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ORIGINAL RESEARCH

Long Troponin T to Separate Troponin Elevations Among Patients With Atrial Fibrillation Versus Myocardial Infarction

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BACKGROUND: Elevated troponin levels are a frequent finding in patients presenting with atrial fibrillation or atrial flutter (AF) to the emergency department but are seldom caused by myocardial infarction (MI). The current high-sensitivity cTnT (cardiac troponin T) assay measures both the intact and highly fragmented cTnT forms (total cTnT) and detects cTnT elevations in conditions causing myocardial injury or MI without distinction between the 2.

METHODS: The SuperTROPO (Better Diagnostics of Myocardial Infarction With a Test for Special Forms of Troponin) study included 521 consecutive patients with AF only and 188 patients with MI only (139 Type 1 MI), all with a total cTnT value ≥14 ng/L at emergency department admission. Intact and long forms of cTnT (long cTnT) were analyzed from the first plasma samples using a novel immunoassay. The diagnostic performance of long cTnT and total cTnT assays was compared in these cases with elevated total cTnT.

RESULTS: Long cTnT was superior to total cTnT in discriminating troponin elevations in patients with MI from those in patients with AF (area under the curve for type 1 MI: 0.879 versus 0.783; for any MI: 0.864 versus 0.779; both $P<0.001$) when measured from the first blood sample without a significant effect of sex, age, estimated glomerular filtration rate, or total cTnT <200 ng/L. The difference in long cTnT levels was most notable in patients presenting within 12 hours of symptom onset.

CONCLUSIONS: The long cTnT immunoassay shows that the troponin release in AF is composed mainly of smaller troponin fragments. This novel test holds promise that measuring long cTnT forms could help to separate troponin elevations caused by AF from those of acute Type 1 MI from a single sample with better accuracy than the commercial high-sensitivity cTnT test.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT05858112.

Key Words: atrial fibrillation ■ biomarker ■ immunoassay ■ myocardial infarction ■ myocardial injury ■ troponin

Cardiac troponins are often measured in patients with atrial fibrillation or atrial flutter (AF) as a part of the routine diagnostic workup in the emergency department (ED).[1] Mildly elevated troponin levels are common, and the interpretation of their significance is an everyday challenge in patients presenting with chest discomfort or other acute symptoms.[2,3] Minor troponin elevations in AF are only rarely caused by myocardial infarction (MI) but are considered to reflect myocardial injury or stress caused by AF itself or various comorbidities.[3,4]

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CLINICAL PERSPECTIVE

What Is New?

- Intact and minimally fragmented cTnT (cardiac troponin T) forms (long cTnT) were measured using a novel immunoassay in 521 consecutive patients with atrial fibrillation or flutter and in 188 patients with acute myocardial infarction (MI) having elevated (≥14 ng/L) high-sensitivity cTnT levels at emergency department admission.
- Long cTnT was superior to a single measurement of commercial cTnT in separating patients with MI versus atrial fibrillation or flutter (area under the curve for Type 1 MI: 0.879 versus 0.783 and for any MI: 0.864 versus 0.779, both *P* values <0.001).

What Are the Clinical Implications?

- The long cTnT immunoassay shows that the troponin release in atrial fibrillation or flutter is composed mainly of smaller troponin fragments, and this novel test holds promise that measuring long cTnT forms could help to separate troponin elevations caused by atrial fibrillation or flutter from those of acute Type 1 MI from a single admission sample with better accuracy than the commercial high-sensitivity cTnT test.

Nonstandard Abbreviations and Acronyms

long cTnT	longer intact or mildly degraded forms of troponin T
NRI	Net Reclassification Index
total cTnT	commercial high-sensitivity cTnT assay

Approximately 5% to 8% of cellular troponin is unbound in the myocyte cytoplasm.[5,6] Release of small cTnT (cardiac troponin T) fragments seems to be responsible for the troponin elevations in myocardial injury, such as in end-stage kidney disease, heart failure, or after strenuous exercise, whereas longer intact and mildly degraded troponin forms are typical for the irreversible myocardial cell damage in MI.[7,8] Importantly, the commercial cTnT assay measures both the intact and long cTnT forms and the highly fragmented short cTnT forms without distinction between the 2 and detects cTnT elevations in conditions causing myocardial injury (total cTnT).[9] Thus, the inability of current commercial cTnT assay to differentiate between molecule lengths may contribute to its limited specificity for MI.

At present, there are no data on the composition of troponin elevations in AF. Our hypothesis was that the troponin release in AF consists mainly of highly fragmented cTnT forms. Based on this background, we assessed whether measuring the longer intact or mildly degraded forms of troponin T (long cTnT) using our novel highly sensitive immunoassay could provide additional value in distinguishing cTnT elevations in AF from those of acute MI, especially Type 1 MI.[10] For this purpose, we selected patients with elevated (≥14 ng/L) commercial total cTnT levels at ED admission and analyzed whether long cTnT was superior to commercial total cTnT in separating patients with MI versus AF when measured from a single ED admission sample.

METHODS

Study Cohort

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patients meeting the inclusion criteria provided written informed consent either in the hospital ward or via mail if discharged early from the ED. Retrospective consent was deemed ethically acceptable due to the health status of potential study patients at the ED entry stage and the use of only leftover samples for research purposes. The study complies with the Declaration of Helsinki, as revised in 2024, and the study protocol was approved by the Medical Ethics Committee of the Wellbeing Services County of Southwest Finland (VARHA/487/13.02.02/2023).

The prospective SuperTROPO (Better Diagnostics of Myocardial Infarction With a Test for Special Forms of Troponin) study (ClinicalTrials.gov Identifier: NCT05858112) recruited consecutive patients with commercial high-sensitivity cTnT levels above the upper reference limit (≥14 ng/L) at the ED of Turku University Hospital, Turku, Finland, between May 8, 2023, and May 30, 2024 (with a summer pause in recruitment from June 22, 2023, to September 1, 2023). The inclusion criteria in the SuperTROPO study were an ED visit, age >18 years, a high-sensitivity cTnT value ≥14 ng/L upon arrival at the first blood sample, and signed informed consent. Exclusion criteria included inability to provide informed consent, a vulnerable condition (delirium, dementia, or critical illness), pregnancy, and prior participation in the study. The cardiac package of laboratory tests, including the high-sensitivity cTnT measurement, which was used to screen for study inclusion (≥14 ng/L), was ordered at the discretion of the attending clinician. This package is typically ordered in the clinical practice of Turku University Hospital ED when a cardiac event is suspected or when symptoms are unclear, and the possibility of a cardiac event cannot be ruled out. In addition to high-sensitivity cTnT, it includes complete blood count, plasma C-reactive protein, sodium, potassium, and creatinine.

This substudy focused on comparing patients with AF only (including atrial fibrillation or atrial flutter) at presentation to the ED with those who had an acute MI (Figure S1). Consequently, patients without MI or AF were excluded. More specifically, in the main analysis, we compared patients who had only AF with those who had only acute MI, and thus those with both conditions were excluded from the main analysis. Each analysis included patients with the MI type of interest and patients with AF, so that when Type 1 MI was the outcome of interest, patients with Type 2 MI were excluded. Additionally, we performed analyses to compare patients who had both AF and MI versus patients with AF only (Figure S1). Patients with AF were analyzed as a single group and separately for atrial fibrillation and flutter. Patients were classified as having AF based on their initial ECG at the ED.

Defining symptom status in clinical practice can be challenging, and minor troponin elevations present diagnostic difficulties, particularly in patients without clear symptoms or findings suggestive of MI. Therefore, in our study, the primary analysis focused on all patients presenting to the ED with elevated cTnT levels, regardless of the type of acute symptoms. Additionally, we assessed the performance of cTnT assays separately in patients presenting to the ED with chest pain or dyspnea.

Definition of Myocardial Infarction

The main outcome, Type 1 MI, was defined in accordance with current clinical guidelines.[1,11] In brief, Type 1 MI was defined as a clinical setting leading to acute myocardial ischemia from an acute coronary atherothrombosis. Type 2 MI was defined as acute myocardial ischemia resulting from a mismatch between oxygen supply and demand in the myocardium, unrelated to acute coronary atherothrombosis. Any MI included patients with Type 1 and Type 2 MI. No single algorithm for total cTnT measurement was mandated as serial cTnT sampling was performed at the discretion of the treating clinicians when deemed necessary, but the national guidelines on acute coronary syndromes advocate the use of the 0/1 hour or 0/2 hour algorithms when clinically applicable. The final MI diagnosis was reviewed from electronic patient records of the index hospitalization and adjudicated by 2 cardiologists based on all available clinical data: routine laboratory tests (including dynamic changes in high-sensitivity cTnT levels in the ED and during hospitalization), ECG, echocardiography, and imaging findings (including cardiac computed tomography angiography and invasive coronary angiography). A third cardiologist was consulted to resolve any disagreements. All adjudicators were blinded to long cTnT values.

Blood Sampling

The first routine laboratory samples were collected as soon as possible after admission to the ED. These samples were analyzed fresh for total cTnT as part of the normal clinical practice. Leftover lithium heparin plasma samples obtained during routine testing were used as the study samples. The centrifuged plasma samples were aliquoted into coded vials and frozen at –70 °C within 12 hours of sampling. A stability study was conducted to confirm analyte stability in room temperature and in +4 °C during the maximum period of 12 hours between sampling and sample freezing.

Total Cardiac Troponin T Assay

All plasma samples were analyzed for total cTnT with the Elecsys Troponin T high-sensitivity kit using the Cobas 8000 system (e801 module) (Roche Diagnostics GmbH, Mannheim, Germany). The Elecsys Troponin T high-sensitivity assay uses 2 monoclonal antibodies, which specifically target the central part of human cTnT and detect intact, mildly, and heavily fragmented cTnT forms (Figure S2). For this assay, as reported by the assay manufacturer in the package insert, the measuring range is 3 to 10000 ng/L with a limit of detection of 3.0 ng/L and a limit of quantitation (coefficient of variation ≤10%) of 13 ng/L.

Long Cardiac Troponin T Assay

Our novel highly sensitive 2-step heterogeneous sandwich-type immunoassay based on upconversion luminescence was used for the detection of long (intact and mildly fragmented) molecular forms of cTnT.[2] The assay was performed according to the previously published protocol, except the capture antibody and sample incubations were 60 minutes instead of 30 minutes. These modifications were made to streamline the analysis process, and they were experimentally proven not to affect the performance of the assay.[2] All study samples were analyzed in duplicates in batch format. The anti-cTnT monoclonal antibodies and native human cardiac troponin ITC complex used as a calibrator were obtained from HyTest Ltd (Turku, Finland). The capture and tracer antibodies in the long cTnT assay bind to epitopes on both sides of the C-terminal cleavage region to detect only intact and mildly fragmented forms of cTnT that have not been cleaved at the C-terminal region of amino acid residues 189 to 223, which contains many cleavage sites (Figure S2).[2] The limit of detection and limit of quantitation (coefficient of variation 10%) of this assay are 0.4 ng/L and 1.8 ng/L, respectively.[2] The ratio of long cTnT forms to total cTnT (troponin ratio) was used as the measure of troponin fragmentation.

Statistical Analysis

Continuous variables were compared using the Mann–Whitney *U* test or Student *t* test, as appropriate (Mann–Whitney *U* test for differences in total and long cTnT). Chi-square test was used to compare categorical variables. Receiver operating characteristic curve analyses were conducted to assess the area under the curve (AUC) as a measure of the ability of total cTnT and long cTnT to discriminate patients with the MI type of interest from those with AF. We also assessed the discriminative capacity of the ratio between total cTnT and long cTnT (troponin ratio). Additionally, the proportion of patients with MI was assessed across tertiles of total and long cTnT values. Stratified receiver operating characteristic curve analyses were conducted by sex (men and women), age (<70 or ≥70 years), estimated glomerular filtration rate (<60 or ≥60 mL/min/1.73 m^2; calculated with Chronic Kidney Disease Epidemiology Collaboration 2021 equation), time from symptom onset (<12 or ≥12 hours), heart rate <100 or ≥100 beats per min, as well as by excluding patients presenting with ST-elevation MI. Additional analyses explored the added value of long cTnT in the clinical setting of smaller troponin elevations (total cTnT <200 ng/L). Optimal cutoffs for total cTnT and long cTnT, maximizing sensitivity and specificity, were determined using the Youden Index, and corresponding positive and negative predictive values were calculated based on these thresholds. Additionally, specificity at a sensitivity threshold of 90% was calculated, as prioritizing sensitivity is important to minimize missed MI cases.

To assess the value of long cTnT for clinical decision-making in patients with elevated total cTnT, we used continuous Net Reclassification Index (NRI) and decision curve analyses. The continuous NRI ranges from −2 to 2 and quantifies the number of cases whose predicted risk increases and noncases whose predicted risk decreases with the new model compared with the reference model.[12] Values >0 are in favor of the new model. Decision curve analyses evaluated the net benefit (true positive rate minus the false positive rate) in identifying MIs with cTnT data across a range of possible decision thresholds.[13,14]

In the NRI and decision curve analyses, predicted MI probabilities were calculated using logistic regression models with continuous variables of long cTnT and total cTnT. To mitigate overfitting in the regression models, we performed repeated 10-fold random sample cross-validation, generating model predictions for each 10% data subset using models trained on the remaining 90%. Final predicted probabilities were averaged across 10 repetitions.[15] The NRI accounts for all changes in predicted risk; however, with continuous predictors, there may be very minor shifts in predicted risks, not all of which are clinically relevant. To address this, we also computed continuous NRI by focusing only on the reclassification of patients whose predicted risk changes at least 10% ($NRI_{>10\%}$). Bootstrapping with 1000 iterations was used to obtain the 95% CIs for the NRI. All tests were 2 sided, with statistical significance assessed using a *P* value threshold of 0.05 or the 95% CIs. All analyses were performed with R (version 4.2.2, R Core Team, Vienna, Austria). This study followed the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline and checklist.

RESULTS

The SuperTROPO study included 1811 patients with a total cTnT value of ≥14 ng/L at ED arrival. This substudy focused on 521 (28.8%) patients with AF only (459 atrial fibrillation, 62 atrial flutter) and 188 (10.4%) patients with acute MI consisting of 139 patients with Type 1 MI and 49 Type 2 MI Additionally, there were 17 (0.9%) patients with AF who were diagnosed with acute MI and were not included in the main analysis. Among patients with AF, 108 (20.7%) were experiencing their first documented episode, and the remainder had a history of prior AF. Moreover, 213 patients (40.9%) had persistent or permanent AF. Patients with MI, especially those with Type 1 MI, were younger, more often male, and more likely to present with chest pain, whereas dyspnea was more common in those with AF (Table 1).

Both long and total cTnT levels were lower in patients with AF compared with those in any of the MI categories (all *P* values <0.001, Figure 1). The troponin ratio as a measure of troponin fragmentation was lower in patients with AF than in patients with MI (median ratios 15%, 33%, and 34% in patients with AF, any MI, and Type 1 MI, respectively; all *P* values between AF and MI categories <0.001; Figure S3). In patients with MI presenting with symptoms starting <12 hours before sampling, troponin ratios were slightly higher (median ratios: 36% and 37% in patients with any MI and Type 1 MI, respectively) than in those with a longer delay between symptom onset and sampling. Long and total cTnT values were similar between patients with atrial fibrillation and atrial flutter (Figure S4). Median total and long cTnT values in subgroups of patients with AF are given in Table S1.

Discriminative Performance

Long cTnT was superior to total cTnT in discriminating patients with Type 1 MI and any MI from those with AF (Figure 2 and Table 2). AUC values for long and total cTnT were 0.879 versus 0.783 for Type 1 MI and 0.864 versus 0.779 for any MI, respectively (both *P* values <0.001). The specificity of long cTnT for both Type 1 MI and any MI

Table 1. Characteristics of the Study Cohort

	AF	Any MI	*P* value	Type 1 MI	*P* value	Type 2 MI	*P* value
No.	521	188		139		49	
Mean age, y	78.0 (8.8)	70.1 (13.1)	<0.01	68.5 (13.7)	<0.01	74.8 (9.7)	0.03
Female sex	231 (44.3)	65 (34.6)	0.02	44 (31.7)	<0.01	21 (42.9)	0.84
Chronic kidney disease	71 (13.6)	15 (8.0)	0.04	12 (8.6)	0.12	3 (6.1)	0.14
Coronary artery disease	133 (25.5)	58 (30.9)	0.16	35 (25.2)	0.93	23 (46.9)	<0.01
Diabetes	175 (33.6)	53 (28.2)	0.17	40 (28.8)	0.28	13 (26.5)	0.32
Dyslipidemia	207 (39.7)	102 (54.3)	<0.01	71 (51.1)	0.02	31 (63.3)	<0.01
Heart failure	178 (34.2)	19 (10.1)	<0.01	14 (10.1)	<0.01	5 (10.2)	<0.01
Hypertension	391 (75.0)	130 (69.1)	0.12	92 (66.2)	0.04	38 (77.6)	0.70
Prior MI	52 (10.0)	43 (22.9)	<0.01	27 (19.4)	<0.01	16 (32.7)	<0.01
Prior stroke	65 (12.5)	15 (8.0)	0.10	10 (7.2)	0.08	5 (10.2)	0.64
Mean CHA_2DS_2-VASc	4.2 (1.7)	3.1 (1.9)	<0.01	2.9 (1.9)	<0.01	3.6 (1.8)	<0.01
Mean body mass index, kg/m^2	28.5 (13.8)	27.8 (4.4)	0.52	28.0 (4.3)	0.74	27.0 (4.7)	0.48
Mean estimated glomerular filtration rate, mL/min per 1.73 m^2	63.3 (24.1)	76.4 (23.7)	<0.01	77.8 (24.0)	<0.01	72.5 (22.8)	<0.01
Mean C-reactive protein, mg/L	22 (45)	11 (30)	<0.01	8 (24)	<0.01	19 (45)	0.90
Mean hemoglobin, g/L	123 (22)	136 (21)	<0.01	139 (16)	<0.01	127 (29)	0.88
Mean creatinine, μmol/L	113 (86)	98 (72)	<0.01	99 (81)	<0.01	93 (41)	0.02
Oral anticoagulant use	365 (70.1)	16 (8.5)	<0.01	13 (9.4)	<0.01	3 (6.1)	<0.01
Admitted to hospital	310 (59.5)	188 (100.0)	<0.01	139 (100.0)	<0.01	49 (100.0)	<0.01
Percutaneous coronary intervention performed*	1 (0.2)	116 (61.7)	<0.01	102 (73.4)	<0.01	14 (42.9)	<0.01
Coronary artery bypass graft planned	1 (0.2)	25 (13.3)	<0.01	19 (13.7)	<0.01	6 (12.2)	<0.01
Median heart rate, beats per min	98 (46)	76 (23)	<0.01	75 (26)	<0.01	77 (21)	<0.01
Known symptom onset time	290 (55.7)	175 (93.1)	<0.01	132 (95.0)	<0.01	43 (87.8)	<0.01
Median time from symptom onset, h†	14.9 (63.8)	6.1 (20.8)	<0.01	5.1 (24.1)	<0.01	7.6 (15.7)	0.11
Chest pain	109 (20.9)	168 (89.4)	<0.01	129 (92.8)	<0.01	39 (79.6)	<0.01
Dyspnea	247 (47.4)	49 (26.1)	<0.01	28 (20.1)	<0.01	21 (42.9)	<0.01
Median total cTnT, ng/L	27 (24)	81 (181)	<0.01	71 (186)	<0.01	92 (168)	<0.01
Median long cTnT, ng/L	4.4 (4.3)	24.6 (67.0)	<0.01	25.5 (80.1)	<0.01	18.0 (60.6)	<0.01
Median long/total cTnT ratio	0.15 (0.15)	0.33 (0.40)	<0.01	0.34 (0.52)	<0.01	0.30 (0.35)	<0.01

Values are presented as counts (percentages) for categorical variables, and as means±SD for normally distributed continuous variables, or medians (interquartile range) for nonnormally distributed continuous variables, as specified. *P* values are compared with patients with AF. AF indicates atrial fibrillation and atrial flutter; cTnT, cardiac troponin T; and MI, myocardial infarction.

*During index hospitalization.

†In patients with known symptom onset time.

was markedly higher than that of total cTnT (57% versus 40% and 53% versus 31%, respectively) at the threshold corresponding to 90% sensitivity (Table 2). Long and total cTnT performed similarly (AUC values 0.822 versus 0.766, *P*=0.19) in distinguishing between patients with Type 2 MI and patients with AF (Table 1).

When patients were divided into tertiles based on their cTnT values, 1.4% of those in the lowest long cTnT tertile had a Type 1 MI and 4.2% had any MI. In contrast, the proportion of patients with either Type 1 MI or any MI increased markedly across higher long cTnT tertiles. Among those in the highest tertile for both total and long cTnT, 63.3% had a Type 1 MI and 73.0% had any MI (Figure 3).

AF Patients With MI

The long and total cTnT levels were higher in the 17 patients with AF and acute MI than in patients with only AF and no MI (mean long cTnT 42.0 ng/L and median long cTnT 11.7 ng/L, p-value for difference to patients with AF and no MI, *P*<0.001; mean total cTnT 181.1 ng/L and median total long cTnT 99.0 ng/L, *P* value for difference to patients with AF and no MI, *P*<0.001). The performance of long and total cTnT did not differ significantly in distinguishing AF patients with MI from those with AF only (AUC 0.833 versus 0.824; *P*=0.66). Long cTnT values were higher than the 90% sensitivity cutoff for Type 1 MI (4.8 ng/L) in 89% of these patients.

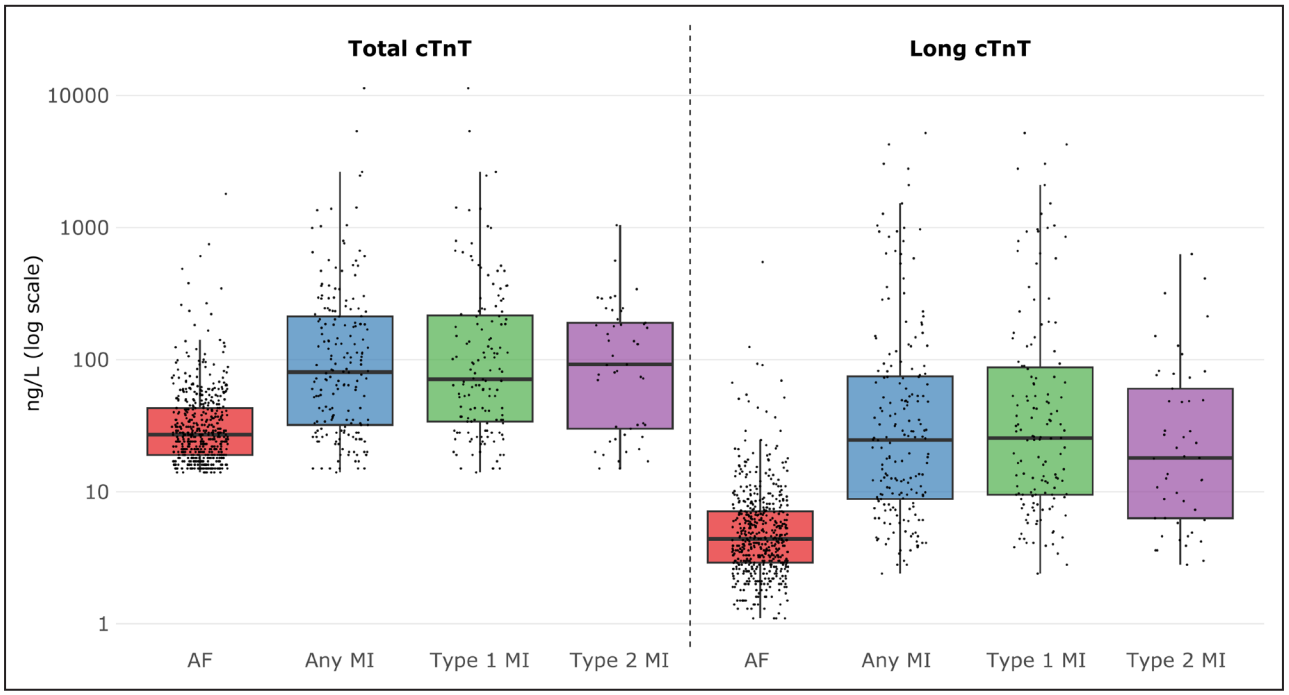

Figure 1. Distribution of total and long cTnT in patients with atrial fibrillation or atrial flutter and those with myocardial infarction.
All *P* values <0.001 when comparing MI categories to patients with AF. AF indicates atrial fibrillation and atrial flutter; cTnT, cardiac troponin T; and MI, myocardial infarction.

When these 17 patients with AF were included in the MI group of the main analysis, the findings were again consistent with the main analysis (AUC values for long cTnT and total cTnT 0.876 and 0.786 for Type 1 MI, and 0.862 and 0.783 for any MI, respectively, *P* values for both differences <0.001).

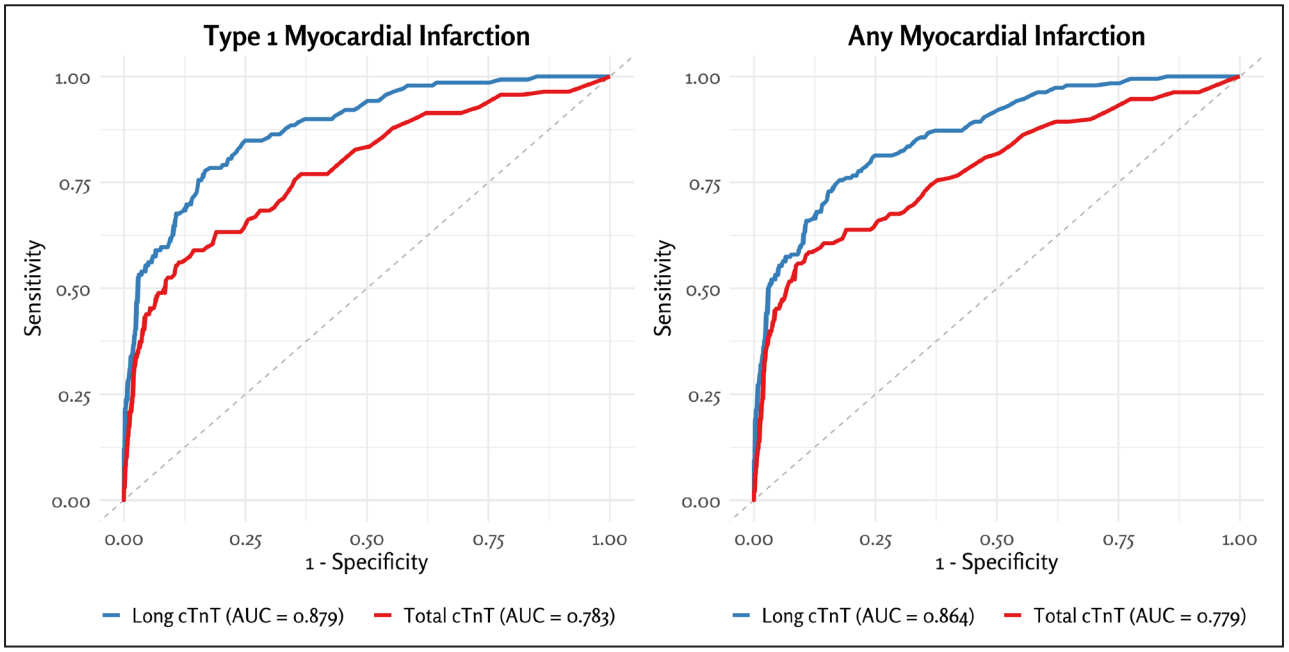

Figure 2. Receiver operating characteristic curves depicting the discriminative performance of total and long cTnT for differentiating Type 1 MI and any MI from atrial fibrillation or atrial flutter.
AUC indicates area under the curve; cTnT, cardiac troponin T; and MI, myocardial infarction.

Table 2. Diagnostic Accuracy of Total cTnT, Long cTnT, and Troponin Ratio (Proportion of Long cTnT to Total cTnT) for Myocardial Infarction

	AUC	Optimal threshold	Sensitivity	Specificity	PPV	NPV	Threshold at 90% sensitivity	Specificity at 90% sensitivity
All patients								
Type 1 MI (n=139)								
Total cTnT	0.783 (0.737–0.830)	61.5 ng/L	56 (48–79)	89 (65–94)	57 (37–70)	88 (86–93)	22.5 ng/L	38 (20–49)
Long cTnT	0.879 (0.847–0.911)	8.9 ng/L	78 (71–89)	83 (73–89)	55 (44–65)	93 (91–96)	5.0 ng/L	57 (46–75)
Troponin ratio	0.768 (0.721–0.816)	0.26	68 (60–82)	79 (66–85)	47 (36–55)	90 (88–94)	0.10	29 (20–48)
Type 2 MI (n=49)								
Total cTnT	0.766 (0.679–0.853)	69.5 ng/L	65 (50–79)	92 (89–94)	42 (31–54)	97 (95–98)	18.5 ng/L	22 (2–46)
Long cTnT	0.822 (0.757–0.887)	9.8 ng/L	65 (51–84)	86 (67–96)	31 (18–60)	96 (95–98)	3.9 ng/L	43 (33–61)
Troponin ratio	0.659 (0.562–0.755)	0.33	49 (40–82)	87 (58–89)	26 (13–35)	95 (93–98)	0.04	4 (1–25)
Any MI (n=188)								
Total cTnT	0.779 (0.737–0.821)	63.5 ng/L	58 (51–68)	89 (81–93)	66 (55–76)	86 (83–89)	18.5 ng/L	27 (20–46)
Long cTnT	0.864 (0.834–0.895)	8.7 ng/L	76 (68–85)	82 (74–90)	61 (52–72)	90 (88–94)	4.6 ng/L	53 (45–67)
Troponin ratio	0.740 (0.695–0.785)	0.26	64 (56–77)	79 (67–85)	53 (44–61)	86 (83–90)	0.09	23 (12–37)
Patients with chest pain or dyspnea								
Type 1 MI (n=133)								
Total cTnT	0.759 (0.707–0.811)	61.5 ng/L	55 (46–80)	87 (62–94)	64 (46–79)	82 (78–89)	22.5 ng/L	32 (17–45)
Long cTnT	0.873 (0.837–0.909)	8.9 ng/L	77 (70–90)	83 (72–90)	67 (56–77)	89 (87–94)	5.7 ng/L	60 (43–74)
troponin ratio	0.774 (0.724–0.824)	0.26	68 (56–82)	80 (66–90)	59 (48–71)	85 (81–91)	0.11	34 (21–47)
Type 2 MI (n=47)								
Total cTnT	0.756 (0.664–0.848)	69.5 ng/L	66 (51–80)	90 (87–94)	51 (38–84)	95 (92–97)	18.5 ng/L	18 (0–47)
Long cTnT	0.820 (0.752–0.889)	9.8 ng/L	66 (51–85)	86 (66–97)	43 (26–76)	94 (92–97)	4.2 ng/L	43 (25–64)
Troponin ratio	0.659 (0.559–0.760)	0.33	49 (40–82)	88 (59–91)	39 (20–52)	92 (89–96)	0.04	5 (1–25)
Any MI (n=180)								
Total cTnT	0.758 (0.711–0.805)	67.0 ng/L	55 (49–67)	90 (79–93)	76 (63–83)	77 (73–82)	19.5 ng/L	23 (15–41)
Long cTnT	0.859 (0.825–0.983)	9.1 ng/L	74 (65–85)	84 (73–92)	73 (64–83)	84 (81–90)	4.6 ng/L	51 (40–67)
Troponin ratio	0.744 (0.696–0.792)	0.26	64 (52–78)	80 (67–90)	65 (55–78)	79 (75–85)	0.09	23 (9–38)

AUC differences between long cTnT and total cTnT were significant (P<0.001), except for Type 2 MI in all patients (P=0.19) and symptomatic patients (P=0.17). Differences between the troponin ratio and total cTnT were nonsignificant for Type 1 MI, Type 2 MI, and any MI in all patients (P=0.68, 0.16, and 0.26) and in symptomatic patients (P=0.71, 0.23, and 0.70). Each analysis included patients with the MI type of interest and patients with AF. 95% CIs in parentheses. Optimal threshold based on the Youden Index. Sensitivity, specificity, PPV and NPV reported as %. AUC indicates area under curve; cTnT, cardiac troponin T; MI, myocardial infarction; NPV, negative predictive value; and PPV, positive predictive value.

Subgroup Analyses

In the subcohort of patients with chest pain or dyspnea, long cTnT outperformed total cTnT in identifying Type 1 MI and any MI, aligning with findings from the overall cohort (Table 2 and Figure S5). Similarly, long cTnT was superior to total cTnT also when analysis was restricted to patients with only mildly elevated (<200 ng/L) total cTnT (AUC values for Type 1 MI 0.847 versus 0.719, P<0.001), as well as when patients with ST-elevation MI were excluded from the analysis. Long cTnT was superior to total cTnT both in men and women. AUC values were numerically higher for long cTnT than total cTnT in patients both <70 and >70 years, but the difference was smaller and not statistically significant in those <70. Long cTnT displayed superior discrimination compared with total cTnT in patients presenting within 12 hours of symptom onset, but among patients with longer symptom duration, the AUC difference favoring long cTnT was smaller and not statistically significant (Table S2).

NRI and Decision Curve Analyses

Long cTnT demonstrated improved accuracy in classifying patients with Type 1 MI or any MI from those with AF compared with total cTnT. This was observed in the continuous NRI that captured all changes in predicted risk, as well as when considering only changes greater than 10%, which may reflect more clinically meaningful shifts in risk stratification (Table S3). This finding was supported by decision curve analyses, where long cTnT yielded higher net benefit than total cTnT in identifying patients with MI across a range of risk threshold probabilities (Figure S6).

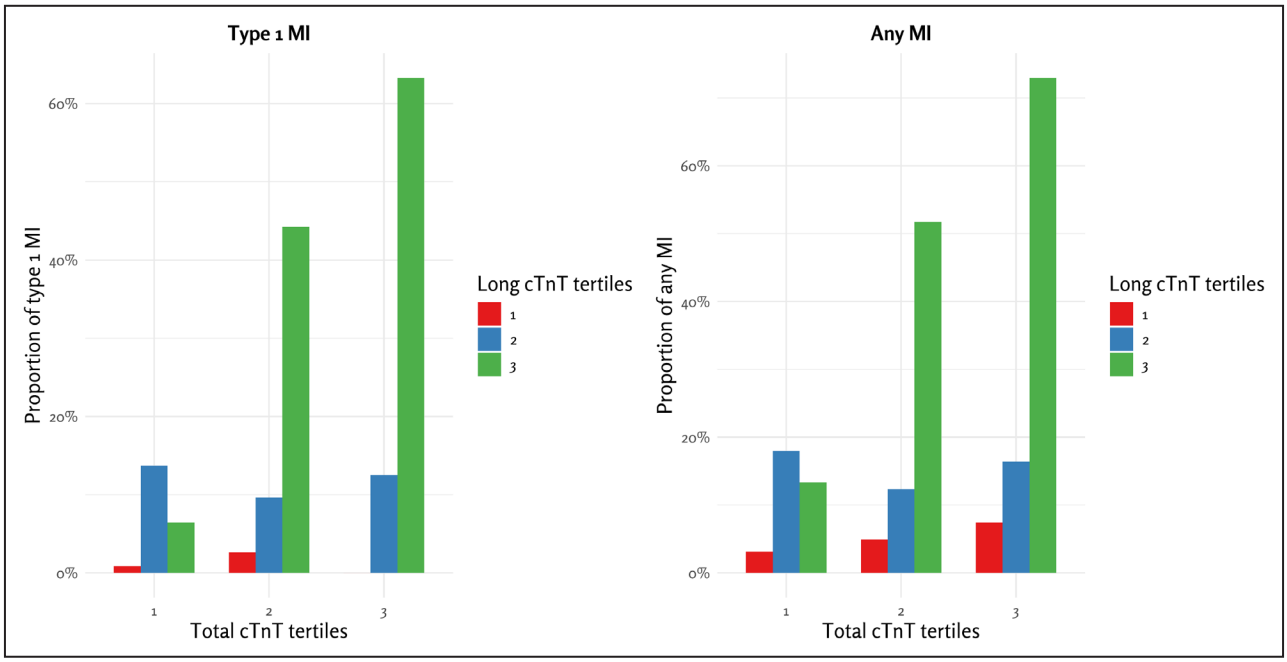

Figure 3. Proportion of patients with Type 1 MI or any MI according to total and long cTnT tertiles (1=lowest tertile, 3=highest tertile).
cTnT indicates cardiac troponin T; and MI, myocardial infarction.

DISCUSSION

Mildly elevated troponin levels are a frequent finding in patients with AF, and the interpretation of their significance is an everyday challenge in patients presenting to the ED with chest discomfort. Our prospective study showed that short cTnT fragments are the predominant (~85%) form of cTnT release in patients with AF, in contrast to those with Type 1 MI, where long (intact and mildly fragmented) molecular forms of cTnT are more commonly found in the circulation after MI. Consequently, measuring the long cTnT forms from a single blood sample at ED admission outperformed the current commercial high-sensitivity cTnT in the discrimination of patients with AF with elevated cTnT levels from those with acute MI. Long cTnT could improve diagnostic accuracy and aid clinical decision-making, particularly regarding Type 1 MI.

The difference in discriminative performance between long and total cTnT was slightly more pronounced for Type 1 MI compared with any MI and the performance of these assays was comparable for Type 2 MI. This finding is of clinical importance, because Type 1 MI requires accurate diagnosis and early active treatment strategy, whereas Type 2 MI or myocardial injury have not been shown to benefit from aggressive antithrombotic treatments or early invasive treatment strategy.[1] The discriminatory capacity of long cTnT was consistent and remained high across all studied subgroups. In line with the earlier observations, total cTnT was only slightly elevated in most patients with AF.[3,16] It was of particular importance that long cTnT also showed a good discriminatory ability between AF and MI in this challenging patient group (total cTnT <200ng/L). The difference favoring long cTnT was diluted and was not statistically significant in patients presenting >12 hours after symptom onset. This finding was not unexpected because the fragmentation of troponins in the circulation is a continuous process after MI.[17] Overall, these findings suggest that measuring long cTnT could enhance diagnostic accuracy for MI, particularly Type 1 MI, across diverse patient populations.

AF is a common comorbidity or primary diagnosis in the ED. Elevated cTnT levels were found in up to 34.4% of patients with AF in biomarker substudies of pivotal randomized trials.[18,19] In the ED, troponins are measured in up to 90% of patients presenting with AF and 14% to 72% of these patients have elevated troponin levels.[2,3,16,20] However, only 5% to 14% of patients with AF with mildly elevated troponins are diagnosed with acute coronary syndrome.[3,20] In line with these findings, almost 30% of the patients with elevated cTnT in the ED had AF, but only 3.2% of these patients with AF had an acute MI in the present study.

This is the first study to show that small troponin fragments are the predominant component of high-sensitivity cTnT elevation in patients with AF without MI. The troponin composition in AF was similar to that previously observed in Takotsubo syndrome and after

strenuous exercise.[21–23] The pathophysiology of this finding may involve the release of free troponin and troponin fragments in the myocyte cytoplasm after myocyte injury or strain through several mechanisms including membranous blebs and increase in membrane permeability.[9] Importantly, myocardial ischemia or necrosis may not be the principal mechanism behind minor and brief troponin elevations or sustained troponin elevations, such as in patients with chronic kidney disease.[24,25] The factors responsible for troponin elevations in AF are largely obscure and probably multifactorial. Atrial cardiomyopathy or high atrial rate may increase troponin release, whereas heart failure and other comorbidities may also contribute in some patients (Table S3).[26,27] High ventricular rate may lead to troponin release in AF, similar to heavy exercise or supraventricular tachycardia, and myocardial ischemia is not a prerequisite for troponin release.[28,29]

Considering the clinical utility of long cTnT testing, it is important to note that all patients with AF presented with suspected cardiac event and elevated high-sensitivity cTnT level on ED admission. However, MI was diagnosed in only 3.2% of them. In view of these prerequisites, one clinically important finding was that a low long cTnT level (<3.9 ng/L) could rule out Type 1 MI with ~99% certainty in one third of this challenging group of patients with AF. This finding may have significant implications for streamlining the management of patients with AF with nonspecific cTnT elevations in real-life clinical practice. At the other end of the spectrum, long cTnT values in the highest tertile substantially increased the likelihood of MI, even among those with high total cTnT levels (Figure 3 and Table S1). Correspondingly, long cTnT demonstrated superior net benefit as compared with total cTnT in the NRI and decision curve analyses, indicating that long cTnT could improve decision-making in the diagnosis of MI from a single admission blood sample.

Our study has some limitations. First, our study included only patients with cTnT levels >99th percentile upper reference limit. However, the current cohort enables the evaluation of the added value of long cTnT in the clinically relevant and challenging context of mildly elevated high-sensitivity troponin levels among ED patients. Second, this was a single-center study, and the number of patients with MI was limited. Further studies are needed to confirm our findings in diverse patient populations, as well as to establish optimal decision thresholds for long cTnT. We did not obtain consent from a relatively large proportion of patients discharged early from the ED and contacted by mail, but most in-hospital patients consented to participate in the study (22 patients refused; Figure S1). This may have introduced selection bias in the group with AF. Finally, we compared troponin values from a single admission blood sample. Guideline-recommended algorithms incorporating troponin dynamics would likely improve the accuracy of total cTnT, although the specificity of serial troponin assays for the diagnosis of Type 1 MI among patients presenting to ED with chest pain is reduced in the presence of AF.[1,30] According to the European Society of Cardiology 2023 guideline, sex-specific upper reference limits of total cTnT were not applied in the study inclusion criteria, but we assume this had no meaningful impact on our results, given their consistency in sex-stratified analyses.[1]

CONCLUSIONS

In conclusion, our novel highly sensitive long cTnT immunoassay shows that the troponin release in AF is composed mainly of smaller troponin fragments. The test holds promise that measuring long cTnT forms could help to separate troponin elevations caused by AF from those of acute Type 1 MI from a single sample with better accuracy than the commercial high-sensitivity cTnT test. 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.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S3
Figures S1–S6

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