Proteolytic Digestion of Serum Cardiac Troponin I as Marker of Ischemic Severity

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Background: The serum troponin assay is the biochemical gold standard for detecting myocardial infarction (MI). A major diagnostic issue is that some believe troponin levels can rise with reversible injury, in the absence of radiologically detectable infarct.

Hypothesis: Because cell death activates intracellular proteases, troponin released by irreversible infarct will be more proteolyzed than that released by milder processes. Our goal was to quantify proteolytic digestion of cardiac troponin I in patients with varying degrees of myocardial injury.

Methods: Serum or plasma samples from 29 patients with cardiac troponin I elevations were analyzed for proteolytic degradation, using 3 different sandwich ELISAs designed to specifically detect the N-terminal, core, or C-terminal regions of cardiac troponin I.

Results: As predicted, the degree of proteolytic digestion increased with increasing severity of injury, as estimated by the total troponin level, and this trend was more pronounced for C-terminal (vs N-terminal) degradation. The highest degree of proteolytic digestion was observed in patients with ST-elevation MI; the least, in type 2 MI (supply–demand ischemia rather than acute thrombus formation).

Conclusions: The proteolytic degradation pattern of cardiac troponin I may be a better indicator of clinically significant MI than total serum troponin level. Distinguishing between intact and degraded forms of troponin may be useful for (*a*) identifying those patients with clinically significant infarct in need of revascularization, (*b*) monitoring intracellular proteolysis as a possible target for therapeutic intervention, and (*c*) providing an impetus for standardizing the epitopes used in the troponin I assay.

IMPACT STATEMENT

Cardiac troponin measurement is the gold standard diagnostic test for myocardial infarction. However, troponin elevations do not always correlate with clinically significant infarct, creating uncertainty for the treatment of troponin-positive patients. We demonstrate that severe infarct releases cardiac troponin I that is more proteolytically digested than that released by milder ischemic conditions. Quantifying troponin degradation products might lead to the development of a novel biomarker that better informs about the underlying cardiac pathology.

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The 2012 Third Universal Definition defines myocardial infarction (MI)[5] as myocardial necrosis in the setting of ischemia (*1*), divided into 5 distinct subtypes. Type 1 MI is the classic "heart attack," in which a coronary vessel is occluded by acute thrombus. Type 1 MI can be further subdivided into ST-elevation MI (STEMI) and non–ST-elevation MI (NSTEMI), based on electrocardiographic findings. STEMI is associated with complete major vessel occlusion (*2*), necessitating urgent revascularization using thrombolytics or invasive procedures (*3*). Management of NSTEMI, associated with partial vessel occlusion, is less clear: An early invasive strategy is favored in high-risk patients, whereas a more conservative ischemia-guided approach is reserved for low-risk patients (*4*).

In clinical practice, myocardial necrosis is detected using serum biomarkers; cardiac troponin has emerged as the gold standard (*1*, *5*). However, recent observations have questioned whether serum troponin elevations necessarily indicate irreversible myocardial cell death (*6*, *7*). Troponin elevations can occur in the absence of infarct detectable by cardiac MRI (the gold standard for imaging) (*8*) and in healthy individuals after strenuous exercise (*9*) or atrial pacing-induced tachycardia (*10*). Moreover, any condition that creates a vascular supply–demand imbalance (tachyarrhythmias, anemia, or shock) can cause a troponin elevation, a situation classified as a type 2 MI (*11*). In the seriously ill patient, it is vitally important to distinguish whether an observed troponin elevation is because of generalized cardiac strain or a critically stenotic lesion in need of urgent revascularization.

The problem with conventional troponin measurements is that they do not distinguish between a focal area of intense infarct and more diffuse injury. It is known that troponin is proteolytically degraded in the setting of ischemia and/or infarction (*12*, *13*) through the activity of proteases (*14*) such as caspases (*15*), calpains (*16*), and intracellular matrix metalloproteinase-2 (*17*). Furthermore, the degradation of the cardiac troponin I subunit (cTnI) in particular is known to produce a heterogeneous mixture of degradation products that complicates its standardization and measurement (*13*). We hypothesize that the degree of proteolysis correlates with severity of ischemic injury. Therefore, we propose to quantify degradation of cTnI as a marker of the ischemic state (ranging from mild injury to irreversible necrosis) of the cells from which it originated.

METHODS

Design of troponin I degradation assay

To quantify proteolytic degradation of cTnI, we designed a series of 3 sandwich ELISAs using commonly used antibodies (HyTest): 19C7 (targeting troponin I core residues 41–49) as the capture antibody and detection antibodies, M18 (N-terminal residues 18–28), 560 (core residues 83–93), and MF4 (C-terminal residues 190–196) (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol3/issue3).

Polystyrene 96-well plates were coated with 2 μg/mL 19C7 antibody in PBS overnight at 4 °C and then blocked with 7% skim milk in PBS for 1 h. Patient plasma or serum samples were added undiluted or at 2-, 5-, or 10-fold dilution and incubated at room temperature for 2 h. Two to 4 μg/mL of biotin-conjugated detection antibodies (EZ-link™ biotinylation kit, Thermo Fisher Scientific) in

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[5] **Nonstandard abbreviations:** MI, myocardial infarction; STEMI, ST-elevation myocardial infarction; NSTEMI, non–ST-elevation myocardial infarction; cTnI, cardiac troponin I.

Superblock (Tris-buffered saline) blocking buffer (Thermo Fisher Scientific) was then added and incubated for 90 min at room temperature. Detection was achieved using NeutrAvidin-horseradish peroxidase conjugate (Thermo Fisher Scientific) and 3,3',5,5'-tetramethylbenzidine substrate (Sigma-Aldrich). Extensive washing (5 times) with PBS/0.05% Tween 20 buffer was performed between every step. Every patient sample was run in duplicate for every capture–detect antibody pairing. As a control, cTnI levels were measured on all clinical specimens using the Beckman Access system.

Patient sample collection

All patients provided written informed consent, and all protocols were approved by the University of Alberta Health Research Ethics Board. Patients with positive plasma cTnI readings >1 ng/mL were identified. The most recent (<24 h) plasma sample for each study patient was retrieved from the hospital laboratory and frozen at −80 °C. An additional serum sample from each patient was collected with the next scheduled blood draw. cTnI concentrations were determined using the Beckman Coulter Access system. For each patient, the sample with the higher value—serum or plasma—was used and reported in this study.

RESULTS

Patients

We assayed cTnI degradation in 29 patients classified by the hospital admitting service or cardiology consult service independently of the study investigators as follows: 12 had a STEMI, 5 were classified as NSTEMI, 1 patient had a type 4a MI following an elective percutaneous coronary intervention for chronic stable angina, 1 patient had myocarditis (confirmed by cardiac MRI), and 10 patients were considered to have type 2 MI (see Table 1 in the online Data Supplement).

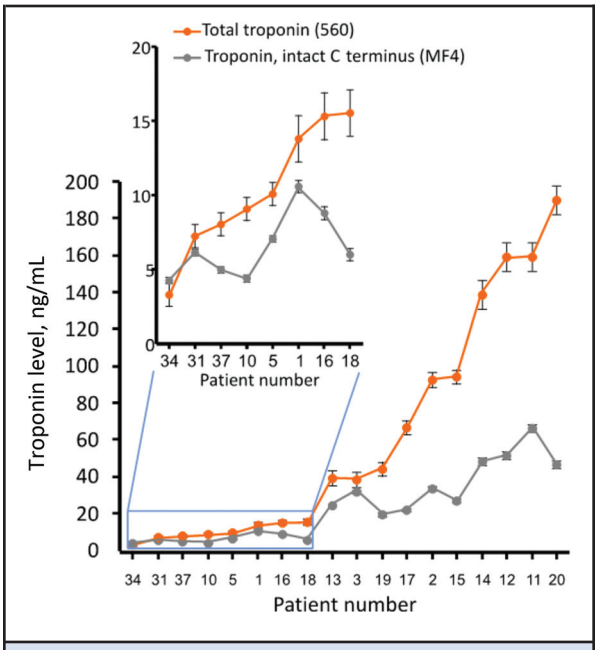
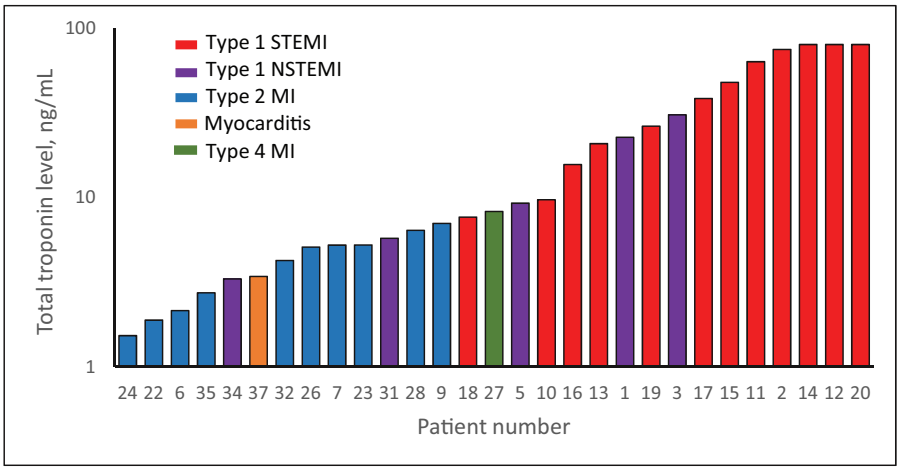


Fig. 1. Plasma/serum troponin I levels in patients with type 1 MI (plus 1 patient, 37, with myocarditis).
Troponin levels measured using 2 different detection antibodies: 560 (core region) vs MF4 (C-terminal region). Error bars represent the average SD between duplicate measurements.

cTnI is extensively degraded in STEMI patients

Three sandwich ELISAs using 3 different detection antibodies yielded troponin readings that correlated well with the Beckman Access system (r = 0.92–0.96).

Fig. 1 shows that as cTnI levels increase in type 1 MI patients, there is proportionately less detection by MF4 antibody at the C-terminal region compared with detection by 560 antibody at the core region, suggesting that the C-terminal degradation of cTnI increases with increasing infarct severity. (Compare this with the less consistent degradation at the N terminus by the M18 antibody shown in Fig. 2 of the online Data Supplement.) Thus, the remainder of our analysis will center on C-terminal degradation of cTnI.



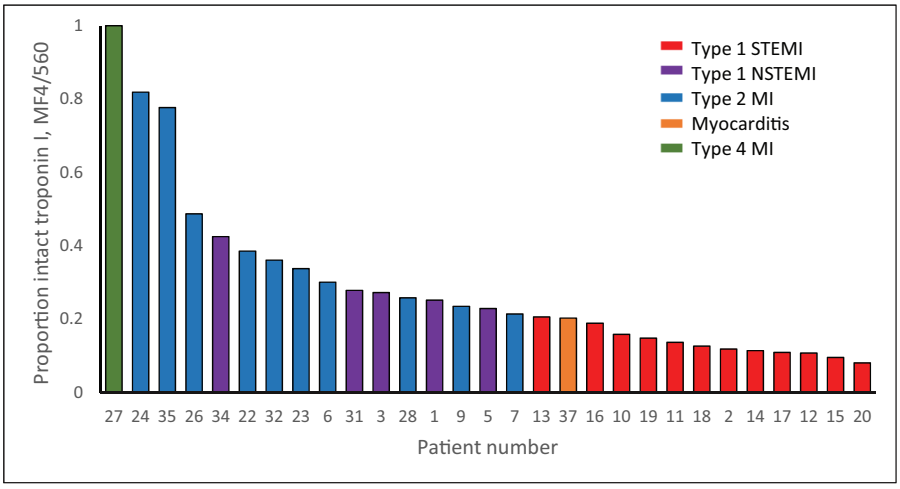


Fig. 2. Patients ordered according to total troponin I level (measured by the Beckman Access system; top panel), and patients ordered according to the proportion of troponin I degraded, determined by the ratio of troponin level determined using MF4 detection antibody (corresponding to intact troponin) to that determined using 560 detection antibody (corresponding to total troponin) (bottom panel).
The ratios were normalized to patient 27, assuming the patient who showed the least amount of degradation had no degradation.

NSTEMI patients (patients 34, 31, 5, 1, and 3) showed less cTnI degradation than STEMI patients, consistent with the expectation that complete vessel occlusion leads to a more focal and intense area of infarction and proteolytic degradation. As shown in Fig. 2, STEMI patients tend to have higher cTnI levels, but the total cTnI level does not reliably differentiate between STEMI and NSTEMI. In contrast, when patients are ordered according to the proportion of intact (vs proteolytically degraded) cTnI, a clear distinction between STEMI and NSTEMI can be seen (Fig. 2).

Of note, the lone patient in our study with acute myocarditis (patient 37) showed cTnI degradation levels comparable with STEMI patients.

Degradation of cTnI is variable in type 2 MI patients

Type 2 MI patients consistently showed less cTnI degradation than type 1 STEMI patients (see Fig. 3 in the online Data Supplement). However, a clear distinction could not be made between NSTEMI and type 2 MI, although there was a trend toward less degradation in type 2 MI patients (compare purple and blue bars in Fig. 2).

The lone type 4a MI patient, patient 27, showed significant improvement of chronic angina and improved exercise capacity after percutaneous coronary intervention, suggesting that the observed postprocedural cTnI elevation was benign. This patient showed the least cTnI degradation (see Fig. 2), although the conventional cTnI test could not differentiate the clinical scenario from a more serious type 1 MI.

DISCUSSION

Our pilot study showed that proteolytic degradation of cTnI appears to correlate with ischemic severity, with type 1 STEMI patients showing the highest degree of degradation, followed by NSTEMI patients. Consistent with clinical experience, patients labeled as having type 2 MI were a heterogeneous group showing varying degrees of degradation. Those with severe medical illness and noncritical atherosclerotic disease show the release of relatively intact cTnI, whereas those with more severe underlying atherosclerotic lesions had cTnI degradation levels comparable with type 1 NSTEMI patients (see Table 1 in the online Data Supplement).

Our study suggests that most commercial cTnI assays, which utilize at least one N- or C-terminal epitope, may favor detection of more intact forms of cTnI released with milder injury. This may explain the observation that total troponin levels correlate poorly with radiologically determined infarct size in NSTEMI patients (*8*). Further studies are needed to determine whether specific quantification of degraded troponin leads to improved correlation with infarct size.

Thus, accurate quantification of degraded troponin may be more useful in guiding clinical management than a single troponin level obtained by a conventional assay. Our proposed cTnI degradation assay could be easily implemented because it uses antibodies that are already routinely used. One potential side benefit of measuring cTnI degradation and standardizing the epitopes used for its quantification is that it may also become possible to achieve universal standardization of cTnI assays (*18*, *19*).

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

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