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ORIGINAL SCIENTIFIC PAPER
Biomarkers

Novel troponin fragmentation assay to discriminate between Takotsubo syndrome and acute myocardial infarction

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Aims

Cardiac troponin levels are elevated in Takotsubo syndrome (TTS) with significant overlap to acute myocardial infarction (MI). Long and intact cardiac troponin T (cTnT) forms are typical for MI. This study sought to assess whether the fragmentation composition of cTnT release in TTS differs from MI.

Methods and results

The concentration of long molecular forms of cTnT (long cTnT) was measured with a novel upconversion luminescence immunoassay and total cTnT with a commercial high-sensitivity cTnT assay in 24 TTS patients and in 84 Type 1 MI patients. The ratio of long to total cTnT (troponin ratio) was determined as a measure of cTnT fragmentation. Troponin ratio was lower in TTS patients [0.13 (0.10–0.20) vs. 0.62 (0.29–0.96), $P < 0.001$]. In the receiver operating characteristic curve analyses, troponin ratio showed a better predictive power than total cTnT in discriminating TTS and MI patients {area under the curve [AUC] 0.869 [95% confidence interval (CI) 0.789–0.948] vs. 0.766 [95% CI 0.677–0.855], $P = 0.047$}. When restricting the analysis to patients with total cTnT below 1200 ng/L (maximal value in TTS patients), the respective AUC values for total cTnT and troponin ratio were 0.599 (95% CI 0.465–0.732) and 0.816 (95% CI 0.712–0.921) ($P = 0.003$). At a cut-off point of 0.12, troponin ratio correctly identified 95% of MI patients and 50% of TTS patients.

Conclusion

In contrast to Type 1 MI, only a small fraction of circulating cTnT in TTS exists in intact or long molecular forms. This clear difference in troponin composition could be of diagnostic value when evaluating patients with cTnT elevations and suspicion of TTS.

Clinical trial registration

URL: https://www.clinicaltrials.gov; Unique identifier: NCT04465591

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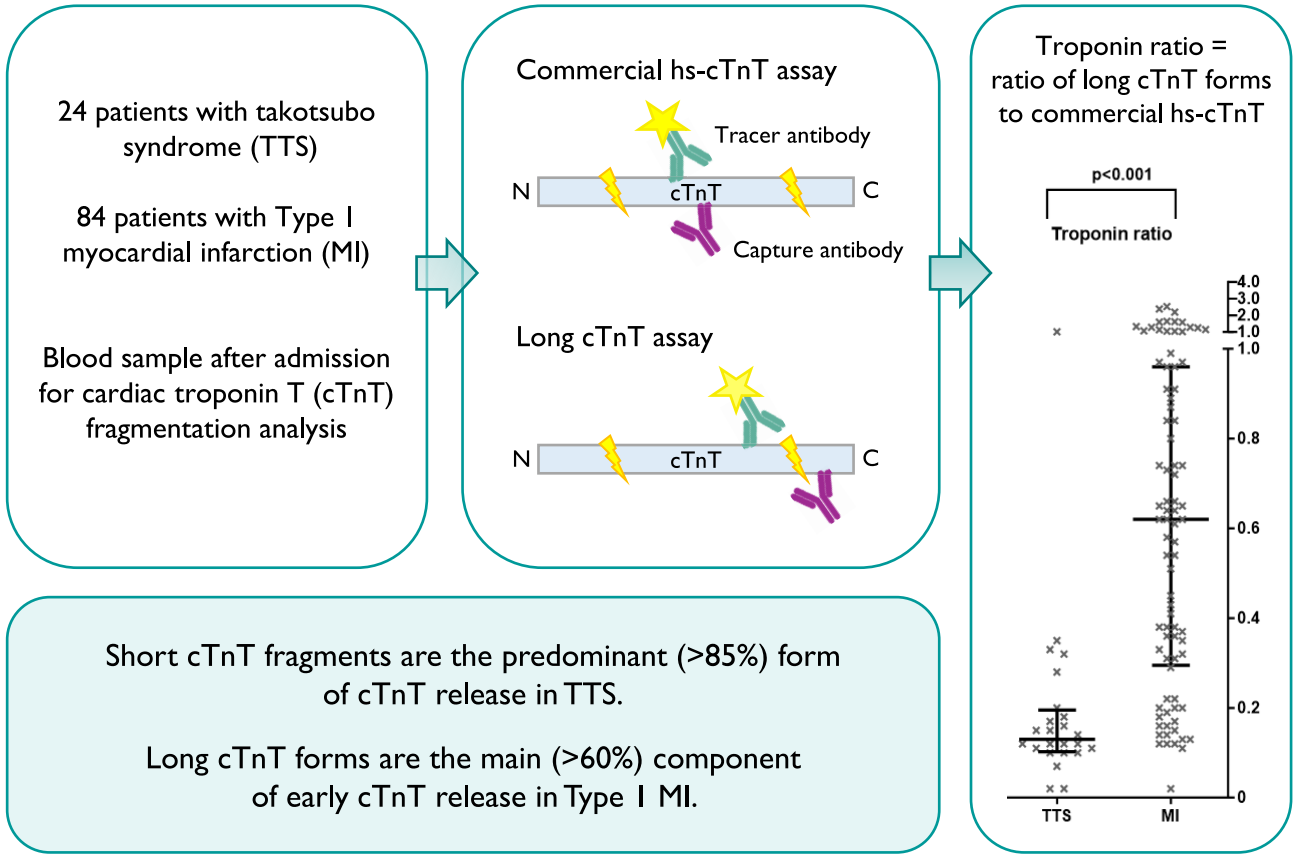
Graphical Abstract



Keywords Troponin • Myocardial infarction • Myocardial injury • Takotsubo syndrome • Biomarker

Introduction

Takotsubo syndrome (TTS) is a rapidly reversible heart failure syndrome that usually mimics the symptoms of acute myocardial infarction (MI). Early diagnosis of TTS is often challenging as the current biomarkers and electrocardiography are unable to reliably differentiate TTS from acute MI and non-invasive imaging may also be inconclusive.[1–3] When patients present with ST elevations, rapid coronary angiography is usually needed to establish the diagnosis and exclude ST elevation MI (STEMI) without delays. In patients without ST elevations, there is more time for diagnostic workup, but laboratory biomarkers that could help to discriminate TTS from Type 1 non-STEMI (NSTEMI) do not exist.[3]

Cardiac troponin levels are elevated in TTS with significant overlap to MI, although peak troponin values are usually lower in TTS than in acute MI.[1,4] Approximately 5–8% of cellular troponin is unbound in the myocyte cytoplasm.[5,6] Release of small cardiac troponin T (cTnT) fragments seems to be responsible for the troponin elevations in myocardial injury, e.g. after strenuous exercise or end-stage renal failure, while longer intact and mildly degraded troponin forms are typical for the irreversible myocardial cell damage in MI.[7–11] Importantly, the commercial cTnT assay measures long cTnT forms and the highly fragmented short cTnT forms without distinction from one another and detects cTnT elevations in conditions causing myocardial injury.[12] We call this assay the 'total cTnT' assay.

At present, there is no information on the composition of troponin elevations in TTS. Our hypothesis was that the troponin release in TTS consists mainly of fragmented cTnT forms. We have recently developed a novel immunoassay, which is much simpler and remarkably more sensitive than previously used laboratory methods for studying cTnT fragmentation.[9,13] Based on this background, we assessed whether the measurement of troponin fragmentation with our troponin fragmentation test could separate TTS from acute MI better than the commercial high-sensitivity cTnT test.[13]

Methods

Patients

We evaluated cTnT fragmentation in 24 patients with TTS and 84 patients with Type 1 MI (24 NSTEMI and 60 STEMI). Patients were recruited among patients admitted to Turku University Hospital within two similar study protocols. In the first protocol, blood samples were drawn only after admission to the Heart Centre (ClinicalTrials.gov Identifier: NCT04465591), but

in the latter protocol (ClinicalTrials.gov Identifier: NCT05858112), blood samples drawn on admission could be used for the study purposes.

Coronary angiography was performed in all included TTS patients to exclude significant coronary artery stenosis or coronary occlusion related to wall motion abnormality. Diagnosis of TTS was based on the InterTAK diagnostic criteria.[3]

Culprit lesion was documented in all included MI patients with coronary angiography, and all MI patients were treated with primary or urgent percutaneous coronary intervention. The only exclusion criteria were estimated glomerular filtration rate < 30 mL/min/1.73m^2 and a delay between symptom onset and blood sampling > 24 h.

All participants provided written informed consent. The study complies with the Declaration of Helsinki as revised in 2013, and the study protocol was approved by the Medical Ethics Committee of the Hospital District of Southwest Finland.

Blood sampling

Lithium-heparin plasma samples were collected for the study at the earliest possible opportunity after hospital admission. The samples were analysed fresh for total cTnT. Plasma for the long cTnT analysis was aliquoted and stored at −70 °C until analysis.

Laboratory methods

Total cardiac troponin T assay

All plasma samples were analysed for total cTnT with Elecsys Troponin T hs kit using Cobas 8000 system (e801 module) (Roche Diagnostics GmbH, Mannheim, Germany). The Elecsys Troponin T hs assay uses two monoclonal antibodies (mAb), which specifically target the central part of the human cTnT. For this assay, the measuring range is 3–10 000 ng/L with the limit of detection 3.0 ng/L and quantitation (coefficient of variation [CV] 10%) 13 ng/L.

Long cardiac troponin T assay

Our novel highly sensitive two-step heterogeneous sandwich-type immunoassay using upconversion luminescence for signal production was used for the detection of long (intact and mildly fragmented) molecular forms of cTnT.[13] The anti-cTnT mAb and human cardiac troponin ITC complex used as a calibrator were obtained from HyTest Ltd (Turku, Finland). The capture antibody (7E7 mAb) and the tracer antibody (1C11 mAb) bind to amino acid residues (aar) 223–242 and 174–190 of cTnT, respectively. The C-terminal region of cTnT between these two epitopes (aar 189–223) contains several cleavage sites, and thus, the ability of the assay to detect long forms of cTnT is based on targeting all cTnT molecules that are not degraded at aar 189–223. The limit of detection and limit of quantitation (CV 10%) of this assay are 0.4 ng/L and 1.8 ng/L, respectively.[13]

Troponin fragmentation

The ratio of long cTnT forms/total cTnT (troponin ratio) was used as the measure of troponin fragmentation.[9]

Statistical analysis

Continuous variables were reported as median (interquartile range) and compared with the Mann–Whitney *U* test. χ^2 or Fisher's exact test was used to compare categorical covariates in the study groups. Correlation between continuous variables was estimated using the Spearman's test. Linear regression analysis was used to identify factors significantly relating to total and long cTnT levels and their ratio in TTS and MI patients. All covariates with $P < 0.1$ in univariate analysis were included in the final multivariate linear regression models. Receiver operating characteristic (ROC) curve analyses were performed to estimate the area under the curve (AUC) to measure the discriminative capacity of total cTnT, long cTnT, and the troponin ratio between TTS and MI patients. Additional analyses were performed to assess their discriminative capacity between the TTS and MI patients with total cTnT levels < 1200 ng/L based on the maximum total cTnT value in the TTS group. All tests were two sided, and the limit of statistical significance was set at $P < 0.05$. SigmaPlot 15 (Inpixon, CA) was used for the comparison of ROC curves, and IBM SPSS Statistics software version 26.0 was used to perform all other analyses.

Table 1 Baseline characteristics, total cardiac troponin T, long cardiac troponin T, and their ratio (troponin ratio) in the study groups

	TTS patients (*n* = 24)	MI patients (*n* = 84)	*P*
Age, years	67 (62–79)	66 (58–74)	0.125
Female gender	24 (100)	19 (22.6)	<0.001
Hypertension	11 (45.8)	39 (46.4)	1.0
Diabetes	2 (8.3)	14 (16.7)	0.515
Heart failure	0 (0)	2 (2.4)	1.0
Atrial fibrillation	2 (8.3)	4 (4.8)	0.613
Creatinine, µg/L	67 (62–83)	75 (65–86)	0.188
Total cTnT, ng/L	191 (90–304)	835 (226–2373)	<0.001
Long cTnT, ng/L	22.3 (8.8–36.5)	385 (69–1810)	<0.001
Troponin ratio	0.13 (0.10–0.20)	0.62 (0.29–0.96)	<0.001
Sampling delay (h)[a]	15 (2–23)	17 (14–22)	0.569

Values are medians (25th–75th percentile) for continuous variables and *n* (%) for categorical variables.
Long cTnT, long molecular forms of cTnT; MI, myocardial infarction; Total cTnT, commercial high-sensitivity cardiac troponin T; troponin ratio, ratio of long cTnT forms to total cTnT; TTS, Takotsubo syndrome.
[a]Time from symptom onset to blood sample. No data in four TTS patients due to vague onset of symptoms.

Results

Baseline characteristics of Takotsubo syndrome patients

All 24 TTS patients were female. Seven patients had ST elevations in the admission electrocardiogram. In coronary angiography, eight patients had mild atherosclerosis including one patient with coronary artery stenosis. Wall motion abnormality was apical in 19 patients, midventricular in four patients, and focal in one patient. The N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels were elevated in all but one TTS patient with measurements [median 2100 (1023–4348) ng/L, *n* = 16]. The length of hospital stay ranged from 2 to 8 days. There was no mortality in 30 days after the index event. The clinical characteristics of the study groups are summarized in *Table 1*.

Total cardiac troponin T, long cardiac troponin T, and troponin ratio in Takotsubo syndrome and myocardial infarction patents

Both the total and long cTnT values were higher ($P < 0.001$) in MI patients than in TTS patients [835 (226–2373) ng/L vs. 191 (90–304) ng/L and 385 (69–1810) ng/L vs. 22.3 (8.8–36.5), respectively; *Table 1* and *Figure 1*]. Importantly, the ratio of long to total cTnT (troponin ratio) was lower in TTS patients compared with MI patients [0.13 (0.10–0.20) vs. 0.62 (0.29–0.96), $P < 0.001$; *Table 1* and *Figure 1*]. When only patients with total cTnT < 1200 ng/L (maximum cTnT for TTS

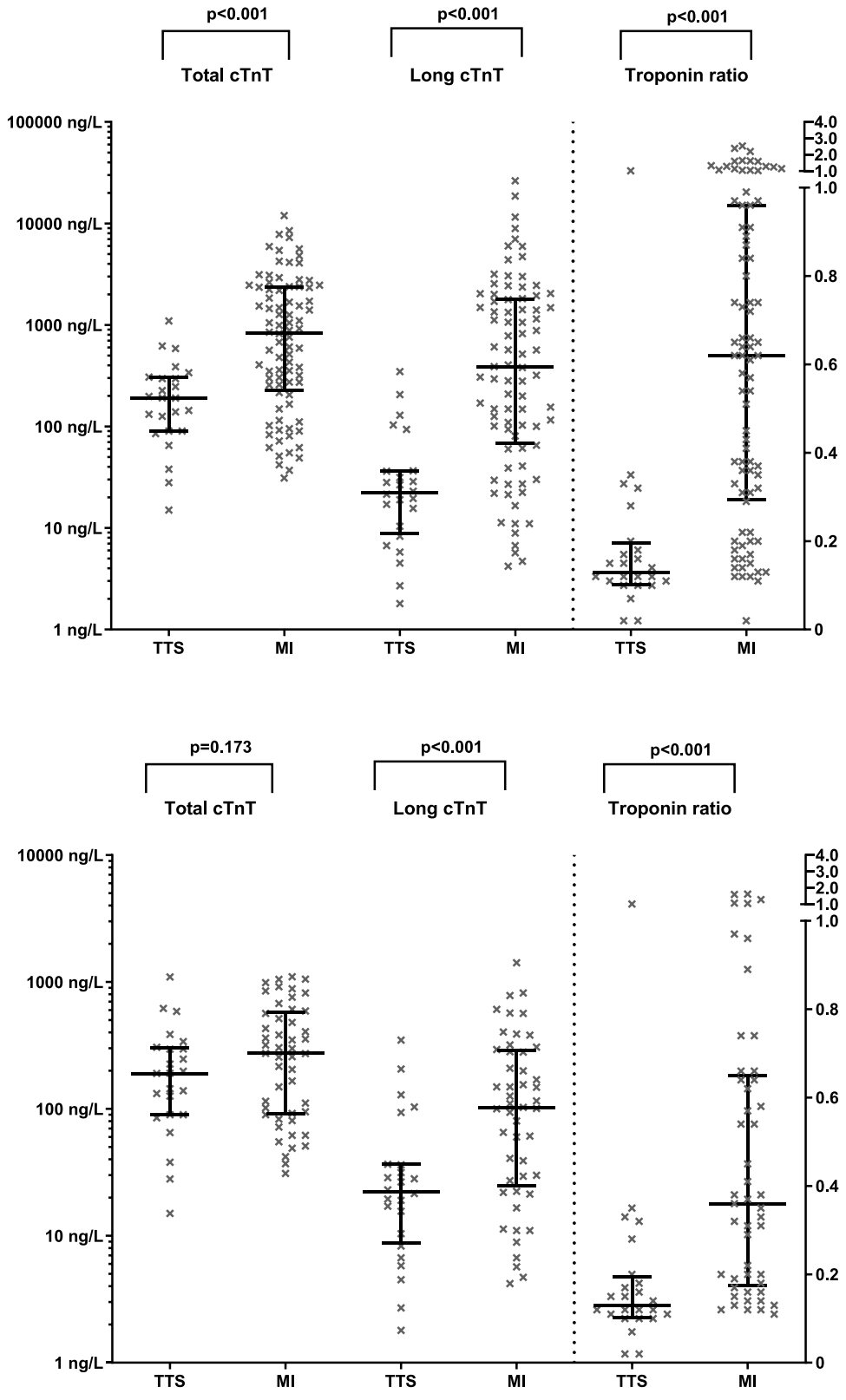


Figure 1 Cardiac troponin T forms in Takotsubo syndrome and Type 1 myocardial infarction patients. Total cardiac troponin T, long cardiac troponin T, and troponin ratio (ratio of long cardiac troponin T to total cardiac troponin T) in all Takotsubo syndrome and myocardial infarction patients (upper panel) and in a subgroup of patients with total cTnT < 1200 ng/L (lower panel). cTnT, cardiac troponin T; MI, myocardial infarction; TTS, Takotsubo syndrome.

group) were included in the comparison, total cTnT did not differ significantly between MI and TTS [274 (91–578) ng/L vs. 191 (90–304) ng/L, $P = 0.173$] while the differences between long cTnT [103 (25–288) ng/L vs. 22.3 (8.8–36.5) ng/L, $P < 0.001$] and troponin ratio [0.36 (0.18–0.65) vs. 0.13 (0.10–0.20), $P < 0.001$] remained significant.

Diagnostic performance of total and long cardiac troponin T and troponin ratio

In the ROC curve analyses, troponin ratio showed a good predictive power in discriminating TTS and MI patients with an AUC of 0.869 [95% confidence interval (CI) 0.789–0.948]. The respective AUC value for total cTnT was significantly lower (0.766, 95% CI 0.677–0.855, $P = 0.047$). For long cTnT, the AUC was between these values (0.844, 95% CI 0.769–0.919, $P = 0.003$ for comparison with total cTnT). At a cut-off point of 0.12, troponin ratio correctly identified 95% of MI patients and 50% of TTS patients (*Figure 2*).

When restricting the analysis in patients with total admission cTnT concentration below 1200 ng/L [49 (58.3%) MI patients and all TTS patients], the respective AUC values for total and long cTnT and troponin ratio were 0.599 (95% CI 0.465–0.732), 0.741 (95% CI 0.625–0.857), and 0.816 (95% CI 0.712–0.921), respectively ($P = 0.001$ and $P = 0.002$ for respective comparisons to total cTnT).

Predictors of troponin ratio

In MI group, troponin ratio increased significantly ($r = 0.565$, $P < 0.001$) with increasing total cTnT release and decreased ($r = -0.276$, $P = 0.011$) with longer delay between symptom onset and plasma sample collection. Troponin ratio was higher in STEMI patients (0.66 vs. 0.21, $P < 0.001$), but there was no significant difference in troponin ratio between men and women (0.65 vs. 0.38, $P = 0.07$). In TTS patients, troponin ratio was not related to total cTnT ($P = 0.676$) or delay between symptom onset and sampling ($P = 0.08$, $n = 20$). Troponin ratio was comparable (0.11 vs. 0.15, $P = 0.21$) in patients with and without atherosclerosis.

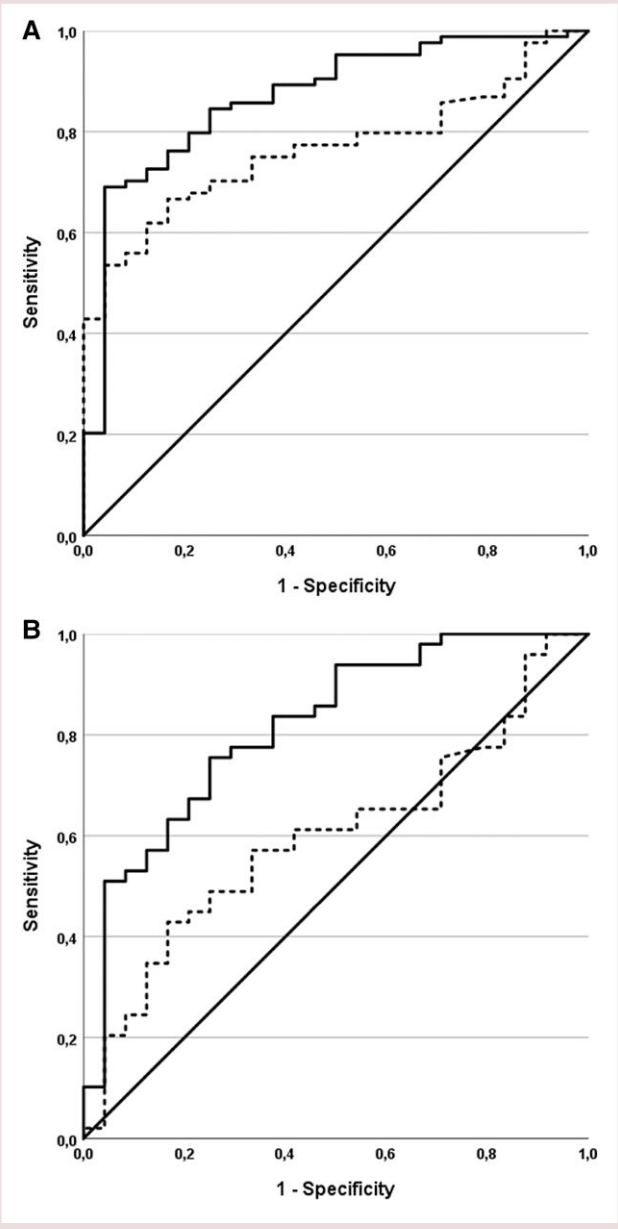


Figure 2 Receiver operating characteristic curves depicting the discriminative capacity of troponin ratio (full line) and total cardiac troponin T (dotted line) between patients with Takotsubo syndrome and myocardial infarction. All patients: area under the curve of 0.869 (95% confidence interval 0.789–0.948) vs. 0.766 (95% confidence interval 0.677–0.855), $P = 0.047$ (*A*); and patients with total cardiac troponin T < 1200 ng/L: area under the curve of 0.599 (95% confidence interval 0.465–0.732) vs. 0.816 (95% confidence interval 0.712–0.921), $P = 0.003$ (*B*).

Discussion

We showed that shorter cTnT fragments are the predominant (>85%) form of cTnT release after TTS in contrast to Type 1 MI, where long cTnT forms are mainly (>60%) found in circulation during the early hours after MI. The present observations on clear difference in troponin composition between Type 1 MI and TTS could be helpful in the early diagnostic workup of TTS.

Earlier research on troponin fragmentation has employed gel filtration chromatography, western blotting, and mass spectrometry. However, these techniques are too complicated and time-consuming for clinical use.[7,11,14] The low analytical sensitivity of these methods is another obstacle for clinical applications. Our sensitive immunoassay technique showed that the magnitude of long cTnT release remained at a low level in TTS patients regardless of the level of total cTnT release. The novel finding on smaller troponin fragments as the predominant component of circulating cTnT in TTS is similar to the troponin composition in patients with end-stage renal failure, although in renal patients, the long cTnT levels are slightly lower than in TTS.[7,9,13] In Type 1 MI, intact and longer forms of troponin were the predominant form of troponin release in patients with extensive myocardial damage, which is in line with previous reports on small groups of patients with large STEMI assessed with gel filtration chromatography, western blotting, and mass spectrometry.[7,11,15] As a novel finding, our sensitive test showed that the troponin composition was related to the magnitude of myocardial injury; that is, that the proportion of long cTnT was higher in patients with higher total cTnT release in MI patients.

Our results provide further evidence that immunoassays for specific forms of cTnT can provide important additional information compared with current cTnT assays. For cTnI, the situation is more complicated, as current commercial cardiac troponin I (cTnI) immunoassays are already utilizing various combinations of antibodies targeting different parts of cTnI as summarized in the online Biomarkers Reference Tables of the Committee on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine (https://ifcc.org/ifcc-education-division/emd-committees/committee-on-clinical-applications-of-cardiac-bio-markers-c-cb/biomarkers

-reference-tables/) and thus detecting different forms while claiming to give similar information.

The InterTAK Diagnostic Score based on simple clinical parameters was the first attempt to provide a diagnostic tool for TTS.[16] Small biomarker studies have suggested that the ratio of natriuretic peptides to troponin or creatinine kinase might also help to separate TTS from MI with relatively high accuracy.[17–21] Unfortunately, admission values that are crucial for the early diagnostic workup have shown lower accuracy than later peak levels of biomarkers, which is natural, since levels of natriuretic peptides typically rise later during the attack. In line with this pathophysiology, the ratios of these 'old' biomarkers at admission had only a moderate power to distinguish between TTS and acute coronary syndromes with AUC values ranging from 0.51 to 0.67 in a recent large all-comer cohort. Area under the curve values above 0.8 are typically considered to indicate high performance. In the present study, troponin ratio showed AUC value of 0.869 in separating TTS patients from Type 1 MI patients, and only 5% of MI patients had a troponin ratio lower than 0.12. More importantly, AUC of troponin ratio remained at a high level of 0.816 also in a more challenging patient group with admission cTnT below 1200 ng/L.

Pathophysiological mechanisms leading to myocardial injury in TTS patients are incompletely understood. Coronary spasm, microvascular dysfunction, and catecholamine toxicity are considered to underly transient myocardial injury and stunning. Small cTnT fragments can be released from the unbound pool in the cytoplasm through several mechanisms and lead to the observed troponin elevations in TTS.[5,22] Importantly, irreversible myocyte damage is not the prerequisite for the release of these cytosolic small troponin fragments and is also observed, e.g. in renal failure and after strenuous exercise.[7,8]

The main limitations of our study are the single-centre design and the small number of TTS patients, and these novel findings need validation in a larger cohort of TTS and MI patients. In some patients, the long cTnT concentrations were higher than the total cTnT values. This is likely due to differences in the calibration of the assays. It should be noted that fragmentation of troponins in the circulation is a continuous process after MI and troponin ratio was related to sample delay also in the present patient cohort.[10,13,23] Longer delays between MI symptom onset and sampling are likely to dilute the observed differences in troponin composition.

In conclusion, our novel highly sensitive long cTnT immunoassay shows that the troponin release in TTS is composed mainly of smaller troponin fragments. The test holds promise that measuring long cTnT forms could help to separate troponin elevations caused by TTS from those of acute MI in a single sample with better accuracy than the commercial high-sensitivity cTnT test combined with natriuretic peptides. Importantly, the principle of our assay could be applied on automated platforms to allow implementation in clinical care to improve the accuracy and rapidity of laboratory diagnostics of MI and TTS.

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Conflict of interest: J.K.E.A.: research grants from the Finnish Foundation for Cardiovascular Research and Clinical Research Fund of Turku University Hospital, Turku, Finland; lectures for Astra Zeneca, Bayer, and Boehringer Ingelheim; and pending patent application WO2023187258 (A1)—ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T; T.P.: lectures for Astra Zeneca; T.H.: lectures for AstraZeneca, Astellas, and GSK and pending patent application WO2023187258 (A1)—ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T; T.V.: pending patent application WO2023187258 (A1)—ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T; T.T., S.S., H.J., M.S.: none declared; A.L.-P.: research grants from the Finnish Society of Clinical Chemistry; H.P.: lectures for Roche; K.T.: research grants from The Finnish Foundation for Cardiovascular Research, The Finnish Medical Foundation, The Finnish Foundation for Alcohol Studies, and the Finnish State Research Funding; S.J.: lectures for Amgen, Boehringer Ingelheim, and BMS Pfizer; S.W.: research grants from the Finnish Society of Clinical Chemistry, the Finnish Foundation for Cardiovascular Research, the Turku University Foundation and the Varsinais-Suomi Regional Fund of the Finnish Cultural Foundation, research funding from Business Finland, official Finnish government agency for trade, and investment promotion, innovation funding, travel promotion, and talent attraction; and pending patent application WO2023187258 (A1)—ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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