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Composition of cardiac troponin release differs after marathon running and myocardial infarction

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ABSTRACT

Objectives Elevations of cardiac troponin T (cTnT) levels are common after strenuous exercise. We assessed whether the composition of cTnT release after marathon race differs from that of acute myocardial infarction (MI).

Methods Troponin composition was analysed in plasma samples taken from 45 runners after marathon race and from 84 patients with type 1 MI. The concentration of long cTnT (intact and mildly fragmented cTnT) was measured with a novel upconversion luminescence immunoassay, total cTnT with a commercial high-sensitivity cTnT assay, and the ratio of long to total cTnT (troponin ratio) was determined as a measure of troponin fragmentation.

Results Total cTnT exceeded the upper reference limit (>14 ng/L) in 37 (82%) runners. Troponin ratio was lower in runners ((IQR) 0.17 (0.11–0.24) vs 0.62 (0.29–0.96), p<0.001). With increasing troponin release the troponin ratio decreased (r=−0.497, p<0.001) in marathon runners and the concentration of long cTnT remained in all runners below 8.4 ng/L. In contrast to marathon runners, troponin ratio increased (r=0.565, p<0.001) with the increase of cTnT release in patients with MI. The median total and long cTnT concentrations were lower in marathon runners than in patients with MI (25 ng/L vs 835 ng/L and 4.1 vs 385 ng/L, p<0.001 for both).

Conclusion In contrast to type 1 MI, only a small fraction of circulating cTnT exists as intact cTnT or long molecular forms after strenuous exercise and the difference in troponin composition is more pronounced in runners with higher troponin release.

Trial registration number NCT06000930.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Elevations of cardiac troponin T (cTnT) levels are common after strenuous exercise, but there is limited information whether the composition of troponin release differs from that of myocardial infarction (MI).

WHAT THIS STUDY ADDS

⇒ Short cTnT fragments were the predominant (>80%) form of cTnT release after marathon race among 45 runners in contrast to 84 patients with MI where intact cTnT and longer fragments were common (>60%) during the early hours after the attack.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The novel highly sensitive immunoassay holds the promise that measuring long cTnT forms could help to separate benign cTnT elevations after strenuous exercise from those of acute MI.

INTRODUCTION

Cardiac troponins play a key role in the diagnosis of acute myocardial infarction (MI).[1] Minor cardiac troponin elevations are common also after strenuous exercise, causing diagnostic challenges when associated with chest discomfort.[2 3] The exact mechanisms of cardiac troponin rise after strenuous physical activity remains ill-defined, but according to a small gel filtration chromatography study, the released cardiac troponin T (cTnT) in this condition seems to be predominantly in the form of small molecules.[4] Temporary increase in permeability of cell membranes may allow the leakage of these smaller cytosolic troponin fragments into circulation. Importantly, the current commercial high-sensitivity cTnT test detects the small and long troponin fragments and the intact cTnT containing the stable central part of the cTnT molecule and is called here the 'total cTnT' assay.

In a Proof-of-Principle study, we developed a simple time-resolved immunofluorometric assay based on europium chelate labels for the measurement of long cTnT molecules, that is, intact and mildly fragmented cTnT forms. The assay showed a high accuracy in discriminating between cTnT elevations in MI and end-stage renal failure.[5] In the present study protocol, we compared the characteristics of troponin release after a marathon race and

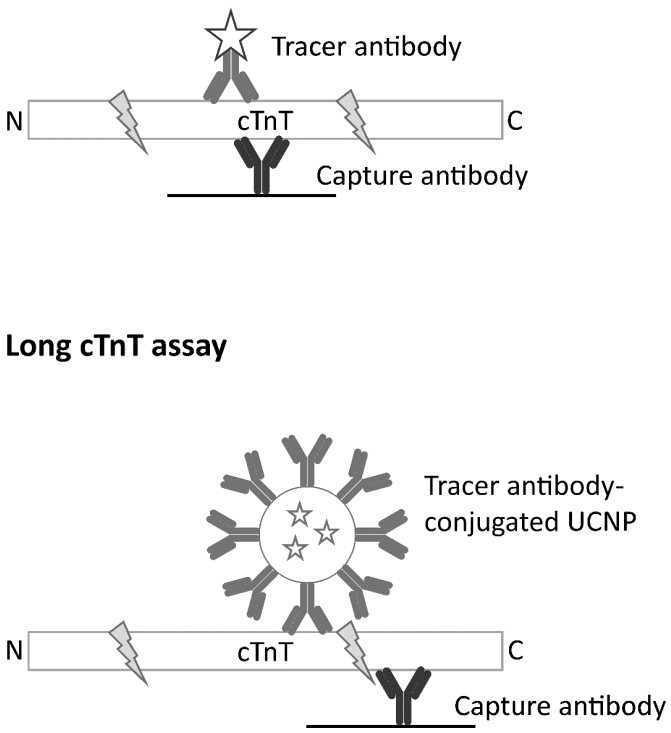

Figure 1 Principles of the commercial hs-cTnT assay ('total cTnT assay') and the novel highly sensitive long molecular forms of cTnT (long cTnT) assay. The commercial hs-cTnT assay targets the stable central part of cTnT and thus, detects both long and short forms of cTnT. The long cTnT assay only detects cTnT molecules that are not degraded at amino acid residues 189–223. Lightnings indicate the major N-terminal and C-terminal cleavage sites at amino acid residues 68–69 and 189–223, respectively. cTnT, cardiac troponin T; hs-cTnT, high-sensitivity cardiac troponin T; UCNP, upconverting nanoparticle.

type 1 MI using an improved version of the long cTnT test and the commercial total cTnT test.[6]

METHODS

Study population and samples

A total of 45 recreational runners (14 female) aged 28–75 (median 35) years participating in the Paavo Nurmi Marathon 2023 in Turku, Finland, accepted an open email invitation and were recruited to the MaraCat2 Study (ClinicalTrials.gov Identifier: NCT06000930) (online supplemental file 1). 18 participants finished the full marathon and 27 the half-marathon. None of the subjects had a history of coronary artery disease. All participants gave a lithium-heparin plasma sample within 60 min after finishing the race.

A control group of 84 patients (19 female) with acute MI (60 ST elevation MI) was recruited among patients admitted to Heart Centre of Turku University Hospital (ClinicalTrials.gov Identifiers: NCT04465591 and NCT05858112). Coronary angiography was performed in all included patients to confirm culprit lesion and the MI diagnosis and all included patients were treated with primary or urgent percutaneous coronary intervention. Only patients with a delay of less than 24 hours from symptom onset to lithium-heparin plasma sample collection and estimated glomerular filtration rate >30 mL/min/1.73m^2 were included.

Table 1 Clinical characteristics and total and long cTnT and their ratio (troponin ratio) in the study groups

	Marathon runners (n=45)	MI patients (n=84)	P value
Age, years	35 (31–61)	66 (58–74)	<0.001
Female gender	14 (31.1)	19 (22.6)	0.299
Hypertension	0 (0)	39 (46.4)	<0.001
Diabetes	1 (2.2)	14 (16.7)	0.019
Heart failure	0 (0)	2 (2.4)	0.542
Atrial fibrillation	1 (2.2)	4 (4.8)	0.657
Creatinine, µg/L	101 (89–124)	75 (65–86)	<0.001
Total cTnT, ng/L	25.0 (16-36)	835 (226–2373)	<0.001
Long cTnT, ng/L	4.1 (2.4–5.8)	385 (69–1810)	<0.001
Troponin ratio	0.17 (0.11–0.24)	0.62 (0.29–0.96)	<0.001

Values are medians (25th–75th percentile) for continuous variables and n (%) for categorical variables.
long cTnT, long molecular forms of cardiac troponin T; MI, myocardial infarction; total cTnT, commercial high-sensitivity cardiac troponin T.

The reporting of this study followed the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. Participants were not involved in the design, conduct, reporting or dissemination of this study.

The samples were analysed fresh for total cTnT. In separate sample tubes the plasma was aliquoted after centrifugation and stored at −70°C (marathon runner samples: <2 months; MI patients: 2 months–2.5 years) until analysis by the long cTnT assay.

Analytical methods

Long cTnT assay

Our novel highly sensitive two-step heterogenous sandwich-type immunoassay using upconversion luminescence for signal production was used for the detection of long (intact and mildly fragmented) molecular forms of cTnT (figure 1). The anti-cTnT monoclonal antibodies (mAb) and human cardiac troponin ITC-complex used as a calibrator were obtained from HyTest (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 long cTnT assay is not affected by N-terminal cleavage of cTnT (figure 1). The limit of detection

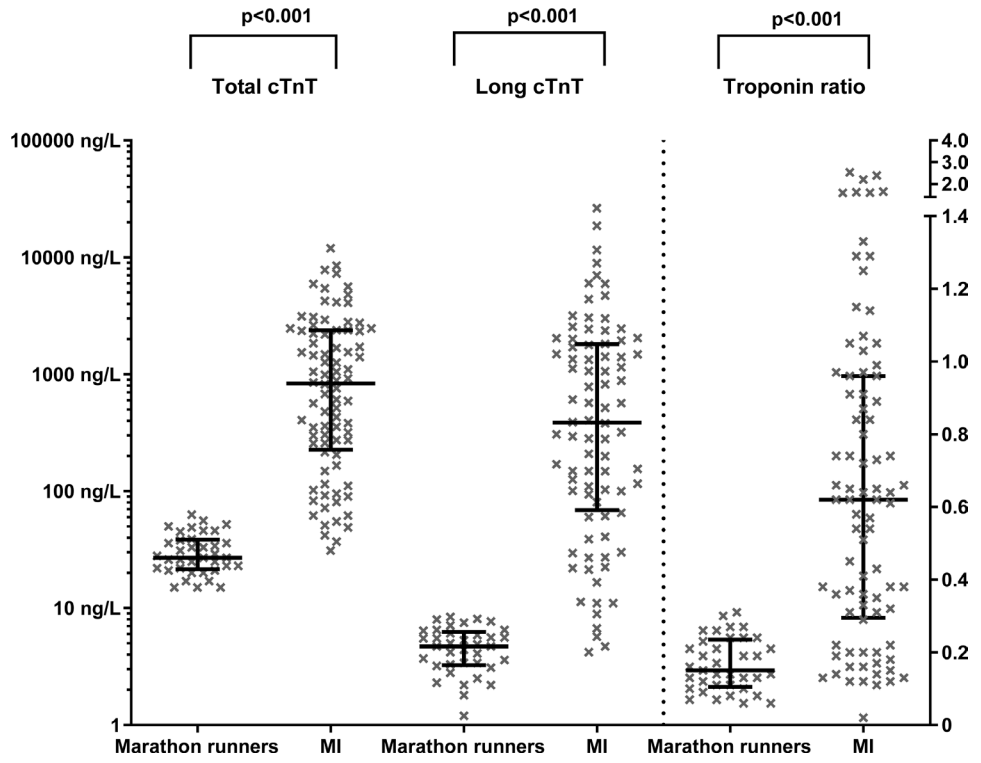

Figure 2 Immunoassay for long cTnT forms to discriminate between marathon runners with total cTnT>14 ng/L and patients with MI. Total cTnT, long cTnT forms (long cTnT) and troponin ratio (ratio of long cTnT forms to total cTnT) in marathon runners with total cTnT>14 ng/L and in patients with MI and <24 hours delay between symptom onset and blood sampling. long cTnT, long molecular forms of cardiac troponin T; MI, myocardial infarction; total cTnT, commercial high-sensitivity cardiac troponin T.

and limit of quantitation of this assay are 0.4 ng/L and 1.8 ng/L, respectively.[6] The inter-day variation of 40 days at 91 ng/L is 4.2%.

Total cTnT assay

All plasma samples were analysed for total cTnT with Elecsys Troponin T hs kit using Cobas 8000 system

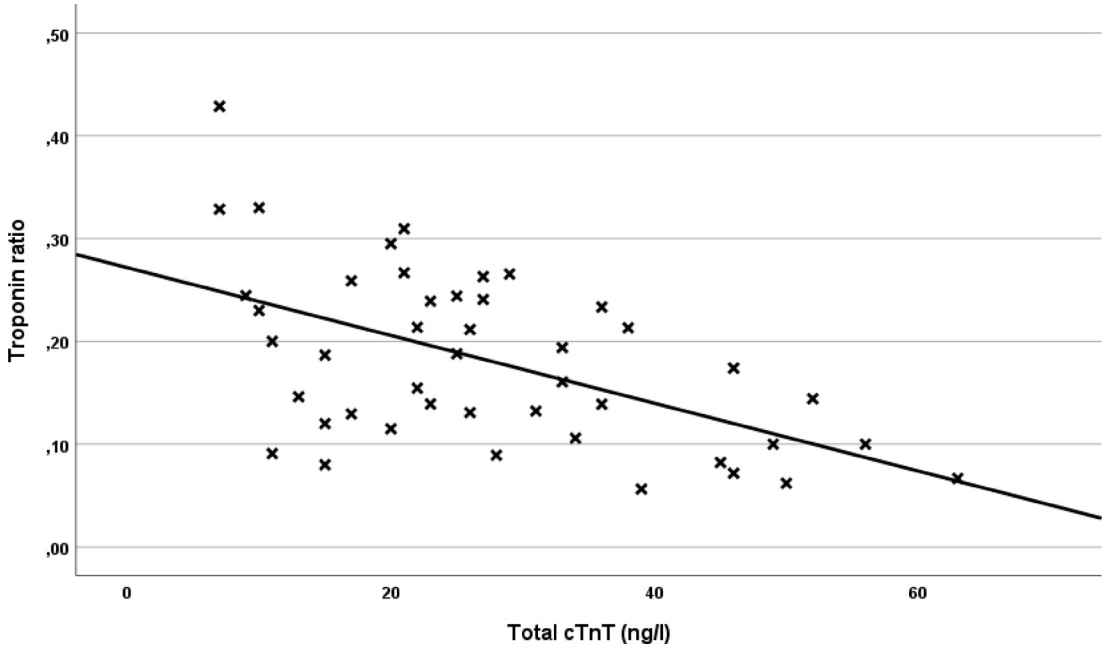

Figure 3 Concentrations of long molecular forms of cardiac troponin T (long cTnT) in relation to the magnitude of commercial high-sensitivity cardiac troponin T (total cTnT) release in marathon runners. The share of long cTnT of total cTnT (troponin ratio) decreased (r=−0.497, p<0.001) with increasing total cTnT release in marathon runners.

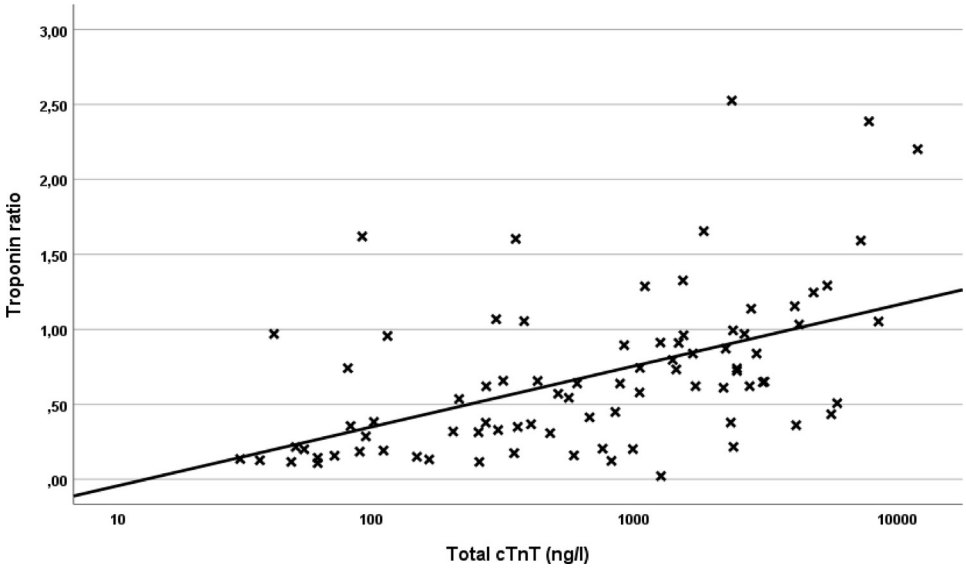

Figure 4 Concentrations of long molecular forms of cardiac troponin T (long cTnT) in relation to the magnitude of commercial high-sensitivity cardiac troponin T (total cTnT) release in myocardial infarction (MI) patients. The share of long cTnT of total cTnT (troponin ratio) increased (r=0.565, p<0.001) with increasing total cTnT release in MI patients.

(e801 module) (Roche Diagnostics GmbH, Mannheim, Germany). The Elecsys Troponin T hs assay uses two mAb, which specifically target the central part of the human cTnT. For this assay, the limits of detection and quantitation are 3 ng/L and 13 ng/L, respectively.

The ratio of long cTnT forms/total cTnT (troponin ratio) was used as the measure of troponin fragmentation.[5]

Statistical analysis

The magnitude of troponin fragmentation in the study populations is not known and the sample size calculation is largely exploratory in nature based on our earlier research with the 1st version of the fragmentation test and earlier small mass spectrometry studies. Continuous variables are reported as median (25th–75th percentiles) and categorical variables as counts (percentage). Mann-Whitney U test was used for group comparisons. χ^2 test and Fisher's exact test were used for categorical variables as appropriate. 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 marathon runners and MI patients. All covariates with a p value<0.1 in univariate analysis were included in the final regression model. All tests were two-sided, and the limit of significance was set at p<0.05. IBM SPSS Statistics software V.26.0 was used to perform the analyses. Receiver operating characteristics (ROC) curve analyses were performed to estimate the area under the curve (AUC) to measure the discriminative capacity of the troponin ratio between marathon runners and MI patients.

RESULTS

None of the runners reported cardiac symptoms after the race. Total cTnT exceeded the upper reference limit (>14 ng/L) in 37 (82%) runners with a median concentration of 25.0 ng/L (range 7–63 ng/L). Median long cTnT concentration was 4.1 ng/L (range 1–8.4 ng/L) (table 1). The total and long cTnT levels were higher after full marathon than after half-marathon ((IQR) 33 (25–47 ng/L) vs 20 (11–27 ng/L) and 5.6 (3.6–7.6 ng/L) vs 3.3 (2.2–4.7 ng/L), p=0.002 for both comparisons), but were not related to age, sex or postrace creatinine level of runners.

As a sign of more marked troponin fragmentation, troponin ratio was significantly lower in marathon runners than in MI patients (0.17 (0.11–0.24) vs 0.62 (0.29–0.96), p<0.001) (figure 2, table 1). Importantly, troponin ratio decreased significantly (r=−0.497, p<0.001) with increasing total cTnT release in marathon runners (figure 3), while in MI patients, troponin ratio increased significantly (r=0.565, p<0.001) with increasing total cTnT release (figure 4). Troponin ratio decreased (r=−0.276, p=0.011) with longer delay between symptom onset and plasma sample collection in MI patients. Troponin ratio was not related to the age of marathon runners (p=0.769) or MI patients (p=0.232). Both the total cTnT and long cTnT concentrations were lower (p<0.001 for all) in marathon runners than in MI patients (figure 2, table 1).

In the ROC curve analysis, troponin ratio showed a good predictive power in discriminating marathon runners and MI patients with an AUC of 0.857 (95% CI 0.794 to 0.920).

DISCUSSION

As expected, the majority of runners had postrace cTnT levels above the rule-in criteria for the diagnosis of acute MI and the troponin release was more marked after running the full marathon.

Most importantly, we showed that short cTnT fragments are the predominant (>80%) form of cTnT release after marathon race in contrast to type 1 MI where long (intact and mildly fragmented) molecular forms of cTnT are more commonly (>60%) found in the circulation during the early hours after MI.

The present findings on troponin fragmentation after exercise are in line with the gel filtration chromatography study by Vroemen *et al* showing 10 runners with a postrace cTnT exceeding 70 ng/L.[4] The results with our sensitive immunoassay technique confirmed this in a larger group of runners and with less profuse troponin release. Importantly, we could show that the magnitude of long cTnT release remained at a very low level (<10 ng/L) in spite of increasing release of total cTnT. The current finding on smaller troponin fragments as the predominant component of circulating cTnT after exercise is similar to the troponin composition seen in patients with end-stage renal failure, although the long cTnT levels in renal patients were slightly lower than the present concentrations after exercise.[5 7] In 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 ST elevation MI.[5 8 9] As a novel finding, our sensitive test showed that the troponin composition was related to the magnitude of the cTnT release and smaller components were more common in patients with milder myocardial injury (ie, total cTnT release).

Proposed mechanisms for exercise-induced cTnT elevations include increased cell membrane permeability or cellular release of proteolytic troponin degradation products during exercise.[3] Enhanced myocyte turnover, necrosis and apoptosis are among other potential mechanisms of cardiac troponin release. The role of temporary increase in cell membrane permeability is supported by small cardiac MRI studies which have detected myocardial oedema, transient increases in mean diffusivity and extracellular volume with a positive correlation to troponin release after marathon race.[10–12]

In clinical practice, problems may arise when subjects present with chest pain to the emergency department shortly after strenuous exercise and have elevated troponin levels. ECG is often normal, but in some cases athlete's heart may complicate the interpretation. The present observations on marked differences in troponin composition between type 1 MI and exercise-induced benign troponin release could be helpful in the future diagnostic workup of the patients in the 'grey zone' of mildly elevated troponin release.

Earlier research on troponin fragmentation has employed gel filtration chromatography, Western blotting and mass spectrometry, techniques that are too complicated and time-consuming for clinical use.[4 8 9] The low analytical sensitivity of these methods is another obstacle for clinical applications.[4 9] In contrast, our novel immunoassay approach is a significantly more sensitive method for the analysis of cTnT fragmentation. 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.

Limitations

The main limitation of the present study was the limited size of study groups. Only few MI patients had minor troponin elevations rendering the analysis of diagnostic performance of the novel assay tentative. Troponin release after the race was less than in many previous reports. We did not collect blood samples before the race, but based on earlier research, chronic elevation of troponins in these healthy subjects is unlikely.[2 3] In some patients the long cTnT concentrations were higher than the total cTnT values in our study. This is likely due to differences in the calibration of the assays. It should be noted that fragmentation of troponins is a continuous process after MI.[5 9] The present study collected blood sample only on single early time point following exercise and within 24 hours from symptom onset in MI patients. Longer delays between MI symptom onset and sampling are likely to dilute the observed differences in troponin composition. It is reasonable to assume that similar troponin fragmentation also continues after strenuous exercise.

CONCLUSION

In conclusion, this novel highly sensitive long cTnT immunoassay shows that the troponin release after strenuous exercise is composed mainly of smaller troponin fragments especially in runners with higher troponin release. The novel test holds promise that measuring long molecular forms of cTnT could help to separate benign cTnT elevations after strenuous exercise from those of acute MI.

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Contributors Contributors JA, TP, TV, SW conceived the present study idea. JA designed the study with inputs from TP, TH, TV, KT, OJH, SJ, SW. TH performed statistical analyses. JA, TP, TV, SW interpreted the findings. JA drafted the manuscript. All authors critically reviewed the manuscript and agreed to its publication. JA is the guarantor and vouches for the accuracy of the analyses and presented results.

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Competing interests JA: research grants from the Finnish Foundation for Cardiovascular Research and Clinical Research Fund of Turku University Hospital, Turku, Finland. Lectures for Astra Zeneca, Bayer, Boehringer Ingelheim, pending patent application WO2023187258 (A1) - ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T. TP: lectures for Astra Zeneca. TH: lectures for AstraZeneca, Astellas and GSK, pending patent application WO2023187258 (A1) - ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T. TV: pending patent application WO2023187258 (A1) - ASSAY FOR LONG FORMS OF CARDIAC TROPONIN T. SS: none. TT: none. AL-P: research grants from the Finnish Society of Clinical Chemistry. H-MP: lectures for Roche. KT: 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. SJ: lectures for Amgen,

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants. The study complies with the Declaration of Helsinki as revised in 2013 was approved by the Medical Ethics Committee of the Hospital District of Southwest Finland (Diary number: VARHA/5618/2023, no ID numbers are provided in the approvals by the ethics committee). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. The data underlying this article will be shared on reasonable request to the corresponding author.

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