



What is new on cardiac troponin degradation?

Johannes Mair

Department of Internal Medicine III-Cardiology and Angiology, Heart Center, Medical University of Innsbruck, Innsbruck, Austria

Correspondence to: Johannes Mair, MD. Department of Internal Medicine III-Cardiology and Angiology, Anichstrasse 35, A-6020 Innsbruck, Austria.

Email: Johannes.Mair@i-med.ac.at.

Comment on: Katrukha IA, Kogan AE, Vylegzhanina AV, *et al.* Thrombin-Mediated Degradation of Human Cardiac Troponin T. *Clin Chem* 2017;63:1094-100.

Received: 09 July 2017; Accepted: 30 July 2017; Published: 09 August 2017.

doi: 10.21037/jlpm.2017.07.06

View this article at: <http://dx.doi.org/10.21037/jlpm.2017.07.06>

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are nowadays the criterion biomarkers for the laboratory diagnosis of acute and chronic myocardial injury due to their very high sensitivities and specificities (1). However, still many aspects of their intramyocardial degradation, their tissue release, and their degradation within and elimination from the human circulation are still incompletely understood. In a recently published manuscript Katrukha *et al.* (2) reported an important novel finding, i.e., degradation of human cTnT by thrombin, the most abundant coagulation enzyme. This editorial aims to give an update on what is known about degradation of human cTnI and cTnT and also points to gaps of current knowledge.

The cardiac troponin (cTn) complex is part of the thin filaments of myocardium, and its biochemistry and pathophysiology have been reviewed recently (3-5). Initially, small (approximately 5% of total content), “cytosolic” cTnI and cTnT pools were reported in myocardium (6,7). But when considering preparation protocols used by the investigators and the poor solubility of cTnI and cTnT in hydrophilic sarcoplasm, a more appropriate term is probably a “loosely bound, rapidly releasable pool”. When not incorporated into myofilaments, e.g., cTnT is rapidly degraded (e.g., by caspase or μ -calpain) within cardiomyocytes to avoid toxic effects (4). Myocardial injury may be caused by a variety of different mechanisms (e.g., inflammatory and immunological processes, trauma, drugs, and toxins), but most frequently by myocardial ischemia. Myocardial necrosis is preceded by a substantial reversible prelethal phase. A variety of factors (e.g., molecular mass, concentration gradient from cardiomyocytes to interstitial space, local blood and lymphatic flow) influences the onset

of release of cardiac markers from injured myocardium, the most important appears to be their intracellular compartmentation (7). In contrast to cytosolic proteins, the release of structurally bound molecules, such as cTns, requires both a leaky plasma membrane and dissociation from or degradation of cellular structures, such as the contractile filaments, which is a timely slower process. Of high significance in this respect is the susceptibility of a structure or protein to degradation by cytosolic proteases, such as the cytoplasmic calpains, which are rapidly activated in case of myocardial injury. By contrast, lysosomes are stable within the first 3–4 hours after onset of cell injury, and, therefore, lysosomal enzymes are not involved in the early degradation of structurally bound proteins. Both cTnI and cTnT are substrates of the cytoplasmic enzyme μ -calpain (8,9), which is activated by increased cytoplasmic calcium, which is an important and early feature of cell injury. This occurs by increased influx and redistribution of calcium from intracellular compartments (e.g., endoplasmic reticulum, mitochondria), and increased calcium in cytoplasm also activates phospholipases and endonucleases. pH dependent dissociation of the troponin complex could be another important factor for early cTn release from injured myocardium. The pathophysiological background of limited N-terminal intramyocardial proteolysis of cTn by caspase and calpain could be that cTns are involved in the sensitivity of cardiac muscle to declines in intracellular pH, and thus this may be an early specific functional adaptive response to myocyte injury rather than just simple destructive breakdown (4).

Once the integrity of the plasmalemma of cardiomyocytes is disturbed, they rapidly release

intracellular macromolecules into the interstitial space. So-called “cytoplasmic blebbing” may be possible also during the reversible phase of cell injury (10). These blebs contain intracellular macromolecules, such as degraded cTn, and detach with resealing of the plasma membrane without cell death in cell culture experiments. By contrast, sarcolemmal disruption with consecutive massive release of intracellular molecules into the interstitial space is believed to indicate morphologically the “point of no return” of cell injury, i.e., necrosis. In summary, cTnI and cTnT manifest rapid release after myocardial injury comparable to cytosolic proteins, such as myoglobin, or CKMB (11). The sustained increase in cTn after myocardial infarction is probably a combination of slow washout and local tissue degradation. Unrestricted blood flow, e.g., from invasive early reperfusion of the infarct-related coronary artery, results in faster extraction and clearance of cTns after myocardial injury (12).

Most proteins released from injured myocardium including cTns appear to be catabolized in tissues with a high metabolic rate, such as the liver, pancreas, kidneys or the reticuloendothelial system. In this respect, the recent report of Katrukha *et al.* (2) of cTnT degradation by thrombin, a rather indiscriminate serine protease, probably between R68 and S69 adds important novel information and may explain the heparin interference of a previous cTnT assay generation (13). cTns are cleared from the circulation with a half-life of 1–2 h similar to myoglobin or CKMB (12). Small molecules, such as myoglobin, pass the renal glomerular filtration membranes and can be found in urine (14). These proteins are mainly reabsorbed and subsequently metabolized in tubular epithelial cells. Thus, in case of impaired clearance from blood (e.g., renal or hepatic failure, hypothyroidism) prolonged biomarker increases are found. Intact cTnT is too large for glomerular filtration, but smaller cTn fragments would be small enough for renal clearance and a renal tubular component may contribute to excretion as well.

Given this very complex biochemical and pathophysiological background with post-translational modifications (e.g., phosphorylation), intramyocardial N-terminal proteolysis of cTnI and cTnT by caspase and calpain in response to myocardial injury and complex formation as well as degradation in blood (15–19), cTnI and cTnT present substantial challenges for measurement by immunoassay technology. Intact cTn rapidly disappear from the circulation during the early hours after acute myocardial infarction, but immunoreactive fragments persist

longer (15–19). The central part of cTnI appears to be stable (20). The practical relevance of complex formation, posttranslational cTn modifications, and cTn degradation are that they may lead to changes in the availability of specific epitopes and thus varying recoveries of cTn variants in different cTn assays. Most currently commercially available assays detect intact cTn (free or complex-bound) and a varying mixture of cTn degradation products (21). The novel finding of Katrukha *et al.* (2) nicely demonstrates how heparin treatment or blood collection in heparinized tubes may interfere with cTnT assays by blocking thrombin activity and thrombin mediated proteolysis of cTnT. The currently commercially available cTnT assay, however, utilizes antibodies which target to the aaar 125–147 fragment (21,22) and is not affected by thrombin-mediated cTnT degradation.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Rong-Rong Jing (Center of Laboratory Medicine, Affiliated Hospital of Nantong University, Nantong, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/jlpm.2017.07.06>). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Thygesen K, Mair J, Katus H, et al. Recommendations for the use of cardiac troponin measurement in acute cardiac care. *Eur Heart J* 2010;31:2197-204.
2. Katrukha IA, Kogan AE, Vylegzhanina AV, et al. Thrombin-Mediated Degradation of Human Cardiac Troponin T. *Clin Chem* 2017;63:1094-100.
3. Parmacek MS, Solaro RJ. Biology of the troponin complex in cardiac myocytes. *Prog Cardiovasc Dis* 2004;47:159-76.
4. Katrukha IA. Human cardiac troponin complex. Structure and functions. *Biochemistry (Mosc)* 2013;78:1447-65.
5. Wei B, Jin JP. Troponin T isoforms and posttranscriptional modifications: Evolution, regulation and function. *Arch Biochem Biophys* 2011;505:144-54.
6. Remppis A, Scheffold T, Greten J, et al. Intracellular compartmentation of troponin T: release kinetics after global ischemia and calcium paradox in the isolated perfused rat heart. *J Mol Cell Cardiol* 1995;27:793-803.
7. Bleier J, Vorderwinkler KP, Falkensammer J, et al. Different intracellular compartmentations of cardiac troponins and myosin heavy chains: a causal connection to their different early release after myocardial damage. *Clin Chem* 1998;44:1912-8.
8. Di Lisa F, De Tullio R, Salamino F, et al. Specific degradation of troponin T and I by μ -calpain and its modulation by substrate phosphorylation. *Biochem J* 1995;308:57-61.
9. Feng J, Schaus BJ, Fallavollita JA, et al. Preload induces troponin I degradation independently of myocardial ischemia. *Circulation* 2001;103:2035-7.
10. Piper HM, Schwartz P, Spahr R, et al. Early enzyme release from myocardial cells is not due to irreversible cell damage. *J Mol Cell Cardiol* 1984;16:385-8.
11. Mair J, Morandell D, Genser N, et al. Equivalent early sensitivities of myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T for acute myocardial infarction. *Clin Chem* 1995;41:1266-72.
12. Starnberg K, Jeppsson A, Lindahl B, et al. Revision of the troponin T release mechanism from damaged human myocardium. *Clin Chem* 2014;60:1098-104.
13. Gerhardt W, Nordin G, Herbert AK, et al. Troponin T and I assays show decreased concentrations in heparin plasma compared with serum: lower recoveries in early than in late phases of myocardial injury. *Clin Chem* 2000;46:817-21.
14. Klocke FJ, Copley DP, Krawczyk JA, et al. Rapid renal clearance of immunoreactive canine plasma myoglobin. *Circulation* 1982;65:1522-8.
15. Wu AH, Feng YJ, Moore R, et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. *Clin Chem* 1998;44:1198-208.
16. Katrukha AG, Bereznikova AV, Esakova TV, et al. Troponin I is released in bloodstream of patients with acute myocardial infarction not in free form but as complex. *Clin Chem* 1997;43:1379-85.
17. Labugger R, Organ L, Collier C, et al. Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. *Circulation* 2000;102:1221-6.
18. Peronnet E, Becquart L, Poirier F, et al. SELDI-TOF MS analysis of the Cardiac Troponin I forms present in plasma from patients with myocardial infarction. *Proteomics* 2006;6:6288-99.
19. Streng AS, de Boer D, van Doorn WP, et al. Identification and characterization of cardiac troponin T fragments in serum of patients suffering from acute myocardial infarction. *Clin Chem* 2017;63:563-72.
20. Katrukha AG, Bereznikova AV, Filatov VL, et al. Degradation of cardiac troponin I: implication for reliable immunodetection. *Clin Chem* 1998;44:2433-40.
21. IFCC. Troponin assay analytical characteristics. Available online: <http://www.ifcc.org/ifcc-scientific-division/documents-of-the-sd/troponinassayanalyticalcharacteristics/>
22. Giannitsis E, Kurz K, Hallermayer K, et al. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem* 2010;56:254-61.

doi: 10.21037/jlpm.2017.07.06

Cite this article as: Mair J. What is new on cardiac troponin degradation? *J Lab Precis Med* 2017;2:55.