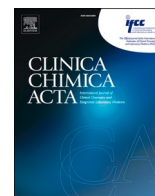




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The ratio of cardiac troponin T to troponin I may indicate non-necrotic troponin release among COVID-19 patients

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ABSTRACT

Background: Although cardiac troponin T (cTnT) and troponin I (cTnI) are expressed to similar amount in cardiac tissue, cTnI often reach ten-times higher peak levels compared to cTnT in patients with myocardial necrosis such as in acute myocardial infarction (MI). In contrast, similar levels of cTnT and cTnI are observed in other situations such as stable atrial fibrillation and after strenuous exercise.

Objective: Examine cTnT and cTnI levels in relation to COVID-19 disease and MI.

Methods: Clinical and laboratory data from the local hospital from an observational cohort study of 27 patients admitted with COVID-19 and 15 patients with myocardial infarction (MI) that were analyzed with paired cTnT and cTnI measurement during hospital care.

Results: Levels of cTnI were lower than cTnT in COVID-19 patients (TnI/TnT ratio 0.3, IQR: 0.1–0.6). In contrast, levels of cTnI were 11 times higher compared to cTnT in 15 patients with MI (TnI/TnT ratio 11, IQR: 7–14). The peak cTnI/cTnT ratio among the patients with MI following successful percutaneous intervention were 14 (TnI/TnT ratio 14, IQR: 12–23). The 5 COVID-19 patient samples collected under possible necrotic events had a cTnI/cTnT ratio of 5.5 (IQR: 1.9–8.3).

Conclusions: In patients with COVID-19, cTnT is often elevated to higher levels than cTnI in sharp contrast to patients with MI, indicating that the release of cardiac troponin has a different cause in COVID-19 patients.

1. Introduction

Cardiac-specific troponins, troponin T (cTnT) and troponin I (cTnI) are biomarkers that are primarily used for diagnosis of acute myocardial infarction (MI) [1].

cTnT and cTnI form an obligate 1:1 protein complex as the individual proteins are insoluble [2]. cTnT and cTnI are therefore expressed to similar amounts in cardiac tissue. Once released into the circulation

cTnT and cTnI are cleared by the liver and kidneys with similar kinetics [3,4]. Despite this fact, the level of cTnI often reach ten-times higher peak levels and decrease more quickly compared to cTnT following myocardial infarction [5–7].

Experimental studies from our group indicate that this difference is due to fast cleavage and release of cTnI in necrotic cardiac tissue and that most cTnT remains bound to the insoluble muscle filaments. cTnT is therefore for the most part locally degraded without reaching the

Abbreviations: MI, Myocardial infarction; cTnT, cardiac troponin T; cTnI, cardiac troponin I; CT, X-ray computed tomography; PCI, percutaneous coronary intervention.

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systemic circulation [3,5,8]. The faster and more complete release of cTnI result in considerably higher cTnI levels compared to cTnT levels in patients with MI [5–7], a fact also experienced by our local clinicians when the hospital lab changed from cTnT to cTnI assays.

In contrast, in individuals with stable troponin elevations such as seen in the general population [9], chronic kidney disease [10,11] and stable patients with atrial fibrillation [11], levels of cTnT are often higher than of cTnI. Following exercise, cTnT and cTnI levels increase to similar levels [17]. This may indicate that stable troponin elevations and transient troponin elevations following exercise have other causes and release mechanisms, and is not primarily due to myocardial necrosis. In addition, inflammation and accumulation of immune cells in the absence of myocardial damage may be a separate troponin release mechanism in COVID-19 [12].

cTnT or cTnI elevation above the diagnostic cut-off in the absence of overt ischemia is defined as myocardial injury according to the latest definition of myocardial infarction [13]. Myocardial injury is linked to increased risk of death and cardiovascular disease, not only in patients with MI, but also among emergency room patients that does not have MI [14–16]. An exception is transient troponin elevations seen after strenuous exercise, in which most data indicate that the troponin elevations are not linked to poor prognosis [17]. Because troponin elevations after exercise may not be indicative of poor prognosis, it may be clinically helpful to distinguish troponin elevations due to necrosis from other causes.

Since the start of the COVID-19 pandemic in early 2020, several studies have shown that elevation of cTnT and cTnI is common in patients who are hospitalized with COVID-19 and are strongly associated with increased risk of death [18–20]. Myocardial injury in patients with COVID-19 disease has been suggested to be due to COVID-19-associated myocarditis [21], concomitant myocardial infarction (MI) [22] or stress-induced cardiac injury (reviewed in [23]). However, no one has previously studied the patterns of cTnT and cTnI simultaneously in COVID-19 patients.

In an attempt to better understand the troponin elevations in this new disease we analysed both cTnT and cTnI on patients with COVID-19 and compared the levels to patients with MI.

2. Material and methods

2.1. Study design and populations

The study was conducted at Sahlgrenska University Hospital, Gothenburg Sweden, during the period of April to August 2020 and included hospitalized patients with COVID-19 or type I MI undergoing percutaneous coronary intervention.

2.2. Patients with COVID-19

Covid-19 was diagnosed using either a rapid SARS-Cov-2 antigen test verified by a SARS-Cov-2 PCR test or solely a SARS-Cov-2 PCR test. Patients with verified COVID-19 but with limited symptoms and adequate oxygenation on air were sent home and not admitted. Therefore, only COVID-19 patients failing to oxygenate only on air and requiring oxygen therapy were admitted to the hospital and could be part of the study. In case of respiratory deterioration, the patients were admitted to the intensive care unit, intubated and treated with the intention to follow updated guidelines [24]. Of the COVID-19 patients included in the study 52% were intubated and 48% were not intubated (Table 1). We collected 67 blood samples from 27 hospitalized COVID-19 patients that were analyzed with cTnT and cTnI on the same day. The study was approved by the Swedish Ethical Review Authority (Registration number 2020–01771 and 2010-755-09).

All journals of the 27 COVID-19 patients and levels of cTnT, creatinine, NTproBNP and CRP were examined by OH, RS and SS in search for possible necrotic events that could explain troponin elevations [13,23].

Table 1

COVID-19 patients with paired cTnI and cTnT samples.

	All	Patients with events†
n	27	5
Age	63 (55–69)	(60–73)
Male sex	67%	60%
BMI	27 (25–30)	28 (27–30)
Diabetes	15%	40%
Heart disease	26%	60%
Stroke	7%	0%
Asthma	0%	0%
Kidney disease	15%	0%
COPD	0%	0%
Other complicating condition	30%	0%
Symptom time before ED (days)	10 (7–14)	10 (6–11)
Hospital time (days)	21 (9–42)	41 (26–54)
Non-survivors	19%	40%
Intubated	52%	80%
cTnI (ng/L)	15 (9–34)	539 (232–901)
cTnT (ng/L)	71 (55–10)	108 (97–121)
I/T ratio	0.3 (0.1–0.6)	5.5 (1.9–8.3)

† Possible necrotic events as defined in materials and methods. Median (interquartile range). ED – Emergency room; COPD – Chronic Obstructive Pulmonary Disease; BMI – Body Mass Index.

A possibly necrotic event included myocardial infarction, atrial fibrillation or other tachycardia with a heart rate ≥ 120 beats per minute for more than 24 h, pulmonary embolism diagnosed with X-ray computed tomography (CT), ventilation problems with hypoxia over 24 h, increased pulmonary pressure estimated by UCG, pneumothorax diagnosed by ultrasound or CT, stroke by CT, heart failure or dilated right ventricle by UCG.

According to the assessment guidelines determined in advance a possible necrotic events was defined as MI if there was a cTnT rise and fall $\geq 20\%$. If the possible necrotic events did not result in a transient cTnT elevation it was labeled “non-MI possible necrotic events”. The 5 patient samples collected during events were due to stroke, persistent tachycardia with ST-depression on ECG, MI the day before and one sample collected before death with ongoing intensive care treatment. The remaining 62 samples were collected at a time during the hospital stay where no possible necrotic events were found up to 5 days before sample collection.

2.3. Patients with myocardial infarction

cTnT and cTnI were also analyzed in blood samples from patients who underwent percutaneous coronary intervention (PCI) due to confirmed type 1 myocardial infarction, and who presented to the hospital within 6 h of symptom onset. All journals of the 15 MI patients were examined to find the type of MI (NSTEMI or STEMI), time of symptom, time of PCI and other variables of interest (Table 2).

2.4. Laboratory methods

All laboratory analyses during the hospital stay were obtained from the local clinical chemistry database for each patient. cTnT was measured using the Elecsys® cTnT immunoassay on a fully automated Modular® Analytics E170. The within-run, between-run and long-term coefficients of variation (CV) have been published previously [25] and were 4.9% at a median cTnT level of 4.6 ng/L, 4.9% at a median cTnT level of 9.5 ng/L and 1–1.4% at a median cTnT levels above 14 ng/L. cTnT values above the limit of quantification (LoQ) of 5 ng/L were reported to clinicians throughout the study. The attending clinician made all the decisions about the number and the timing of cTnT measurements. Troponin I was measured using the Abbott high-sensitive assay on Alinity with a LoB of 2 ng/L and the CV was 6% at a median cTnI level of 8 ng/L, CV was 6.5% at a median cTnI level of 11 ng/L, CV was 4.9% at a median cTnI level of 90 ng/L and CV was 3% at a median cTnI level

Table 2
Patients with myocardial infarction.

n	15
Age	67 (56–72)
Male sex	60%
BMI	28 (26–29)
NSTEMI	33%
Diabetes	60%
Heart disease	25%
Stroke	6%
Asthma	0%
Kidney disease	6%
COPD	0%
Other complicating conditions	0%
Symptom to hospital time (h)	2 (0–4)
Symptom to PCI time (h)	6 (4–19)
Hospital time (h)	56 (36–72)
Sampling time (h)†	25 (11–34)
cTnI (ng/L)	3672 (133–21773)
cTnT (ng/L)	673 (78–2751)
cTnI/cTnT ratio	11 (7–14)
Max cTnI (ng/L)	35 086 (17 000–95 645)
Max cTnT (ng/L)	2835 (1036–4686)
Max cTnI/Max cTnT ratio	14 (11–22)

Median (interquartile range). PCI – Percutaneous Coronary Intervention; COPD – Chronic Obstructive Pulmonary Disease; BMI – Body Mass Index. †Time after symptom.

of 1000 ng/L. During the study cTnI levels were not reported to the clinician.

3. Results

A total of 67 blood samples from 27 COVID-19 patients (Table 1) had paired measurements of cTnT and cTnI. Four intubated COVID-19 patients under intensive care had serial paired cTnT and cTnI measurements (Fig. 1). Levels of cTnT were often higher than cTnI and sometimes with distinct dynamic change. In contrast, in patients with MI (Table 2) the opposite relation was observed with significantly higher levels of cTnI compared to cTnT (Table 2). Median cTnI/cTnT ratio were 0.3 (IQR: 0.1–0.6) in the COVID-19 patients and 11 (IQR: 7–14) in patients with myocardial infarction. During the troponin peak after PCI median cTnI/cTnT ratio were 14 (IQR: 11–22). When cTnI and cTnT levels were plotted, COVID-19 patients and MI patients clustered differently in relation to the unity line. MI patients tended to cluster below the unity line (Fig. 2A) whereas COVID-19 patients often had

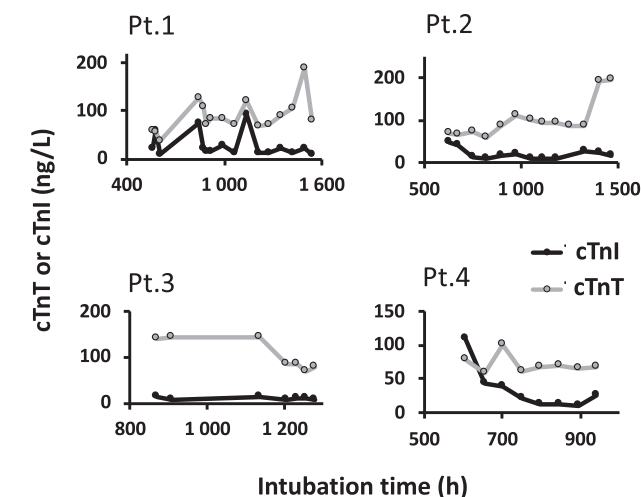


Fig. 1. Serial measurement of cTnT and cTnI during intensive care in four COVID-19 patients. The first sample in patient 4 (pt. 4) at 600 h of intubation was identified as an “event” as defined in materials and methods.

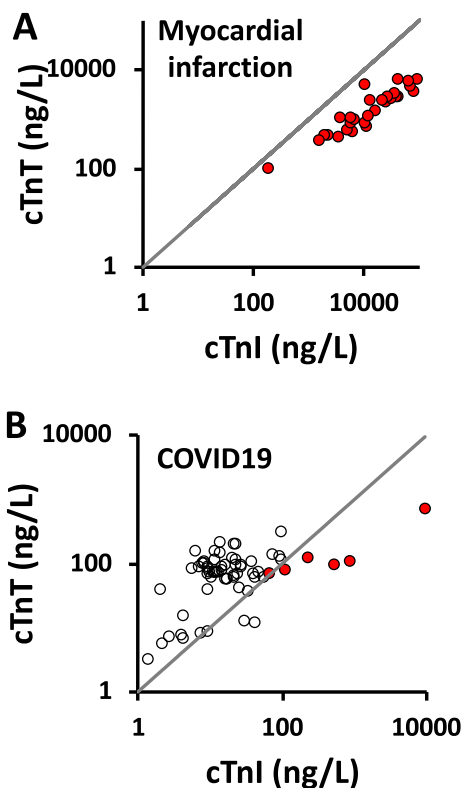


Fig. 2. Paired cTnT and cTnI measurements included in this study plotted against each other. (A) Patients with myocardial infarction described in Table 1. Samples were collected after PCI up to 80 h after PCI. (B) COVID-19 patients described in Table 2. Red dots are samples collected during possible necrotic events as described in materials and methods.

higher cTnT levels compared to cTnI (Fig. 2B). The 5 COVID-19 patients samples collected during potentially necrotic events had a median cTnI/cTnT ratio of 5.5 (IQR: 1,9–8,3) (Red dots in Fig. 2B, Table 1).

4. Discussion

We observed that many COVID-19 patients slowly developed stable cTnT elevations during intensive care as has been described before [26] and similar to studies of troponin elevations seen during acute respiratory distress syndrome by other causes [27]. In fact, cTnT elevations are a common observation in intensive care patients with non-cardiac diagnoses [28]. The late and stable cTnT elevations evoked the question of the underlying mechanism especially as cTnT elevations signifies poor prognosis (reviewed in [29]).

In paired measurements, cTnT levels were often higher compared to cTnI in patients with COVID-19. In addition, cTnT and cTnI levels sometimes had different dynamic change, as seen in the four patients with serial measurements. In contrast, cTnI levels in patients with type 1 MI were often ten-fold higher compared to cTnT [5–7] and cTnT and cTnI had similar kinetic of change with a peak following the event and a slow decline.

The fact that cTnT levels were higher than cTnI and that the dynamic change of cTnI and cTnT sometimes did not overlap in patients with COVID-19 point to the possibility that the late troponin elevations we observed was not primarily due to cardiac necrosis. One possibility is that troponin elevations were due to long-term cardiac stress following high ventilation pressures, right ventricular strain [30], right ventricular Takotsubo syndrome [31], fast atrial flutter or other sustained periods of tachycardia (reviewed in [23]). This would fit with several observations.

First, prolonged, but not transient, endurance exercise causes

skeletal muscle fibers to release creatine kinase, myoglobin and other muscle proteins into the circulation [32–34]. Muscle biopsy studies indicates that this delayed release of muscle proteins is not due to necrosis [35,36].

Second, a recent study show that cTnT increased to similar or higher levels compared to cTnI levels during controlled myocardial ischemia that most likely did not induce necrosis by inflating a balloon in the left anterior descending artery for 0.5–1.5 min [37].

Third, the late elevation of creatine kinase and myoglobin from skeletal muscle after high-load, long duration exercise is mirrored by the cTnT and cTnI elevations observed in young athletes [17,38]. Participants in extreme races often develop troponin elevations that are ten times the assay specific cutoff's that become more prominent after long bouts of exercise [39] and is related to duration of elevated heart rates [40]. Most studies have not excluded necrotic events, but necrosis is unlikely to be the only release mechanism as most participants that develop troponin elevations are young and fit. Thus, several studies with paired measurements of both cTnT and cTnI consistently find that cTnI reach similar levels as cTnT after exercise [40–42]. In contrast, cTnI levels are considerably higher than cTnT in patients with MI [5–7]. Our finding that the cTnI/cTnT ratio is very different in patients with MI and after long-term cardiac strain in COVID-19 is therefore not without precedent.

Another possibility behind the troponin release in our COVID-19 patients is myocardial inflammation. In a multicenter cardiovascular pathology study of autopsies of 21 consecutive COVID-19 patients that died [12], troponin elevation was present in several cases without myocardial damage, myocarditis or thrombus. These authors noted that 86% of the cases had extensive interstitial macrophage infiltration without clearly associated myocyte injury.

It is known that interstitial macrophages may induce membrane defects in skeletal muscle fibers via excess production of cytolytic free radicals (reviewed in [43]). For example, it has been shown that immunodepletion of macrophages reduces signs of membrane injury both in mice with muscular dystrophy and in normal mice after exhaustive exercise [44]. Importantly, both skeletal and cardiac myocytes appear to be able to survive transient elevations in the permeability of the sarcolemma [35,45] (Reviewed in [46]). The scenarios of increased membrane permeability caused by strain and by inflammation are not mutually exclusive and may be complementary [47].

No matter of the underlying release mechanism, before we knew the mechanisms of cTnT and cTnI release and clearance after necrotic events [3,4,11,48,49] it might be understandable that the different cTnI/cTnT ratio following exercise and MI have been overlooked. For instance, there is no obvious reason to believe that the ratio between cTnT and myoglobin or CK-MB would add information as the relative abundance, release kinetics and clearance are different.

The amount of cTnT and cTnI on the other hand is highly linked as they are part of a 1:1 protein complex that can only be separated if denatured or degraded [2]. In addition, clearance of cTnT and cTnI are identical once they reach circulation [3]. The vast difference in cTnI/cTnT ratios between MI and exercise, transient ischemia or COVID-19 might therefore signify different release mechanisms.

Possibly, if the cTnI/cTnT ratio is < 1 non-necrotic events could be the primary suspicion especially if the symptom time is short. It is however important to note that exercise can induce necrotic events in susceptible hearts [42] and thus blur the picture if analysis of the cTnI/cTnT ratio is done without careful characterization of the individual event [50]. As necrosis result in ten-fold higher levels of cTnI any small region of necrosis will likely make cTnI levels dominant over cTnT levels. This in turn indicates that a troponin elevation where cTnI/cTnT ratio < 1 is unlikely to be dominated by necrosis.

This study has several limitations. The study group was small. The group of patients with COVID-19 were heterogenous. It was a service evaluation of retrospective data and the preliminary findings concerning the cTnI/cTnT ratio found in this study must be verified in a prospective

study where blood samples are taken before, during and after controlled ischemia. The possibility that isolated cTnT elevations were from skeletal muscle [51] were not excluded, although creatine kinase or myoglobin were not elevated in four patients with cTnT elevations (data not shown). Finally, it is unlikely that central labs will provide both cTnT and cTnI assays for clinical service. The clinical utility cTnI/cTnT ratio is therefore unclear.

In summary, we find that troponin elevations in COVID-19 patients have lower cTnI/cTnT ratio compared to patients with MI indicating that troponin elevations in patients with COVID-19 may be due to non-necrotic mechanisms.

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Ethics committee approval

The study was approved by the Ethics Committee at the University of Gothenburg, and the study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki (Registration number 2020–01771 and 2010-755-09).

CRediT authorship contribution statement

Ola Hammarsten: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration. **Pontus Ljungqvist:** Investigation, Data curation. **Björn Redfors:** . **Mathias Wernbom:** Conceptualization. **Hannes Widing:** Conceptualization. **Bertil Lindahl:** Conceptualization. **Sabin Salahuddin:** Investigation, Data curation. **Ruwada Sammantar:** . **Sandeep Jha:** Investigation, Data curation. **Annica Ravn-Fisher:** Conceptualization. **Magnus Brink:** Conceptualization, Investigation. **Magnus Gisslen:** Conceptualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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