the estimated bioequivalent concentrations between assays were used as cutoff values.

Results: There was no major difference in prognostic accuracy between cTnT and cTnI analyzed as continuous variables. The correlation between cTnT and cTnI was high ($r=0.724-0.785$), but the cTnT assay diagnosed 3.9–4.5 times more patients with having CMI based on the sex-specific URLs (TnT, $n=207$; TnI Abbott, $n=46$, TnI Siemens, $n=53$) and had higher clinical sensitivity and AUC at the URL.

Conclusions: The prevalence of CMI is highly assay-dependent. cTnT and cTnI have similar prognostic accuracy for mortality or CV events when measured as continuous variables. However, a CMI diagnosis according to cTnT has higher prognostic accuracy compared to a CMI diagnosis according to cTnI.

Keywords: cardiac troponin T; cardiac troponin I; chronic myocardial injury; 99th percentile; prognostic accuracy

Introduction

Cardiac troponin T and I (cTnT and cTnI) assays have a high and similar accuracy in identifying acute myocardial infarction (AMI) [1, 2]. In patients without AMI, elevated cTn

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concentrations signals increased risk of future cardiovascular disease and mortality [3–8]. The risk is proportional to cTn concentrations, and patients with stable cTn concentrations exceeding the 99th percentile have the highest risk of future adverse events [8, 9]. This condition is known as chronic myocardial injury (CMI) [9] and may be caused by a variety of conditions such as heart failure, left ventricular hypertrophy, cardiac fibrosis, and cardiac exposure to metabolic risk factors [10–13]. There is yet no consensus on specific treatments or follow-up for CMI, but these high-risk patients may be a future target for increased cardioprotective therapy [14].

One major challenge with the current definition of CMI is the non-linear relationship and low to moderate concordance between concentrations of cTnT and cTnI measured with high-sensitivity (hs-cTn) assays in patients without acute myocardial injury [15–18]. Vestergaard et al. and Árnadóttir et al. compared cTnT and cTnI in hospitalized patients and found that cTn more frequently were elevated above the 99th percentile measured by a cTnT assay compared to cTnI [19, 20]. Large differences in CMI prevalence can affect risk stratification and preventive treatment offered by healthcare institutions that use different hs-cTn assays for analysis.

The objective of this prospective analysis was to assess whether CMI identified by different hs-cTn assays serve as a uniform and relevant marker of elevated cardiovascular risk. We evaluated the correlation between cTn concentrations measured by three different hs-cTn assays, prevalence of CMI, and long-term outcome in patients diagnosed with CMI by any one or all three assays. We also compared the prognostic accuracy of assay-equivalent cutoff values (calculated based on leveled pairs of cTn) or optimal cutoff values assessed by Youden Index, and the accuracy when using the limit of detection, the 90th, 95th, or 97.5th percentiles as cutoff limits for risk stratification.

Materials and methods

Study design, population, and data inclusion

Patients without acute myocardial injury were extracted from the Aiming Towards Evidence-Based Interpretation of Cardiac Biomarkers in Patients Presenting with Chest Pain (WESTCOR) study, a prospective and cross-sectional observational study (Clinical Trial NCT02620202) [21]. The study was approved by the regional Ethics Committee (REC number 2014/1365) and was carried out according to the Declaration of Helsinki. The current analysis contains patients from Haukeland University Hospital only.

The WESTCOR study included patients ≥ 18 years consecutively admitted with symptoms suggesting acute coronary syndrome and has

been described elsewhere [21]. The final diagnoses were adjudicated by two independent cardiologists based on all clinical information including routine laboratory result (using the hs-cTnT assay and sex-neutral cutoff values). In cases of disagreement, a third adjudicator was consulted.

CMI was defined as cTn concentration at presentation above the sex-specific 99th percentile URL by any cTn assay, without rise and/or fall of more than 20 % in subsequent blood samples. Since cTn usually reaches a plateau phase 10–15 h after an AMI, patients with symptom debut >12 h before admission were considered late presenters.

Patients who had acute myocardial injury based on the sex-specific cutoff values of any assay were excluded from the current analysis, and coronary artery disease was hence defined as unstable or stable angina pectoris. Non-coronary cardiac diseases included diseases such as pericarditis, myocarditis, and heart failure. Noncardiac chest pain included myalgia, esophageal disease, and pleural diseases.

Outcomes

The prevalence of CMI was calculated for all three assays based on the assays' sex-specific cutoff values. The primary prognostic endpoint was a composite of cardiovascular death, AMI after discharge, or revascularization. The secondary endpoint was all-cause mortality, AMI, revascularization, or hospitalization for heart failure or stroke. We also evaluated the risk of all-cause mortality (tertiary endpoint) as death from any cause is the most used endpoint in existing head-to-head comparisons of cTnT and cTnI assays [20, 22, 23]. Information on mortality was collected through the Norwegian Cause of Death Registry, and readmittances, diagnoses and procedures were collected through the Norwegian Patient Registry.

Cardiac troponin analysis

cTnT concentrations (Roche Diagnostics, Basel, Switzerland) were analysed in fresh material using Cobas 602 (up to July 2017) and Cobas 801 (from August 2017). cTnI concentrations were analyzed from bio-banked material stored at -80 degrees Celsius during two different time points using the Architect platform (approximately 2/3 of patients) and Alinity platform (approximately 1/3 of patients) by Abbott Diagnostics (Illinois, USA) and the Atellica platform by Siemens Healthineers (Erlangen, Germany). The method comparison done locally (at Stavanger University Hospital) between the Abbott Architect and Alinity platform showed good agreement, in line with earlier published data [24]. The long-term stability of the cTnI assays have been shown to be acceptable [25, 26]. Analytical details are provided in the Supplementary Material.

The recommended 99th percentile URLs differ by region, and the cutoff values used in this study are consistent with manufacturers' recommendations outside USA [27]. The 99th percentiles are based on data from presumable healthy volunteers (numbers of subjects are given in Supplementary Material, Table S1). The additional percentiles (90th, 95th and 97.5th) were reported by the manufacturers on request from the authors of the current study.

Statistical analysis

Baseline characteristics were reported as means (± 2 SD) for normally distributed data, median with 25- and 75 percentiles for nonnormally

distributed data and frequencies with percentages for categorical data. Differences between groups were compared using the 2-sample t-test or Mann–Whitney U test for continuous variables and Pearson's chi-square test for categorical data.

The correlation (r) between cTn measured by different assays was assessed by Pearson's correlation test of log-transformed cTnT and cTnI values at presentation. Agreement between CMI diagnoses by different assays were assessed by Cohen's kappa coefficients. The prognostic performances of cTn as continuous variables were assessed by the receiver operating characteristics curve (ROC) for all three endpoints. The Youden Index was used to identify the optimal cTn cutoff points for risk stratification of the primary endpoint. Differences between area under the curves (AUCs) were analyzed by DeLong test (unadjusted analysis).

Linear regression of log-transformed cTn values were used to calculate between-assay equivalent cTn cut-off values for the different percentiles, using the assay with the highest prevalence of CMI as reference (cTnT). cTn concentrations by all three assays at the same time point were analyzed for women and men separately with the formula $e^{(\beta + \epsilon \times \ln[\text{cTnT percentile}])}$. Sensitivity, specificity, predictive values, and odds ratio (OR) were calculated for the primary and secondary endpoints using the between-assay equivalent cutoff values, the calculated optimal cutoff values (Youden Index), the LoD, 90th, 95th, 97.5th, and 99th percentiles as provided by the manufacturer. AUC for cTn at these distinct cutoff values were used to assess the balance between sensitivity and specificity and proximity between the chosen cutoff value and the calculated optimal prognostic values. Multivariate analyses were assessed using Cox proportional hazard regression model adjusted for age, sex and eGFR.

Hypothesis testing were 2-tailed, and p-values <0.05 were considered statistically significant. The analyses were performed using IBM SPSS Statistics version 26.0.0.1 and Medcalc version 17.6.

Results

More men than women had detectable cTn (cTnT Roche, women/men: 55.8/75.3%; cTnI Abbott, 59.1/71.2%; cTnI Siemens 90.4/97.5%, all p-values for diff. <0.001). The baseline characteristics are shown in Table 1. Three or more blood samples were collected in 86.3% of patients with symptom onset <12 h before presentation and 90.6% of patients with symptom onset >12 h before presentation.

The correlation between cTnI concentrations measured by Abbott and Siemens assays was high, $r=0.876$ (0.849–0.904), see Figure 1. A High, but weaker correlation was found between the cTnT and the two cTnI assays, ranging from $r=0.724$ (0.684–0.763) for cTnT vs. cTnI Siemens to $r=0.785$ (0.751–0.819) for cTnT vs. cTnI Abbott, see Supplementary Material, Table S2a. The correlations appeared higher in patients with cardiac disease, for instance $r=0.907$ (0.861–0.973) vs. $r=0.841$ (0.806–0.877) in patients with coronary artery disease vs. non-cardiac chest pain when the two cTnI assays were compared, see Supplementary Material Table S2b.

Table 1: Baseline characteristics.

	All (n=1,147)	CMI ^a (n=218)	No CMI (n=929)	p- Value
Age, median	61 (51–71)	76 (68–84)	58 (49–67)	<0.001
Female gender, n (%)	458 (40.0 %)	111 (50.9 %)	347 (37.4 %)	<0.001
Cardiovascular risk factors				
Obesity ^b , n (%)	159 (26.7 %)	27 (25.7 %)	132 (26.9 %)	0.806
Active smoker, n (%)	215 (18.7 %)	32 (14.7 %)	183 (19.7 %)	0.087
Former smoker, n (%)	504 (43.9 %)	102 (46.8 %)	402 (43.3 %)	0.346
Hyperlipidemia, n (%)	228 (19.9 %)	58 (26.6 %)	170 (18.3 %)	0.006
Diabetes mellitus, n (%)	128 (11.2 %)	44 (20.2 %)	84 (9.0 %)	<0.001
Insulin-dependent, n (%)	35 (3.1 %)	19 (8.7 %)	16 (1.7 %)	<0.001
Hypertension, n (%)	454 (39.6 %)	122 (56.0 %)	332 (35.7 %)	<0.001
Family history of CAD, n (%)	212 (18.5 %)	22 (10.1 %)	190 (20.5 %)	<0.001
Medical history				
Previous AMI, n (%)	204 (17.8 %)	64 (29.4 %)	140 (15.1 %)	<0.001
Previous PCI, n (%)	214 (18.7 %)	55 (25.2 %)	159 (17.1 %)	0.006
Previous CABG, n (%)	67 (5.8 %)	31 (14.2 %)	36 (3.9 %)	<0.001
Atrial fibrillation, n (%)	120 (10.5 %)	53 (24.3 %)	67 (7.2 %)	<0.001
Previous stroke, n (%)	30 (2.6 %)	12 (5.5 %)	18 (1.9 %)	0.003
Renal failure ^c , n (%)	119 (10.4 %)	79 (36.2 %)	40 (4.3 %)	<0.001
Peripheral arterial disease, n (%)	24 (2.1 %)	10 (4.6 %)	14 (1.5 %)	0.004
Known heart failure, n (%)	33 (2.9 %)	21 (9.6 %)	12 (1.3 %)	<0.001
Clinical and laboratory parameters				
Systolic blood pressure, mmHg	145 (144–146)	147 (144–150)	145 (144–146)	0.140
Diastolic blood pressure, mmHg	84 (83–85)	82 (79–85)	84 (84–85)	0.012
Pulse, bpm	74 (73–75)	78 (75–80)	73 (72–74)	<0.001
Total cholesterol, mmol/L	4.9 (4.8–4.9)	4.5 (4.3–4.7)	5.0 (4.9–5.0)	<0.001
LDL cholesterol, mmol/L	3.1 (3.0–3.2)	2.7 (2.5–2.9)	3.2 (3.1–3.3)	<0.001
HDL cholesterol, mmol/L	1.4 (1.4–1.5)	1.5 (1.4–1.6)	1.4 (1.4–1.5)	0.034
Hemoglobin, g/dL	14.2 (14.1–14.4)	13.5 (13.3–13.7)	14.4 (14.3–14.5)	<0.001
Glucose, mmol/L	6.2 (6.1–6.3)	6.9 (6.6–7.2)	6.1 (6.0–6.2)	<0.001
CRP, mg/L, median	2.0 (0.7–4.0)	2.0 (0.9–4.5)	1.0 (0.7–4.0)	0.020
proBNP, ng/L, median	81 (34–230)	88 (32–310)	80 (35–226)	0.263
eGFR, mL/min/1.37 m ²	84.7 (83.5–85.8)	65.9 (62.7–69.0)	88.7 (87.7–89.7)	<0.001
hs-cTnT (Roche), median	6 (3–11)	20 (15–29)	5 (3–7)	<0.001
hs-cTnI (Abbott), median	2.7 (1.5–5.5)	9.7 (5.3–19.8)	2.2 (1.5–3.8)	<0.001
hs-cTnI (Siemens), median	4.4 (2.7–8.8)	15.3 (8.0–36.9)	3.8 (2.4–6.3)	<0.001

Table 1: (continued)

	All (n=1,147)	CMI ^a (n=218)	No CMI (n=929)	P- Value
Outcome within follow-up				
All-cause mortality, n (%)	91 (7.9 %)	53 (24.3 %)	38 (4.1 %)	<0.001
Cardiovascular death, n (%)	26 (2.3 %)	15 (6.9 %)	11 (1.2 %)	<0.001
AMI, n (%)	35 (3.0 %)	16 (7.7 %)	19 (2.0 %)	<0.001
Revascularization, n (%)	58 (5.1 %)	10 (4.6 %)	48 (5.2 %)	0.725
Heart failure, n (%)	26 (2.3 %)	20 (9.2 %)	6 (0.6 %)	<0.001
Stroke, n (%)	1 (0.1 %)	1 (0.5 %)	0 (0.0 %)	0.039

CMI, indicates chronic myocardial injury; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein. ^aStable cTnT concentration above the sex-specific 99th percentile of any assay. ^bBMI >30, calculated for 596 patients only. ^ceGFR <60 mL/min/1.37 m².

Diagnostic inconsistencies using URLs by manufacturers

A total of 218 patients (19.0 %) of the patients had CMI by any assay. The prevalence was 4.5 times (95 % CI 3.3–6.1) higher according to the cTnT assay than the cTnI assay by Abbott

and 3.9 times (95 % CI: 2.9–5.2) higher than the prevalence found by the Siemens cTnI assay. As shown in Figure 1, more patients had elevated concentrations of cTnT and non-elevated cTnI (upper left quadrants in plot B and C) than non-elevated concentrations of cTnT and elevated cTnI (lower right quadrants in plot B and C). Only 29/218 patients (13.3 %) had CMI according to all three assays, see Figure 2. The kappa coefficients for diagnostic agreement were moderate to good for the two cTnI assays (0.652), yet only 50 % (33/66) of patients with CMI diagnosed by a cTnI assay were identified by both assays. The kappa coefficients were fair for cTnT vs. the cTnI assays (0.277 for cTnT vs. cTnI Abbott; 0.278 for cTnT vs. cTnI Siemens).

Patients with CMI diagnosed by a cTnI but not the cTnT assay were more often men, had higher concentrations of NT-pro-BNP and less often a history of hypertension, see Supplementary Material, Table S3. The prevalence of reduced renal function was similar in patients diagnosed with CMI either based on cTnT or cTnI, and none of the patients had known musculoskeletal disease.

CMI and risk of reaching an endpoint

During a median of 4.1 years (1,504 days, range 7–2,208) follow-up, 93 patients (8.1 %) reached the primary endpoint,

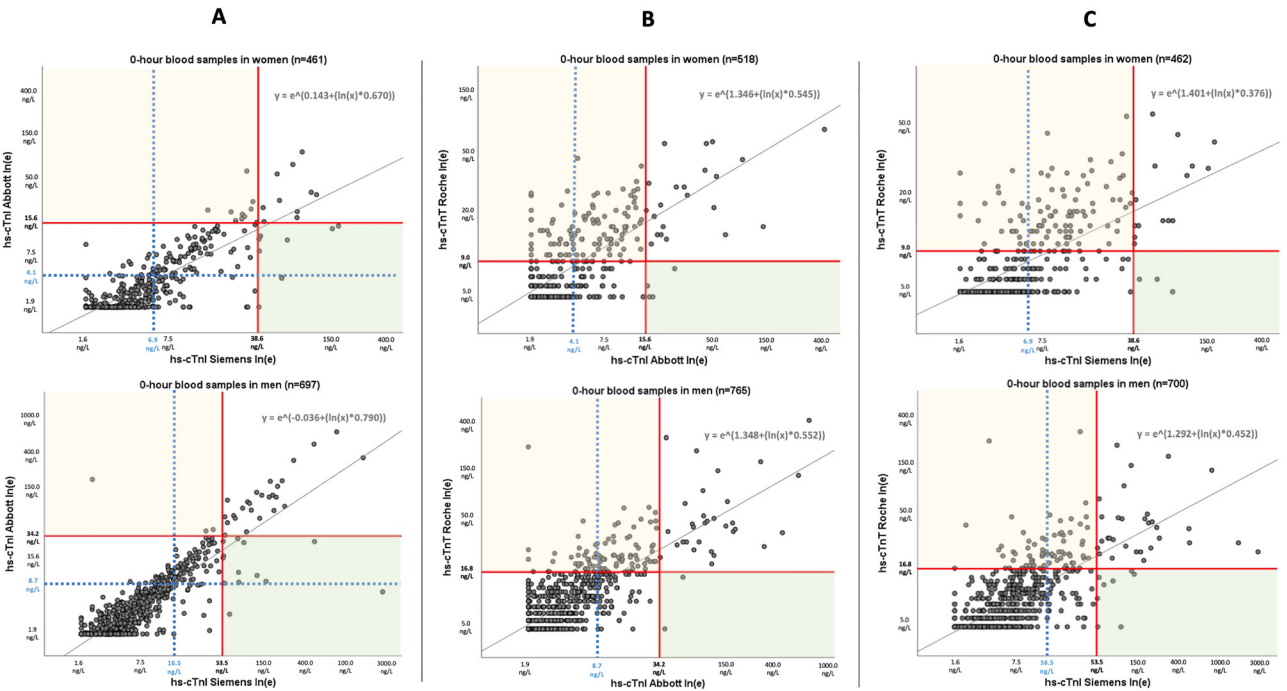


Figure 1: Distribution of cardiac troponin T and I (cTnT and cTnI) under and above the 99th percentile upper reference limit (red line) provided by manufacturers in women and men without acute myocardial injury comparing (A) cTnI Abbott vs. cTnI Siemens, (B) cTnT Roche vs. cTnI Abbott and (C) cTnT Roche vs. cTnI Siemens. Regression lines are colored in grey while the dashed blue lines mark the calculated equivalent cTnI values (relative to cTnT). The formula for the linear regression is provided in each scatter plot.

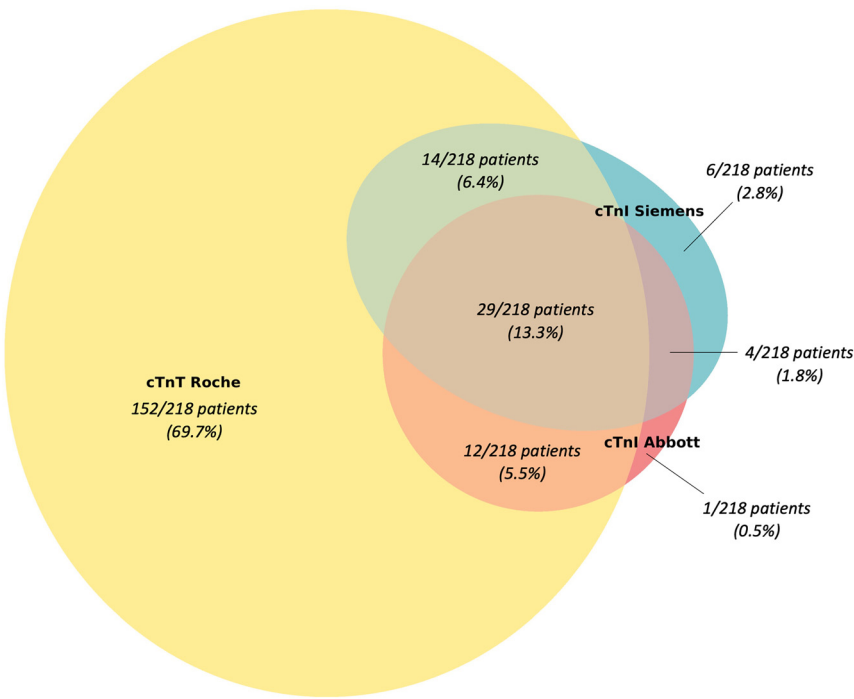


Figure 2: Distribution of patients diagnosed with chronic myocardial injury (n=218) according to three different cTn assays based on the accepted 99th percentiles provided by the assay manufacturers.

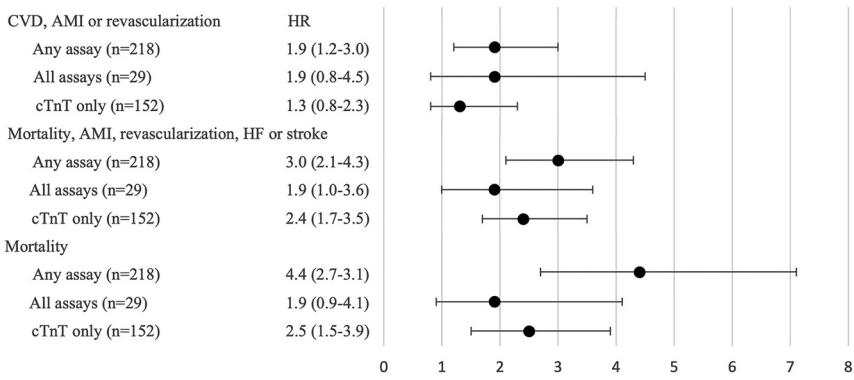


Figure 3: Hazard ratio for reaching the primary, secondary, or tertiary endpoints based on chronic myocardial injury by any assay, all assays or the cTnT assay only, adjusted model.

162 patients (14.1 %) reached the secondary endpoint, and 91 patients (7.9 %) reached the tertiary endpoint.

Univariate and multivariate analyses showed that CMI by any assay was associated with cardiovascular death, AMI, and revascularization, as well as secondary and tertiary endpoints, see Figure 3. The large group of patients with elevated cTnT and cTnI below the 99th percentile (n=152) had a significant increase in hazard ratio for reaching all three endpoints (unadjusted analysis). In the adjusted analysis, increased cTnT, but cTnI below the 99th percentile, was significantly associated with reaching the secondary and tertiary endpoints, but not the primary endpoint. Outcome in patients with elevated cTnI based on the Siemens or Abbott assay exclusively was not calculated due to the small number of patients.

Prognostic accuracy at the calculated optimal cutoff value

The optimal cutoff value for risk stratification based on the primary endpoint was 8 ng/L (women/men 8/9 ng/L) for the Roche cTnT assay, 2.9 ng/L (women/men: 2.9/3.4 ng/L) for the Abbott cTnI assay and 3.5 ng/L (women/men: 3.6/3.5) for the Siemens cTnI assay. At these cutoff levels there were no difference in prognostic accuracy between the three cTn assays. AUCs were significantly higher compared to AUCs at the 99th percentile cutoff levels used to diagnose myocardial injury ($p<0.001$ for all), see Table 2 and Figure 4.

Table 2: Prognostic precision at the optimal cut-off value calculated by the Youden index.

	True pos	False pos	True neg	False neg	Odds ratio	NPV (95 % CI)	Sens (95 % CI)	PPV (95 % CI)	Spes (95 % CI)	>Perc. ^a	AUC
Primary endpoint ^b											
Troponin T (Roche)	57	295	759	36	4.1	95.5	61.3	16.2	72.0	30.7	0.667 ^e
8.0/9.0 ng/L (w/m)					(2.6–6.2)	(94.2–96.5)	(50.6–71.2)	(13.8–18.9)	(69.2–74.7)		(0.638–0.694)
Troponin I (Abbott)	69	415	639	24	4.4	96.4	74.2	14.3	60.6	42.2	0.674 ^e
2.9/3.4 ng/L (w/m)					(2.7–7.2)	(95.0–97.4)	(64.1–82.7)	(12.6–16.1)	(57.6–63.6)		(0.646–0.701)
Troponin I (Siemens)	82	621	433	11	5.2	97.5	88.2	11.7	41.1	61.3	0.646 ^e
3.6/3.5 ng/L (w/m)					(2.7–9.9)	(95.7–98.6)	(79.8–94.0)	(10.8–12.6)	(38.1–44.1)		(0.618–0.674)
Secondary endpoint ^c											
Troponin T (Roche)	114	238	747	48	7.5	94.0	70.4	32.3	75.8	30.7	0.731 ^e
8.0/9.0 ng/L (w/m)					(5.2–10.8)	(92.5–95.2)	(62.7–77.3)	(29.1–35.6)	(73.0–78.5)		(0.704–0.757)
Troponin I (Abbott)	128	347	638	34	6.9	94.9	79.0	27.0	64.8	41.4	0.719 ^e
3.1/3.4 ng/L (w/m)					(4.6–10.3)	(93.0–96.5)	(71.9–85.0)	(23.0–31.2)	(61.7–67.8)		(0.692–0.745)
Troponin I (Siemens)	107	303	682	55	4.4	92.5	66.1	26.1	69.2	35.7	0.676 ^e
6.2/6.4 ng/L (w/m)					(3.1–6.2)	(90.4–94.3)	(58.2–73.3)	(21.9–30.6)	(66.3–72.1)		(0.649–0.703)
Tertiary endpoint ^d											
Troponin T (Roche)	76	276	780	15	14.3	98.1	83.5	21.6	73.9	30.7	0.787 ^e
8.0/9.0 ng/L (w/m)					(8.1–25.3)	(97.0–98.8)	(74.3–90.5)	(19.4–24.0)	(71.1–76.5)		(0.762–0.810)
Troponin I (Abbott)	74	342	714	17	9.1	97.7	81.3	17.8	67.6	36.3	0.745 ^e
3.1/4.1 ng/L (w/m)					(5.3–15.6)	(96.3–98.6)	(71.8–88.7)	(14.2–21.8)	(64.7–70.4)		(0.718–0.770)
Troponin I (Siemens)	70	333	723	21	7.2	97.2	76.9	17.4	68.5	35.1	0.727 ^e
6.7/6.4 ng/L (w/m)					(4.4–12.0)	(95.7–98.2)	(66.9–85.1)	(13.8–21.4)	(65.6–71.3)		(0.700–0.753)

w indicates women; m, men; NPV, negative predictive value; PPV, positive predictive value; AUC, area under the receiver operator characteristics curve.

^aPercent of patients with high-sensitivity cardiac troponin concentration at presentation above given cutoff value. ^bCardiovascular death, acute myocardial infarction or revascularization. ^cAll-cause mortality, AMI, revascularization, or hospitalization due to heart failure or stroke. ^dAll-cause mortality.

^eSignificantly different compared to the 99th percentile provided by manufacturer, $p < 0.001$.

Continuous variables and optimal cTn cutoff values for predicting prognosis

When evaluating cTn concentration as continuous variables, the prognostic accuracy for reaching the primary composite endpoint was highest for the Abbott cTnI assay (AUC 0.718, 95 % CI: 0.691–0.744) followed by the Roche cTnT assay (AUC 0.697, 95 % CI: 0.670–0.724) and the Siemens cTnI assay (AUC 0.662, 95 % CI: 0.634–0.689), see Figure 4 and Supplementary Material, Table S4. The AUC for cTnI by Abbot was significantly higher than the AUC for cTnI by Siemens (p -value < 0.001). Except in some subgroups, there were no significant differences between AUCs for the cTnT assay compared to those for the cTnI assays.

For the secondary endpoint, there were no differences in AUC between the Abbott cTnI assay (AUC 0.762, 95 % CI: 0.736–0.786) and the cTnT assay (AUC 0.770, 95 % CI: 0.745–

0.794), $p = 0.600$, but both had significantly higher AUC than the Siemens cTnI assay (AUC 0.714, 95 % CI 0.687–0.740). For the tertiary endpoint (all-cause mortality), the cTnT assay had higher prognostic value than the cTnI assays, see Supplementary Material, Table S4.

Outcome prediction using the LoD or 90th – 99th percentiles as cutoff values

At the 99th percentile URL, sensitivity for the primary and secondary endpoint was higher for the cTnT assay than the cTnI assays (primary endpoint: cTnT, 33.3 % [23.9–43.9]; cTnI Abbott 9.7 % [4.5–17.6]; cTnI Siemens, 8.6 % [3.8–16.3]) while the cTnI assays had higher specificity (cTnT, 83.3 % [80.9–85.5]; cTnI Abbott, 96.5 % [95.2–97.5]; cTnI Siemens, 95.7 % [94.3–96.9]). AUC increased for all assays when cutoff values were

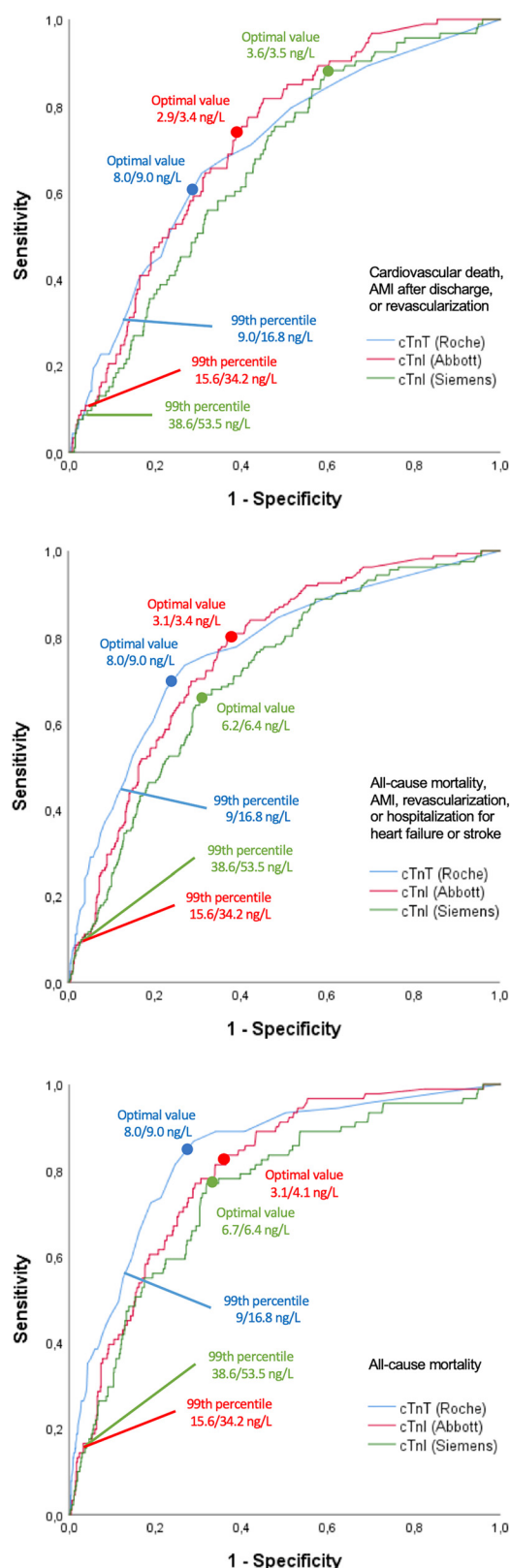


Figure 4: Receiver-operating characteristics curve for the primary, secondary, and tertiary endpoints. The 99th percentiles that help diagnose chronic myocardial injury (CMI), appears unharmonized and are numerically higher than the prognostic optimal value found by the Youden's index. Hence, sensitivity and specificity for reaching an endpoint vary between assays.

lowered from the 99th to the 90th percentile (Supplementary Material, Tables S5–S7) as the cutoff concentrations moved closer to the optimal value found by the Youden's index (Table 2 and Figure 4).

Using the LoD as cutoff value improved sensitivity to 86–97 % for the primary, secondary and tertiary outcomes but specificity was reduced, see Supplementary Material, Tables S5–S7. The cTnT assay and the Abbott cTnI assay had significantly higher AUC than the Siemens cTnI assay at the LoD for all endpoints ($p < 0.001$ for differences).

CMI prevalence and diagnostic performance for bioequivalent cut-off values

If the concentration of cTnI found to be equivalent of the cTnT 99th percentile URL (cTnI Abbott, 4.1/8.7 ng/L for women/men; cTnI Siemens, 6.9/16.5 ng/L) were used as cutoff values for CMI, the number of diagnosed patients increased by a factor of 5.2 (cTnI Abbott) and 4.2 (cTnI Siemens). The sensitivity for the primary endpoint increased from <10 to 38.7 % (Abbott) and <10 to 29.0 % (Siemens), becoming similar to the sensitivity at URL by the cTnT assay (33.3 %) see Supplementary Material, Table S8.

Discussion

In a prospective study assessing the prevalence and prognostic implications of CMI diagnosed with cTnT and cTnI in the same cohort, we demonstrate important similarities and differences between the cTnT and cTnI assays. More patients had cTnT above the URL compared to cTnI, and accordingly, CMI is diagnosed several times more often by the cTnT assay. The optimal cTn cutoff value for predicting future CV events was lower than the 99th percentile URL, and much lower for cTnI compared to cTnT. The risk assessment based on the presence or absence of CMI was of greater utility for cTnT than for any of the cTnI assays. There were no consistent differences in prognostic accuracy when cTnT and cTnI were compared as continuous variables, clearly indicating that differences in prognostic ability shown between assays were related to the absolute cut-offs (URL) chosen for diagnosing CMI. Hence, troponin URLs must be harmonized, or the diagnostic definition of CMI should be reconsidered to increase concordance between assays.

CMI is a marker of an increased risk of future CV events, but it is not adopted as a condition that warrants specific treatment or follow-up other than treatment of underlying conditions. Few studies have evaluated the effect of

prophylactic treatment based on elevated cTn apart from a study on statin treatment [28] and an observational study on CMI and the number of prescribed cardioprotective medications [14]. Our study does not find that the presence or absence of CMI is ideal for risk assessment or identifying patients who will benefit from preventive treatment, for two reasons. First, the increased risk of future CV events starts at concentrations below the 99th percentile, and risk stratification based on a continuum may be favored. Second, if CMI were to be used for prognostic assessments, the 99th percentiles of the cTnT and cTnI assays are not harmonized, and different patients will be identified depending on assay used.

The 99th percentile was a natural choice as cutoff value for CMI since it was already used in the definition of acute myocardial injury in the Universal Definition of Myocardial Infarction [9]. The calculated 99th percentile URL for different cTn assays is highly dependent on selection of reference group, preanalytical and analytical conditions [29]. For instance, subclinical disease has been extensively studied as a reason why 99th percentiles differ between assays. When echocardiography or biomarkers such as NT-proBNP, eGFR or HbA1C are used for screening before selecting the healthy cohort, the 99th percentile can be reduced by 50 % [30–32]. A recent study demonstrated that macrotroponin formation may cause false high concentrations of cTnI [33]. The mean age and ethnic composition of the reference group can also affect the measured 99th percentile, as well as the statistical analysis [34]. A recent study demonstrated that the statistical uncertainty related to estimating 99th percentiles are substantial, even in very large cohorts [35]. All these issues have led to a call for harmonisation of troponin assay cutoffs. One possibility is to derive the URLs of all assays from the same population [36]. This will improve harmonization between assays [37], but will still yield higher cut-off values compared to those suggested optimal for long-term prognostication [3–8], particularly for the cTnI assays.

A study by Wildi et al. that compared the concentrations of cTnT and cTnI in patients with AMI suggested that 20 % of patients with AMI would have been reclassified using a different cTn assay [38]. Reclassifying CMI is less clinically important, but from a clinical and patient perspective, it is rarely acceptable that a diagnosis with prognostic implications may be given four times more often in institution A compared to institution B depending on laboratory tests. Our study supports previous findings by Vestergaard and Árnadóttir, that the definition of CMI should be harmonized [19, 20], a measure likely to be of importance for future studies exploring treatment options for this high-risk patient group.

Consistent with previous studies, we found the highest AUC for prognostication at a cutoff below the 99th percentile [3–8]. The optimal prognostic threshold for cTnI was as low as 2.9 and 3.5 ng/L for the Abbott and Siemens cTnI assays, respectively. However, using such a low threshold for risk assessment would be controversial as specificity will be low. Also, the combination of biological and analytical variations is 50–60 % at low cTn concentrations [9] which limits the prognostic utility of a single blood sample to assess future risk of cardiovascular events. An alternative pragmatic strategy could be to use a lower percentile, e.g., the 90th or 95th percentile, that would correspond to a higher concentration (with higher analytical precision) and still be closer to the optimal cutoff.

The stronger association between the tertiary endpoint (all-cause mortality) and cTnT compared to cTnI has been shown before [17, 22, 23, 39, 40]. The reason may be intrinsic assay differences and the ability of cTnT to predict mortality in patients with noncardiac diseases, such as kidney failure [41, 42]. It could also be related to the distinct release mechanism of cardiomyocytes [43] or differences in protein degradation and excretion [44–46]. Although all-cause mortality as endpoint is at low risk of being affected by missing data and selection bias, it may be considered a less clinically useful endpoint, since it does not expose modifiable risk factors.

Strengths and limitations

Still, few published studies have evaluated the diagnostic and prognostic accuracy of cTnT and cTnI in the same cohort, as seen in a meta-analysis from 2017 [47]. The current study is conducted in a large cohort of patients admitted to hospital with acute chest pain. More than 80 % of the patients had three or more blood samples collected at presentation and after 3 and 8–12 h, making it unlikely that patients with acute myocardial injury are part of the cohort.

Since all patients presented to hospital with symptoms suggestive of ACS, the cohort does not represent the broad pathophysiological range of CMI including cardiomyopathies, arrhythmias, cardiac remodelling, and fibrosis. With revascularization being part of the primary endpoint, a cohort with a higher rate of patients with coronary artery disease than the general population of CMI would increase the HR for a primary event. The final diagnoses were adjudicated based on the Roche cTnT assay. Only cTnT results were available for physicians who decided on additional measurements in the emergency department, which may have introduced a diagnostic bias if ED physicians referred more

patients with chronically elevated cTnT to cardiac imaging. This bias may have affected HR for reaching the primary and secondary endpoints due to possible increased frequency of revascularizations.

Data on patient outcomes were collected through patient registries, which is less robust than information verified by clinical adjudication. However, the Norwegian Cause of Death Registry and the Norwegian Patient Registry are under Norwegian legislature, institutions are obligated to report all diagnoses, procedures, and the cause of death, and an earlier study has found the data to have acceptable accuracy [48].

Finally, the number of patients with CMI in our study is limited to 218 patients. The possible discrepancy between the assays and the possible effect on risk assessment and treatment should be assessed in a larger study evaluating more hs-cTn assays.

Conclusions

We found no consistent differences in prognostic accuracy between a high-sensitivity cTnT and cTnI assay. CMI was diagnosed four times more often by the cTnT assay than the two cTnI assays. The cTnT assay had a higher performance for risk prediction at the 99th percentile. This indicates that the diagnostic definition of CMI should be reconsidered to reduce assay-dependent differences. Using a lower percentile derived from a healthy cohort, bioequivalent cTn cutoff values, or cutoff values based on prognosis may be considered to harmonize the classification and prognostication within a high-risk patient group.

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Research ethics: The study was approved by the regional Ethics Committee (REC number 2014/1365) and was carried out according to the Declaration of Helsinki.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability: The raw data can be obtained on request from the corresponding author.

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