Differential associations of cardiac troponin T and cardiac troponin I with coronary artery pathology and dynamics in response to short-duration exercise

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ABSTRACT

Background

We aimed to assess the associations between cardiac troponin (cTn) T and I concentrations, physical exercise and the presence and severity of angiographic coronary artery disease (CAD) in patients evaluated for suspected chronic coronary syndrome (CCS).

Methods and results

All patients performed an exercise stress test on a bicycle ergometer and underwent invasive coronary angiography with weighted anatomical evaluation using the Gensini score. Blood samples were collected before and after exercise and analysed with high-sensitivity (hs) cTnT and cTnI assays.

Of 297 patients (median age 62 (Quartile [Q]1-3 56-69) years, 35% female), 46% were categorized as “severe CAD” (Gensini score ≥ 20).

Resting hs-cTnT and hs-cTnI concentrations were detectable in 88% and 100% of patients, with medians of 6 (Q1-3 4-9) ng/L and 1.5 (0.9-2.4) ng/L, respectively.

In adjusted normalized linear regression analyses, higher resting concentrations were associated with increasing Gensini score (hs-cTnT: B 0.19, 95% Confidence Interval [CI] [0.09-0.41], $p < 0.001$; hs-cTnI: B 0.18, [0.06-0.30], $p = 0.002$).

The area under the receiver operating characteristics curve for predicting *severe* CAD was 0.72 (95% CI [0.66-0.78]) and 0.68 (0.62-0.74) for resting hs-cTnT and hs-cTnI, $p = 0.11$ for difference.

The median (Q1-3) relative increase in hs-cTnT and hs-cTnI concentrations were 5 (0-12) % and 13 (3-27) %, respectively, with no significant associations with CAD severity.

Conclusions

In patients with suspected CCS, higher hs-cTn concentrations at rest were associated with increasing angiographic severity of CAD, without any significant differences between the troponin isotypes. Post-exercise hs-cTn concentrations did not have discriminatory power for CAD.

Keywords

Stable coronary artery disease; Chronic coronary syndrome; High-sensitivity; Troponin; Exercise testing

Abbreviations

cTnI - Cardiac troponin isotype I; cTnT - Cardiac troponin isotype T; hs-cTn – High-sensitivity cardiac troponin; CAD – Coronary artery disease; CCS – Chronic coronary syndrome; CV - Coefficient of variation; URL - Upper Reference Limit; LoD - Limit of Detection; LoB - Limit of Blank

1 INTRODUCTION

The two cardiac specific troponin isotypes I and T (cTnI, cTnT) are used interchangeably in the diagnosis of myocardial injury. In the setting of acute coronary syndromes (ACS), elevated and dynamic concentrations above the assay-specific 99th percentile upper reference limit (URL) signify acute myocardial infarction (AMI). In chronic coronary syndromes (CCS), elevated but non-dynamic concentrations signify chronic myocardial injury. [1] In patients with CCS, there is a graded relationship between the concentrations of cTn and the extent and severity of coronary lesions. [2, 3] This is not limited to concentrations above the 99th percentile URL but is also observed within the reference range of normality. [2] In these patients, cTn has been demonstrated to be a robust marker of poor prognosis, both for cardiovascular events and all-cause mortality. [4, 5]

With the advent of high-sensitivity cTn (hs-cTn) assays, it has become possible to detect and quantify cTn at lower concentrations and with greater analytical accuracy than with previous generations of cTn assays. However, due to different epitope specificity of antibodies used in different assays [6] and to different reference populations, there is considerable inter-assay variability in URLs. As such, direct comparison of cTn concentrations between different assays in a clinical setting remains problematic. However, due to the increased analytical accuracy at lower concentrations, previously undetectable increases in cTn are now observed in response to physical exercise [7]. Recent studies indicate that this phenomenon, rather than being benign, is of possible diagnostic and prognostic importance. [8, 9]

Although cTnI and cTnT are used interchangeably in the diagnosis of AMI, there is evidence that the two isotypes are differentially associated with several physiological and pathophysiological processes. [10, 11] For instance, in contrast to cTnI, cTnT shows diurnal variation and seems to be a more robust marker of poor long-term outcome [12-14], whereas cTnI increases earlier and with greater magnitude in the setting of myocardial ischemia [12]

and is less associated with renal function than cTnT. [15] Any clinical implications of these phenomena have yet to be established.

In this study, we aimed to assess the relative strength of associations between resting concentrations of cTnI and cTnT and their dynamic response to short duration exercise and the angiographic extent and severity of coronary artery disease (CAD) in patients with suspected CSS.

2 MATERIALS AND METHODS

2.1 Study design

The current investigation utilizes data from the Markers of Coronary Artery Disease During Exercise Testing (CADENCE) study (NCT01495091). Results from this study have previously been published. [16] Briefly, patients referred for evaluation of suspected CCS, 18 years or older and a Morise score ≥ 9 (i.e. intermediate or high pre-test CAD risk [17]) were eligible for inclusion. The study was conducted at Oslo University Hospital, Ullevål (OUH) and written informed consent was obtained prior to inclusion. Major exclusion criteria included ACS, symptomatic heart failure (HF), moderate to severe valvular heart disease, previous coronary artery bypass graft (CABG), ongoing cardiac dysrhythmias such as atrial fibrillations, renal insufficiency (estimated glomerular filtration rate <45 ml/min/1.73m²) or inability to follow study protocol. All patients underwent a baseline physical examination, cardiac exercise stress test on an upright stationary ergometer bicycle and invasive coronary angiography. History of hypertension was defined as known diagnosis or use of anti-hypertensive medication. History of CAD was defined as previous AMI or coronary revascularization. The study was approved by the Regional Ethics Committee and performed in accordance with the Declaration of Helsinki.

2.2 Cardiac exercise stress test

Cardiac exercise stress test was performed on an upright stationary ergometer bicycle (*CS-200 Excellence, Switzerland or Ergoline, Germany*) supervised by a physician and nurse team. All patients were monitored with 12-lead ECG for the duration of the test. Any dynamic ST-segment elevation, horizontal or downward sloping ST-segment depression >0.1 mV 60 ms after the J-point and/or ischemic symptoms were defined as a positive test. All patients were exercised to exhaustion, starting at 30W for women and 50W for men with a gradual increase of 10W per min. The patient target pedal cadence was set at 65 rotations per min. Blood

pressure was measured manually every 5 min and patients asked to grade their perceived exertion using the Borg scale [18] in addition to the calculated metabolic equivalent of task (MET). The test was terminated if the patients developed ischemic symptoms or signs on ECG, cardiac arrhythmias, inability to maintain exercise intensity or insufficient or exaggerated chronotropic or blood pressure response. Ten percent of test results were assessed by an independent physician with a 100% concordance rate with the primary test physician.

2.3 Invasive coronary angiography

Invasive coronary angiography was performed by trained interventional cardiologists using standard Seldinger technique with primarily radial artery access. All coronary segments were retrospectively described and graded according to a modified version of the American Heart Association coronary segmentation definition using the Gensini score. [19, 20] In cases of doubt (20%) a second blinded interventional cardiologist re-evaluated the angiograms with an inter-observer variability of 7%. The Gensini score provides a graded assessment of the total coronary artery plaque burden based on a weighted grading of separate coronary artery segments. The primary end point of *severe CAD* was defined as a Gensini score ≥ 20 , corresponding in severity to an angiographically verified singular left main coronary artery stenosis $\geq 70\%$. *Non-severe CAD* was defined as Gensini score 1-19 and *No CAD* as Gensini score 0.

2.4 Blood sampling and biochemical assays

Blood samples were obtained by venepuncture prior to the exercise stress test, and within five min after completion of the test. Serum was obtained by centrifugation at 2500 x g for 10 min at room temperature and kept frozen at -80°C until analysed. Routine analyses were performed by conventional laboratory methods. cTnI was measured at Akershus University Hospital with the Singulex Clarity Single Molecule Counting (SMC) ultra-sensitivity cTnI assay. As reported by the manufacturer, this assay has a limit of blank

(LoB) at 0.02 ng/L, Limit of detection (LoD) at 0.08 ng/L and a 99th percentile URL of 8.67 ng/L. The coefficient of variation (CV) was 14.5% at a sample concentration of 0-2.00 ng/L, 11.5% at 10.00 ng/L and 6.8% at 100.00 ng/L. cTnT was analysed at OUH with the Roche fifth generation hs-cTnT assay (Gen 5 STAT cardiac troponin T) on a Cobas e602 platform. Per the manufacturer, this assay has a LoB at 3 ng/L, LoD at 5 ng/L and a 99th percentile URL of 14 ng/L. The inter-assay CV is reported to be 7.8% at a sample concentration of 6.5 ng/L and 4.8% at a sample concentration of 11.5 ng/L. In our laboratory the CV was 6% at a sample concentration of 13 ng/L. To allow for an assumed but unmeasurable distribution, hs-cTnT concentrations below the LoB are imputed as 1.5 ng/L in all analyses. To utilize the high analytical sensitivity and precision of the SMC hs-cTnI assay, concentrations are reported to one decimal place, while hs-cTnT concentrations are reported as whole numbers as recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was utilized to calculate the estimated glomerular filtration rate (eGFR).

2.5 Statistical analysis

Continuous variables are reported as medians with quartiles 1 and 3 (Q1-3), categorical variables as absolute numbers with percentages. We use non-parametric tests when assessing baseline variables; Cuzick's non-parametric trend test for continuous variables and chi-square trend test for categorical variables. Spearman's rank correlation was utilized to assess correlates of each troponin isotype. Predictors of resting hs-cTn, exercise-induced changes in hs-cTn, and the Gensini score were identified using linear regression analyses. Logistic regression models were used to analyse predictors of severe CAD. In adjusted regression analyses, the following variables were selected *a priori*; age, sex, body mass index (BMI), current smoking, systolic blood pressure, history of diabetes, history of CAD and eGFR. All variables were assessed for univariable associations followed by multivariable modelling of

significant predictors utilizing a stepwise backward elimination approach. To account for a right-skewed distribution, we use base 2 logarithmically transformed values of hs-cTnI and hs-cTnT in all regression models. Exercises induced changes in cTn concentrations are expressed as ratios and are also logarithmically transformed when included in regression models. Due to the semi-continuous distribution of the Gensini score, transformation to normality is not possible. Thus, in regression analyses, we modelled the Gensini score either as a dichotomous variable (i.e. zero vs. non-zero), or the non-zero scores as a base 2 logarithmically transformed continuous variable. To compare regression coefficients and odds ratios between hs-cTnI and hs-cTnT we normalized the logarithmically transformed covariates. Finally, to assess the differential performance of hs-cTnI and hs-cTnT in predicting severe CAD we compared the areas under the receiver operating characteristics curves (ROC-AUC), as well as the Continuous Net Reclassification Improvement (cNRI) and Integrated Discrimination Improvement (IDI) with cTn added to a base risk prediction model consisting of age, sex, history of CAD, diabetes, hypertension, low-density lipoprotein (LDL) cholesterol concentrations and current smoking.

All statistical analyses were performed with STATA 15 (StataCorp. 2017. *Stata Statistical Software: Release 15.1*. College Station, TX: StataCorp LLC). *p*-values with a significance level of 5% or confidence intervals (CI) with a confidence level of 95% are used to indicate assumed statistical significance.

3 RESULTS

3.1 Baseline characteristics

In total, 297 patients were included in the study, with median age 62 (Q1-3 56-69) years, 105 (35%) women and 119 (40%) with a history of CAD. The median Gensini score was 17.5 (Q1-3 6.0-43.5). When categorizing patients based on Gensini score, 25 (8%) had no CAD (Gensini score = 0), 135 (46%) had non-severe CAD (Gensini score 1-19) and 137 (46%) had severe CAD (Gensini score ≥ 20). Increasing severity of CAD was associated with older age, male sex, history of hypertension or CAD and use of antiplatelet drugs, statins and beta-/calcium channel blockers. With increasing severity of CAD, patients also had lower LDL-cholesterol, lower eGFR and higher NT-proBNP concentrations. (**Table 1**) The median exercise duration was 9.5 (Q1-3 7.5-12.1) min and the median MET was 6.7 (Q1-3 5.6-8.0); p for trend = 0.02 and 0.04, respectively, with increasing severity of CAD.

Table 1. Baseline characteristics of patients referred for evaluation of suspected chronic coronary syndrome stratified by categories of Gensini score.

	No coronary artery disease (Gensini = 0) (n=25)	Non-severe coronary artery disease (Gensini = 1-19) (n=135)	Severe coronary artery disease (Gensini ≥20) (n=137)	p-value for trend
Age (years)	57 (53, 64)	62 (54, 68)	64 (58, 69)	0.002
Female sex	15 (60%)	67 (50%)	23 (17%)	<0.001
Family history of CAD	12 (48%)	78 (57%)	58 (42%)	0.07
Current smoking	7 (28%)	24 (18%)	22 (16%)	0.24
Body Mass Index (kg/m ²)	28 (26, 31)	26 (24, 29)	27 (25, 30)	0.79
Systolic blood pressure (mmHg)	126 (115, 136)	130 (119, 148)	139 (126, 154)	<0.001
Heart rate	68 (62, 75)	70 (63, 78)	67 (59, 76)	0.10
History of				
Hypertension	12 (48%)	72 (53%)	92 (67%)	0.01
Diabetes type I or II	7 (28%)	22 (16%)	27 (20%)	0.79
Coronary artery disease	0 (0%)	28 (21%)	61 (45%)	<0.001
Medication				
Acetylsalicylic acid (ASA)	9 (36%)	86 (64%)	119 (87%)	<0.001
Clopidogrel	0 (0%)	5 (4%)	14 (10%)	0.01
Warfarin	0 (0%)	2 (2%)	3 (2%)	0.43
Statins	10 (40%)	89 (66%)	113 (83%)	<0.001
Beta blocker	2 (8%)	51 (38%)	70 (51%)	<0.001
Calcium blocker	1 (4%)	15 (11%)	32 (23%)	0.001
ACE-i or ARB	9 (36%)	40 (30%)	59 (43%)	0.08
Exercise duration (min)	10.3 (8.7, 12.7)	9.7 (7.8, 12.2)	9.2 (6.6, 11.9)	0.02
METs	7.0 (6.3, 7.8)	6.9 (5.8, 8.0)	6.3 (5.5, 8.0)	0.04
Hemoglobin concentration (g/dl)	14.5 (14.0, 15.2)	14.3 (13.4, 15.1)	15.05 (14.2, 15.7)	<0.001
White blood cell count (x10 ⁹ /L)	6.8 (5.8, 8.5)	6.4 (5.6, 7.7)	7 (6, 8.3)	0.10
eGFR ckd-epi (ml/min/1.73 ²)	89 (81, 99)	85 (75, 96)	83 (71, 92)	0.02
Low density lipoprotein cholesterol (mmol/L)	3.0 (2.5, 3.8)	2.7 (2.2, 3.9)	2.4 (1.9, 3.1)	<0.001
HbA1c (%)	5.6 (5.3, 6.4)	5.6 (5.4, 6.0)	5.7 (5.4, 6.2)	0.67
NT-proBNP (mmol/L)	42 (21, 67)	64 (37, 112)	70 (39, 153)	0.003

Presented as absolute numbers or medians with percentages or quartiles 1 and 3. p-values are for trend. Abbreviations: eGFR ckd-epi – estimated glomerular filtrations rate using the Chronic Kidney Disease Epidemiology Collaboration equation, ACE-i – Angiotensin converting enzyme inhibitor, ARB – Angiotensin-receptor blocker

3.2 Angiographic coronary artery disease severity and resting concentrations of hs-cTnI and hs-cTnT

All patients had resting hs-cTnI concentrations above the LoD. Two-hundred-sixty-two (88%) patients had hs-cTnT concentrations above the LoB (3 ng/L), of which 190 (64%) had concentrations above the LoD (5 ng/L). When imputing values for hs-cTnT concentrations <LoB, hs-cTnI and hs-cTnT concentrations in the total population as well as in the subset of patients with hs-cTnT concentrations >LoB, correlated significantly (Spearman's rho (r) = 0.66, $p < 0.001$ and $r = 0.60$, $p < 0.001$, respectively). When dichotomizing the cTn concentrations at the assay-specific 99th percentiles the concordance rate between the two assays was 0.93 (95% confidence interval [CI] 0.90-0.96), $p < 0.001$. **Supplemental figure 1** shows a scatterplot visualizing the correlation between concentrations of hs-cTnI and hs-cTnT.

Supplemental figure 1. Scatterplot visualising the correlations between resting cardiac troponin I and T (hs-cTnI; hs-cTnT). Two outliers have been removed in this plot for enhanced readability.

The median (Q1-3) resting hs-cTnI concentrations in the total population, and in women and men separately, were 1.5 (0.9-2.4) ng/L, 1.2 (0.7-1.7) ng/L and 1.8 (1.0-2.9) ng/L, respectively. In adjusted multivariable linear regression models, older age and male sex were associated with greater concentrations of hs-cTnI while current smoking was associated with lower values. These variables accounted for 19% of the variance in hs-cTnI (adjusted $R^2=0.19$). (Table 2).

Table 2. Linear regression analyses of predictors of resting cardiac troponin I and T (hs-cTnI; hs-cTnT).

		Univariable analyses			Multivariable analyses		
		B	95% Confidence interval	p-value	B	95% Confidence interval	p-value
Age (years)	hs-cTnI	0.44	0.29-0.59	<0.001	0.48	0.33-0.62	<0.001
	hs-cTnT	0.46	0.35-0.58	<0.001	0.46	0.32-0.59	<0.001
Male sex	hs-cTnI	0.57	0.29-0.86	<0.001	0.69	0.42-0.97	<0.001
	hs-cTnT	0.75	0.53-0.98	<0.001	0.92	0.73-1.12	<0.001
Current smoker	hs-cTnI	-0.62	-0.98- -0.26	0.001	-0.40	-0.73- -0.06	0.02
	hs-cTnT	-0.50	-0.80- -0.21	0.001	-	-	NS
Body Mass Index (kg/m ²)	hs-cTnI	0.01	-0.02-0.05	0.52	-	-	-
	hs-cTnT	0.02	-0.006-0.05	0.12	-	-	-
History of diabetes	hs-cTnI	-0.19	-0.55-0.17	0.3	-	-	-
	hs-cTnT	0.13	-0.17-0.42	0.39	-	-	-
History of coronary artery disease	hs-cTnI	0.38	0.08-0.69	0.01	-	-	NS
	hs-cTnT	0.42	0.17-0.67	0.001	-	-	NS
Systolic blood pressure (mmHg)	hs-cTnI	0.12	0.05-0.19	0.001	-	-	NS
	hs-cTnT	0.08	0.02-0.14	0.006	-	-	NS
eGFR ckd-epi (ml/min/1.73 ²)	hs-cTnI	-0.02	-0.03- -0.01	<0.001	-	-	NS
	hs-cTnT	-0.02	-0.03- -0.01	<0.001	-0.01	-0.02- -0.001	0.02

Results presented as unadjusted and adjusted coefficients with 95% confidence intervals and p-values. Concentrations of cardiac troponin I and T and Gensini score are log₂-transformed. Age and systolic blood pressure are reported per 10-unit increase. Abbreviations: hs-cTnI - high-sensitivity cardiac troponin I, hs-cTnT - high-sensitivity cardiac troponin T, eGFR ckd-epi – estimated glomerular filtrations rate using the Chronic Kidney Disease Epidemiology Collaboration equation, NS - non-significant

The median (Q1-3) resting hs-cTnT concentrations in the total population, and in women and men separately were 6 (4-9) ng/L, 5 (3-7) ng/L and 7 (5-10) ng/L, respectively. In adjusted multivariable linear regression models, older age, male sex and lower eGFR were associated with greater concentrations of hs-cTnT. These variables accounted for 36% of the variance of hs-cTnT (adjusted $R^2=0.36$).

Supplemental table 1 shows Spearman rank coefficients of correlation between hs-cTnT, hs-cTnI, demographic, clinical and laboratory variables.

Supplemental Table 1. Spearman rank correlation coefficients between cardiac troponin I (hs-cTnI), cardiac troponin T (hs-cTnT), demographic, clinical and laboratory parameters in the total population.

	hs-cTnI	p-value	hs-cTnT	p-value
Age (years)	0.33	<0.001	0.44	<0.001
Sex	0.27	<0.001	0.33	<0.001
BMI (kg/m ²)	0.04	0.47	0.07	0.26
Heart rate	-0.12	0.03	-0.11	0.07
Systolic blood pressure (mmHg)	0.20	<0.001	0.18	<0.001
Current smoking	-0.18	<0.001	-0.18	<0.001
History of coronary artery disease	0.19	<0.001	0.20	<0.001
History of diabetes type I or II	-0.05	0.41	0.07	0.21
Hemoglobin concentration (g/dl)	0.18	<0.001	0.17	<0.001
White blood cell count (x10 ⁹ /L)	-0.01	0.92	0.09	0.13
NT-proBNP (mmol/L)	0.39	<0.001	0.35	<0.001
eGFR ckd-epi (ml/min/1.73 ²)	-0.32	<0.001	-0.32	<0.001
Low density lipoprotein cholesterol (mmol/L)	-0.06	0.28	-0.11	0.07
Gensini score	0.37	<0.001	0.44	<0.001
hs-cTnI (ng/L)	-	-	0.66	<0.001
hs-cTnT (ng/L)	0.66	<0.001	-	-

Abbreviations: eGFR ckd-epi – estimated glomerular filtrations rate using the Chronic Kidney Disease Epidemiology Collaboration equation, hs-cTnI - high-sensitivity cardiac troponin I, hs-cTnT - high-sensitivity cardiac troponin T

In patients with no CAD, non-severe CAD and severe CAD the median (Q1-3) concentrations of hs-cTnI were 1.2 (0.7-1.5) ng/L, 1.3 (0.7-2.1) ng/L and 1.9 (1.4-3.0) ng/L. (**Figure 1, panel A**) The corresponding hs-cTnT concentrations were 4 (2-5) ng/L, 5 (3-8) ng/L and 8 (5-11) ng/L. (**Figure 1, panel B**)

Figure 1. Box plot showing the resting concentrations of hs-cTnI (A) and hs-cTnT (B) in patients stratified by severity of coronary artery disease (CAD). *p*-values are for comparison between two categories.

Among patients with non-zero Gensini scores (n=272, 92%), both hs-cTnI and hs-cTnT were associated with increasing scores in univariable linear regression analyses (standardized B 0.31, 95% CI [0.20-0.42], $p<0.001$] and standardized B 0.37, 95% CI [0.25-0.48], $p<0.001$], respectively) (For doubling of Gensini score per doubling of hs-cTnI concentrations).

These associations remained significant after adjusting for sex, age, BMI, systolic blood pressure, history of diabetes, current smoking and eGFR. hs-cTnI: Standardized B 0.18, 95% CI (0.06-0.30), $p=0.002$; hs-cTnT: Standardized B 0.19. 95% CI (0.09-0.41), $p<0.001$. No significant difference in the strength of the associations was observed ($p=0.97$ for difference).

Both hs-cTnI and hs-cTnT were associated with the presence of severe CAD (n=137) in unadjusted and adjusted logistic regression analyses (hs-cTnI: Unadjusted standardized odds ratio [OR]: 1.88, 95% CI [1.42-2.47], $p<0.001$, adjusted standardized OR: 1.40, 95% CI (1.05-1.87), $p=0.02$; hs-cTnT: Unadjusted standardized OR: 2.43 [1.80-3.28], $p<0.001$, adjusted standardized OR: 1.96, 95% CI (1.43-2.70), $p<0.001$), $p=0.08$ for difference between the adjusted standardized OR.

The ROC-AUC for predicting severe CAD was 0.68 (95% CI [0.62-0.74]) for resting hs-cTnI and 0.72 (0.66-0.78) for resting hs-cTnT, $p=0.11$ for difference. When adding resting hs-cTn concentrations to a base risk model for prediction of severe CAD consisting of age, sex, history of CAD, diabetes, hypertension, low-density lipoprotein cholesterol concentrations and current smoking, hs-cTn reclassified patients to a more correct risk stratum (hs-cTnI: cNRI 36%, 95% CI [5-65], IDI 1% [0-8]; hs-cTnT: cNRI 27% [11-68], IDI 2% [0-9]). Neither hs-cTnI, nor hs-cTnT significantly increased the c-statistics of the base model (0.76, 95% CI [0.70-0.82]). hs-cTnI: 0.76 (0.70-0.82), $p=0.91$; hs-cTnT: 0.77 (0.71-0.83), $p=0.73$.

3.3 Dynamic changes in cTn in response to physical exercise

The median (Q1-3) absolute change in hs-cTnI concentrations in response to exercise was 0.2 (0.0-0.4) ng/L, corresponding to a relative change of 13 (3-27) %, $p<0.001$. Nineteen (7%) patients had ≥ 2 -fold increase (from median 0.9 (Q1-3 0.6-1.5) ng/L to 2.3 (1.4-5.8) ng/L.

(Figure 2).

Figure 2. Comparison of the magnitude of relative change in hs-cTnI and hs-cTnT concentrations in response to physical exercise. A value of two corresponds to a doubling relative to resting cTn concentrations.

The median (Q1-3) absolute change in hs-cTnT concentrations was 0 (0-1) ng/L, corresponding to relative change of 5 (0-12) %, $p<0.001$. Six (2%) patients had ≥ 2 -fold increase (from median 5 (Q1-3 3-8) ng/L to 20 (15-26) ng/L).

There was a significant difference between the exercise-induced relative changes in cTn concentrations between cTnI and cTnT (median 13 (Q1-3 3-27) % vs. 5 (0-12) %, $p<0.001$).

respectively, *p* for difference <0.001). In sensitivity analyses only including patients with both pre- and post-test hs-cTnT above the LoB (n=260) the median (Q1-3) cTn relative changes in concentration were 13 (3-26) % for cTnI and 6 (1-13) % for cTnT, *p* for difference <0.001).

The correlations between absolute and relative changes in hs-cTnI and hs-cTnT concentrations were $r=0.13$, $p=0.03$ and $r=0.09$, $p=0.13$, respectively.

We did not identify any clinical predictors, including age, sex and history of CAD, of greater exercise-induced change in either hs-cTnI or hs-cTnT concentrations, with the exception of current smoking, which was associated with a greater relative increase in exercise-induced hs-cTnI concentrations. (**Supplemental Table 2**).

Supplemental Table 2. Linear regression analyses of relative increase in cardiac troponin I and T (hs-cTnI; hs-cTnT) in response to exercise.

		Univariable analyses		
		B	95% Confidence interval	p-value
Age (years)	hs-cTnI	-0.06	-0.14-0.03	0.20
	hs-cTnT	0.003	-0.04-0.05	0.88
Female sex	hs-cTnI	-0.13	-0.29-0.03	0.12
	hs-cTnT	-0.06	-0.14-0.02	0.16
Current smoker	hs-cTnI	0.28	0.08-0.48	0.005
	hs-cTnT	0.009	-0.09-0.11	0.86
Body Mass Index (kg/m ²)	hs-cTnI	-0.01	-0.03-0.005	0.15
	hs-cTnT	0.007	-0.003-0.07	0.16
History of diabetes	hs-cTnI	-0.08	-0.27-0.12	0.45
	hs-cTnT	0.06	-0.04-0.16	0.25
History of coronary artery disease	hs-cTnI	-0.03	-0.20-0.13	0.70
	hs-cTnT	0.04	-0.04-0.13	0.32
Systolic blood pressure (mmHg)	hs-cTnI	0.02	-0.02-0.05	0.42
	hs-cTnT	-0.003	-0.02-0.02	0.73
eGFR ckd-epi (ml/min/1.73 ²)	hs-cTnI	0.003	-0.002-0.01	0.23
	hs-cTnT	0.0006	-0.002-0.003	0.93
Gensini score	hs-cTnI	0.02	-0.03-0.07	0.37
	hs-cTnT	0.001	-0.02-0.03	0.93
METs	hs-cTnI	0.01	-0.03-0.06	0.52
	hs-cTnT	0.004	-0.02-0.03	0.72
Exercise duration (minutes)	hs-cTnI	-0.008	-0.03-0.01	0.46
	hs-cTnT	0.007	-0.004-0.02	0.21

Results presented as unadjusted coefficients with 95% confidence intervals and p-values. Concentrations of cardiac troponin I and T, Gensini score and the relative cTn increase are log₂-transformed. Age and systolic blood pressure are reported per 10-unit increase. Abbreviations: hs-cTnI - high-sensitivity cardiac troponin I, hs-cTnT - high-sensitivity cardiac troponin T, eGFR ckd-epi – estimated glomerular filtrations rate using the Chronic Kidney Disease Epidemiology Collaboration equation

There was no significant association between relative changes in hs-cTnI and hs-cTnT concentrations and Gensini score ($p=0.37$ and $p=0.93$, respectively). Moreover, relative changes in hs-cTnI and hs-cTnT during exercise did not differ between participants with no CAD, non-severe CAD and severe CAD (**Supplemental Table 3**). The ROC-AUC for relative changes in hs-cTnI and hs-cTnT concentrations in association with severe CAD was 0.50 (0.43-0.57) and 0.53 (0.46-0.60), respectively.

Supplemental Table 3. Exercise characteristics, resting cardiac troponin values and relative increase in response to exercise stratified by categories of Gensini score.

	No CAD (Gensini = 0) n=25	Minimal CAD (Gensini = 1-19) n=135	Severe CAD (Gensini ≥20) n=137	p-value for trend
hs-cTnI at rest (ng/L)	1.16 (0.74, 1.47)	1.26 (0.7, 2.09)	1.88 (1.37, 2.97)	<0.001
hs-cTnI delta ratio	1.18 (1.09, 1.24)	1.11 (1.03, 1.28)	1.13 (1.03, 1.27)	0.63
hs-cTnT at rest (ng/L)	4 (4, 5)	5 (3, 8)	8 (5, 11)	<0.001
hs-cTnT delta ratio	1.03 (1.00, 1.11)	1.05 (1.00, 1.13)	1.06 (1.01, 1.12)	0.31
Exercise duration (min)	10.3 (8.7, 12.7)	9.7 (7.8, 12.2)	9.2 (6.6, 11.9)	0.02
METs	7.0 (6.3, 7.8)	6.9 (5.8, 8.0)	6.3 (5.5, 8.0)	0.04

Presented as medians with quartiles 1 and 3. p-values are for trend. Abbreviations: hs-cTnI - high-sensitivity cardiac troponin I, hs-cTnT - high-sensitivity cardiac troponin T

4 DISCUSSION

4.1 Main findings

There are three main findings in our study investigating the relationship between resting and exercise-induced cTnI and cTnT concentrations and coronary artery disease in patients with suspected CCS.

- 1) There was a graded relationship between concentrations of both for cTnI and cTnT at rest and the severity of CAD with no significant differences between the two isotypes.
- 2) The addition of resting cTn concentrations to a diagnostic model of known predictors of CAD did not significantly enhance the model's diagnostic power, although resting cTn concentrations might provide some reclassification ability.
- 3) Exercise-induced changes in cTn concentrations were not associated with the presence or severity of CAD, even with the enhanced analytical sensitivity of the cTnI assay. Further, we did not identify any relevant clinical predictors of greater exercise-induced changes in cTn concentrations. Although of no diagnostic significance, the increase in cTn concentrations was greater for cTnI than for cTnT

4.2 cTn and myocardial damage

With the increasing utilization of high-sensitivity cTn assays in the last decade, cTn as a biomarker is now understood to not only reflect the presence of ischemia driven acute myocardial necrosis, but also chronic and subclinical disease processes. In support of this, the fourth universal definition of myocardial infarction defines elevated concentrations of cTn in the non-ischemic setting as either chronic or acute myocardial damage. [1]

To what extent the elevated cTn concentrations observed in patients with chronic myocardial damage represent underlying coronary artery pathology or other pathological processes such

as left ventricular (LV) remodelling or chronic kidney disease, is not thoroughly understood. Indeed, LV mass is perhaps the most important determinant of cTn concentrations [21], but greater cTnT concentrations also appears to be independently associated with reversible ischemia in patients with CCS. [22] Furthermore, cTnI is associated with the presence of obstructive CAD and coronary artery calcium score in patients with suspected CCS. [2] Our findings extend these observations to a higher risk population (Morise score >9) and also demonstrate the performance equivalency of cTnI and cTnT, measured with the highest sensitivity assays available. Thus, despite mounting evidence suggesting idiosyncratic biological properties of the cTnI and cTnT isotypes, the association with chronic CAD seems to be comparable.

4.3 Exercise intensity and temporal release kinetics

Recent studies have demonstrated that exercised-induced increases in cTn concentrations are associated with increased cardiovascular risk. [9] In an observational study of 725 older individuals participating in a long-distance long-duration march, exercise-induced increases in cTnI concentration above the 99th percentile URL independently predicted higher all-cause mortality and incidence of cardiovascular events. In a heterogenous and comorbid population, these findings challenges the paradigm of the benign nature of post exercise cTn elevations. In our study, cTn concentrations after a traditional high-intensity short-duration bicycle cardiac stress test with immediate post-test sampling of cTn *did not* associate with coronary pathology. However, many patients had increases in cTn concentrations in response to exercise (a doubling of cTnI and cTnT concentrations were observed in 7% and 2% of patients, respectively), but this was not more prevalent in patients with obstructive CAD. This lack of association may represent a benign response to increased contractility and heart rate. However, the limited duration of exercise and short time to sampling may have influenced the results, as both exercise intensity [23] and temporal aspects [24] of cTn kinetics have

diagnostic and prognostic importance. In a study of 120 middle aged participants in a high-intensity mountain bicycle race, post-exercise cTn elevations were significantly associated with the presence of Coronary Computed Tomography Angiography (CCTA) verified CAD. [25] Notably, while both cTnI and cTnT concentrations peaked around 3 hours after exercise, any significant diagnostic utility was only found in samples obtained 24 h post-exercise. Considering this, the lack of a significant diagnostic signal in our study is not surprising, as our post-exercise samples were obtained immediately after terminations of exercise. While patients in our study performed at a high peak level of intensity, the duration of exercise was limited, resulting in an overall lower burden of physiological stress. Although it is apparent that both intensity and duration of exercise are determinants of inducible cTn concentrations, shorter, high-intensity performance might mimic the physiological stress seen in clinical scenarios such as self-terminating cardiac arrhythmias or episodes of convulsion. As such, our study indicates the low clinical feasibility of repurposing traditional cardiac stress tests for a biomarker driven diagnostic approach, as well as the difficulties associated with interpreting cTn in the setting of acute myocardial damage.

4.4 Isotype-dependent cTn kinetics

Although of uncertain clinical significance, emerging evidence indicate that cTnI and cTnT are differentially associated with both physiological processes and clinical outcomes. With cardiomyocyte damage, cTnI seems to be released earlier and in greater magnitude than cTnT [12] and is also less affected by impaired renal function. [15] Concentrations of cTnT appear to have a diurnal variation that is not apparent for cTnI [14] and also appear more prone to interference by neuromuscular disorders. [26] Prognostically, cTnT appears more strongly associated with all-cause mortality in the general population [10] and after MI [12], while cTnI seems to be more strongly associated with incident coronary heart disease. [27, 28] Furthermore, cTnI and cTnT appear to provide complementary prognostic information in the

general population and in patients with CCS. [5, 27] In a situation of MI, cTnI and cTnT concentrations are strongly correlated and as such does not seem to provide substantial complementary diagnostic information as to rule-in MI. However, differences in isotype specific release kinetics could translate into differences in earlier rule-out of MI, as cTnI appears to be a more sensitive marker of myocardial damage at an earlier time point than cTnT. [12, 29] In our study, the magnitude of cTnI release in response to physiological stress was indeed significantly greater than that of cTnT, although this did not yield any additional diagnostic information. Whether this reflects true biological differences between the isotopes or if it is related to biochemical properties of the assays utilized in the study is unknown, but knowledge of the phenomenon could be useful for clinicians interpreting cTn concentrations in patients admitted with chest pain after physical activity.

4.5 Limitations

The diagnostic precision of a biomarker is dependent on the accuracy of the gold standard definition for the disease being investigated. In our study, all patients underwent invasive coronary angiography and the extent and severity of CAD was estimated using the Gensini score. However, it is uncertain to what extent a pure anatomical estimate of CAD burden is physiologically significant. As such, the lack of measures such as fractional flow reserve (FFR) may attenuate the diagnostic efficacy of cTn and is a major limitation to the study. Furthermore, as cTn is associated with myocardial remodelling, the lack of echocardiographic data limits our ability to control for any effects of subclinical heart failure. However, the low NT-proBNP concentrations (median 64 [Q1-3 37-123] ng/L) suggests that subclinical HF was rare. Further, our study did not include serial sampling of cTn concentrations, and as such does not contain the information needed to opine on the temporal release patterns observed in other studies. [23, 25] Obtaining a single post-exercise sample is a clinically desirable practice, as prompt sampling eliminates the need for prolonged observation. However, it is

evident that the temporal aspect of cTn release is important and the lack of such data is a limitation to our study.

5 CONCLUSION

There was a graded relationship between resting concentrations of cTn measured with high-sensitivity assays and the extent and severity of CAD in patients referred for evaluation of suspected CCS. The clinical and demographic determinants of this relationship were similar for cTnI and cTnT, despite the superior analytical sensitivity of the cTnI assay. cTnI concentrations increased by a larger magnitude than cTnT concentrations in response to short-duration high-intensity exercise. However, elevation in neither isotype was associated with the presence or severity of CAD. Thus, there appears to be a threshold with regards to exercise intensity and timing of cTn sampling that needs to be exceeded for cTn dynamics to reflect the presence of CAD.

6 GLOSSARY

ACE-i – Angiotensin converting enzyme inhibitor

ACS – Acute Coronary Syndrome

AMI - Acute myocardial infarction

ARB – Angiotensin-receptor blocker

BMI – Body Mass Index

CABG - Coronary artery bypass graft

CAD - Coronary artery disease

CADENCE - Markers of Coronary Artery Disease During Exercise Testing

CCTA – Coronary Computed Tomography Angiography

CI – Confidence Interval

CKD-EPI - Chronic Kidney Disease Epidemiology Collaboration equation

cNRI - Continuous Net Reclassification Improvement

CSS - Chronic Coronary Syndrome

cTnI - Cardiac troponin, isotype I

cTnT - Cardiac troponin, isotype T

CV - Coefficient of variation

eGFR - Estimated glomerular filtration rate

FFR - Fractional Flow Reserve

HF – Heart failure

hs-cTn – High-sensitivity cardiac troponin

IDI - Integrated Discrimination Improvement

LDL cholesterol - Low density lipoprotein cholesterol

LoB - Limit of Blank

LoD - Limit of Detection

LV – Left ventricle

MET - Metabolic Equivalent of Task

OR – Odds ratio

OUH - Oslo University Hospital

ROC-AUC- Receiver Operating Characteristics – Area Under the Curve

URL - Upper Reference Limit

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