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Editorial

High-sensitivity cardiac troponin testing during and after ACS: Complexed or not?

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In 1999, the National Academy of Clinical Biochemistry [now known as the American Association for Clinical Chemistry (AACC) Academy], published a document on laboratory recommendations for the use of cardiac biomarkers in coronary artery disease (CAD) [1]. Over these past twenty years, the field has been dominated by cardiac troponin (cTn), because of its reliability of detection and high degree of specificity for the heart. The most recent laboratory recommendations have focused on high-sensitivity cardiac troponin assays (hs-cTn) [2]. From the early clinical and analytical descriptions of the hs-cTn assays nearly a decade ago, there was an appreciation that the low concentrations measured by these assays would enhance detection and enable earlier decision making in patients with possible acute coronary syndrome (ACS) [3,4]. However, there were also concerns that the more sensitive tests could create false positives, leading to unnecessary investigations and interventions [5,6].

In complex, atypical, or unstable patients, clinicians sometimes struggle to differentiate between ACS and other pathologic processes that release cardiac troponin, relying on a combination of clinical presentation, ECG changes, and imaging studies. A tantalizing possibility is that a difference in the forms of cardiac troponin released would allow a biochemical distinction between irreversible infarct versus milder reversible injuries. It has been known since 1998 that cardiac troponin released into the bloodstream is a heterogeneous mixture of complexed and uncomplexed forms [7], as well as proteolyzed and more intact forms [8].

In this issue of *Clinical Biochemistry*, Van Wijk and collaborators explore the possibility that uncomplexed cardiac troponin I (cTnI) is preferentially released from a free cytoplasmic pool in injured but still viable cells [9,10]. In a first, they used high-sensitivity methods to detect total versus complexed forms of cTnI in plasma taken from patients, many of whom had troponin elevations due to non-ACS conditions [9]. The investigators did not consistently deduce free cTnI in any particular population of patients, in agreement with earlier studies indicating that plasma cTnI always exists in a complexed state, either as a binary troponin IC complex or ternary ITC complex [7]. Also, using light microscopy and immunohistochemistry, they could not detect a free cTnI pool in adult cardiomyocytes, though they could detect it in the Golgi/endoplasmic reticulum region of newly differentiated rat myotubes [9]. It is possible that only newly synthesized cTnI is soluble apart from cardiac troponin C (cTnC) and cardiac troponin T (cTnT), while it is still attached to the protein translation machinery or chaperones.

Instead of free cTnI, the Van Wijk team did find elevated concentrations of relatively intact troponin ternary ITC complex earlier in patient clinical courses, consistent with two other studies published in July 2019 [11,12] demonstrating that the amount of troponin ternary ITC complex decreased with time in ACS patients. Moreover, a significant proportion of the early ITC complex was largely intact (early pain onset approximately 6 h post-symptom onset), but at 30 h was largely replaced by highly proteolyzed ternary ITC complex, binary IC complex, and free cTnT fragments (Fig. 1). It is possible that the relatively intact ternary troponin ITC complex represents the sought-after free pool of early-release troponin, rather than cTnI on its own. Certainly, the ternary complex is more soluble than isolated cTnI or cTnT.

Release of sarcomere-bound troponin ternary complex may very well require proteolytic cleavage. Based on studies of tropomyosin binding to cTnT fragments, there is an important tropomyosin binding site located between cTnT residues Ile188 and Arg230, with additional weak binding sites N-terminal to it [13,14]. (Note that alternative splicing of residues 23–32 from the 298- residue form of cTnT leads to different numbering schemes for cTnT. We are using the numbering for the 288-residue cTnT isoform.) Cleavage within this site generates cTnT fragments commonly found in the blood circulation of myocardial infarction patients. Mass spectrometry analysis reveals 16 and 29 kDa cTnT fragments consisting of Ser69-Gln189 and Ser69-Trp287, respectively [15,16]. Cleavage at Gln189-Lys190 might be required to release cTnT from sarcomeric tropomyosin. More often, cTnT is cleaved at Arg68-Ser69 by extracellular thrombin, a well-known protease that is highly present at the infarcted zone, but which is also overwhelmingly activated in the conversion of plasma to serum [17,18].

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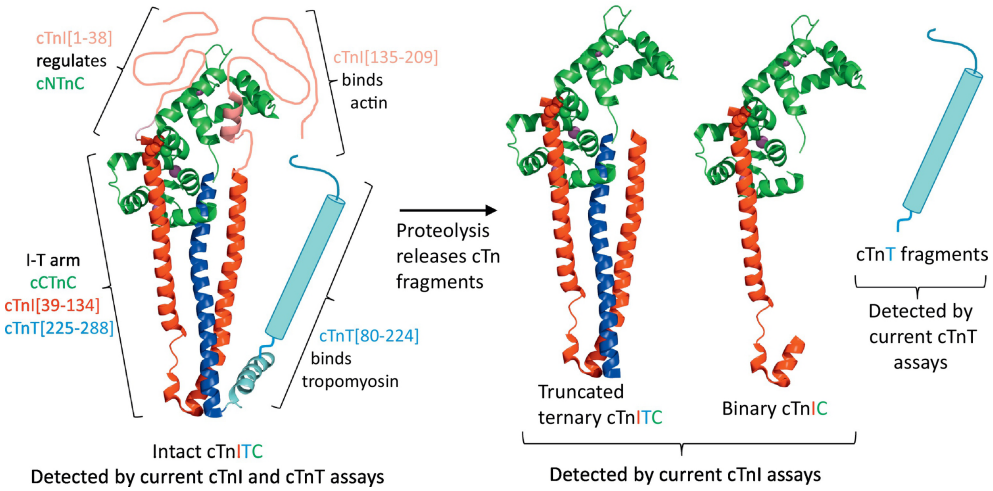

Fig. 1. Intact cardiac troponin complex binds to tropomyosin via cTnT[80-224] and to actin via cTnI[135-209]. Intracellular protease activation associated with cell death cleaves the complex to release truncated ternary complex, binary complex, and N-terminal cTnT fragments. This figure was generated using 1J1E from reference [20]. Regions that were not included in the X-ray crystal structure have been drawn in.

While cTnT is found mainly as free fragments or in cTn ternary complexes, cTnI appears to exist almost exclusively in complex with cTnC, which is not surprising given its very high affinity for cTnC (K_D~1 nM) [19]. cTnI also has a strong tendency to aggregate in the absence of cTnC. The well-structured proteolysis-resistant core of the ternary complex is comprised of the C-terminal domain of cTnC, cTnT[225-288], and cTnI[39-135], which are together known as the I-T arm (Fig. 1) [20]. In the recent Vylegzhanina and colleagues study, binary troponin IC complexes, were shown to possess a very truncated form of cTnI, suggesting that perhaps a proteolytic cleavage within the protease-resistant core of cTnI (cTnI[39-135]) releases cTnT to yield binary IC complex [11] (see Fig. 1). Apart from its protease-resistant core, cTnI has long intrinsically disordered tails at the N- and C-termini (cTnI[1-37] and cTnI[135-209], respectively), both of which are very susceptible to proteolytic cleavage by intracellular matrix metalloproteinase or calpain [21]. Most cTnI fragments found in serum derive from proteolytic cleavages in these N- or C-terminal tails rather than the structured core [22]. Another recent study found that cTnI was more extensively proteolyzed in patients with ACS, particularly those with severe ST-elevation MI versus those with non-ACS conditions [23].

Thus, with the recent expansion in the repertoire of high affinity monoclonal antibodies capable of detecting different epitopes and different complexed forms of cardiac troponin, a more comprehensive picture of cardiac troponin release is beginning to emerge. Early and reversible injury releases relatively intact ternary troponin ITC complexes from a soluble cytoplasmic pool. If the injury progresses to irreversible cell necrosis, then intracellular protease activation results in the release of sarcomere-bound troponin, a mixture of N-terminal cTnT fragments and highly proteolyzed ternary ITC and binary IC complexes. Carefully conducted studies are needed to correct or refine this model further. One major barrier to understanding this process is the lack of universal standardization for cTnI [24], given its many different proteolyzed forms and the many different epitopes that are used in its detection. The issue of large discrepancies in cTnI measurement is only magnified when one considers the many different forms of troponin complexes that include cTnT as well. Another equally challenging issue is the heterogeneity of patient presentations, particularly for non-ACS conditions. Careful and appropriately referenced quantitation of free versus complexed troponin, intact versus proteolyzed troponin, and correlation with clinical presentation are needed to fully understand the different forms of cardiac troponin and how this new information can be used to impact the diagnosis and management of complex cardiac patients. As more sophisticated laboratory algorithms have and are being developed to harness the analytical power of the hs-cTn assays for early risk-stratification and decision-making in the emergency setting [25,26], the ability to detect different cardiac troponin complexes and fragments represents exciting new avenues to explore for improving patient care in the era of high-sensitivity cardiac troponin testing [2].

Declaration of Competing Interest

Dr. Kavsak has received grants/reagents/consultant/advisor/honoraria from the laboratory diagnostic industry, specifically from Abbott Laboratories, Beckman Coulter, Ortho Clinical Diagnostics, Randox Laboratories, Roche Diagnostics and Siemens Healthcare Diagnostics. McMaster University has filed patents with Dr. Kavsak listed as an inventor in the acute cardiovascular biomarker field. Dr. Mingels has received reagents from Abbott Laboratories and Roche Diagnostics.

References

[1] A.H. Wu, F.S. Apple, W.B. Gibler, R.L. Jesse, M.M. Warshaw, R. Valdes Jr., National Academy of Clinical Biochemistry standards of laboratory practice: recommendations for the use of cardiac markers in coronary artery diseases, Clin. Chem. 45 (7) (1999, Jul) 1104–1121.

[2] A.H.B. Wu, R.H. Christenson, D.N. Greene, A.S. Jaffe, P.A. Kavsak, J. Ordonez-Llanos, F.S. Apple, Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion from the academy of the American Association for Clinical Chemistry and the task force on clinical applications of cardiac bio-markers of the International Federation of Clinical Chemistry and Laboratory Medicine, Clin. Chem. 64 (2018) 645–655.

[3] P.A. Kavsak, A.R. MacRae, M.J. Yerna, A.S. Jaffe, Analytic and clinical utility of a next-generation, highly sensitive cardiac troponin I assay for early detection of myocardial injury, Clin. Chem. 55 (3) (2009, Mar) 573–577.

[4] T. Reichlin, W. Hochholzer, S. Bassetti, S. Steuer, C. Stelzig, S. Hartwiger, S. Biedert, N. Schaub, C. Buerge, M. Potocki, M. Noveanu, T. Breidthardt, R. Twerenbold, K. Winkler, R. Bingisser, C. Mueller, Early diagnosis of myocardial infarction with sensitive cardiac troponin assays, N. Engl. J. Med. 361 (9) (2009, Aug 27) 858–867.

[5] A. Mingels, L. Jacobs, E. Michielsen, J. Swaanenburg, W. Wodzig, M. van Dieijen-Visser, Reference population and marathon runner sera assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and I assays, Clin. Chem. 55 (1) (2009, Jan) 101–108.

[6] K. Thygesen, J.S. Alpert, A.S. Jaffe, B.R. Chaitman, J.J. Bax, D.A. Morrow, H.D. White, ESC Scientific Document Group, et al., Eur. Heart J. 40 (3) (2019, Jan 14) 237–269.

[7] A.H. Wu, Y.J. Feng, R. Moore, F.S. Apple, P.H. McPherson, K.F. Buechler, G. Bodor, 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. 44 (6 Pt 1) (1998, Jun) 1198–1208.

[8] A.G. Katrukha, A.V. Bereznikova, V.L. Filatov, T.V. Esakova, O.V. Kolosova, K. Pettersson, T. Lovgren, T.V. Bulargina, I.R. Trifonov, N.A. Gratsiansky, K. Pulkki, L.M. Voipio-Pulkki, N.B. Gusev, Degradation of cardiac troponin I: implication for reliable immunodetection, Clin. Chem. 44 (1998) 2433–2440.

[9] X.M.R. van Wijk, S. Claassen, N.S. Enea, P. Li, S. Yang, M.A. Brouwer, G.E. Cramer, R. Zuk, K.L. Lynch, A.H.B. Wu, Cardiac troponin I is present in plasma of type 1 myocardial infarction patients and patients with troponin I elevations due to other etiologies as complex with little free I, Clin. Biochem. (2019, Jun 25) (pii: S0009-9120(19)30034-7).

[10] P.E. Hickman, J.M. Potter, C. Aroney, G. Koerbin, E. Southcott, A.H. Wu, M.S. Roberts, Cardiac troponin may be released by ischemia alone, without necrosis, Clin. Chim. Acta 411 (2010) 318–323.

[11] A.V. Vylegzhanina, A.E. Kogan, I.A. Katrukha, E.V. Koshkina, A.V. Bereznikova, V.L. Filatov, M.N. Bloshchitsyna, A.P. Bogomolova, A.G. Katrukha, Full-size and partially truncated cardiac troponin complexes in the blood of patients with acute myocardial infarction, Clin. Chem. 65 (7) (2019, Jul) 882–892.
[12] S.A.J. Damen, W.H.M. Vroemen, M.A. Brouwer, S.T.P. Mezger, H. Suryapranata, N. van Royen, O. Bekers, S.J.R. Meex, W.K.W.H. Wodzig, F.W.A. Verheugt, D. de Boer, G.E. Cramer, A.M.A. Mingels, Multi-site coronary vein sampling study on cardiac troponin T degradation in non-ST-segment-elevation myocardial infarction: toward a more specific cardiac troponin T assay, J. Am. Heart Assoc. 8 (14) (2019, Jul 16) e012602.
[13] J.P. Jin, S.M. Chong, Localization of the two tropomyosin-binding sites of troponin T, Arch. Biochem. Biophys. 500 (2010) 144–150.
[14] A.S. Streng, D. de Boer, J. van der Velden, M.P. van Dieijen-Visser, W.K. Wodzig, Posttranslational modifications of cardiac troponin T: an overview, J. Mol. Cell. Cardiol. 63 (2013, Oct) 47–56.
[15] E.P. Cardinaels, A.M. Mingels, T. van Rooij, P.O. Collinson, F.W. Prinzen, M.P. van Dieijen-Visser, Time-dependent degradation pattern of cardiac troponin T following myocardial infarction, Clin. Chem. 59 (7) (2013, Jul) 1083–1090.
[16] A.S. Streng, D. de Boer, W.P. van Doorn, F.G. Bouwman, E.C. Mariman, O. Bekers, M.P. van Dieijen-Visser, W.K. Wodzig, Identification and characterization of cardiac troponin T fragments in serum of patients suffering from acute myocardial infarction, Clin. Chem. 63 (2) (2017, Feb) 563–572.
[17] A.S. Streng, D. de Boer, W.P. van Doorn, J.M. Kocken, O. Bekers, W.K. Wodzig, Cardiac troponin T degradation in serum is catalysed by human thrombin, Biochem. Biophys. Res. Commun. 481 (1–2) (2016, Dec 2) 165–168.
[18] I.A. Katrukha, A.E. Kogan, A.V. Vylegzhanina, M.V. Serebryakova, E.V. Koshkina, A.V. Bereznikova, A.G. Katrukha, Thrombin-mediated degradation of human cardiac troponin T, Clin. Chem. 63 (6) (2017, Jun) 1094–1100.
[19] M.X. Li, P.M. Hwang, Structure and function of cardiac troponin C (TNNC1): implications for heart failure, cardiomyopathies, and troponin modulating drugs, Gene 571 (2015) 153–166.
[20] S. Takeda, A. Yamashita, K. Maeda, Y. Maeda, Structure of the core domain of human cardiac troponin in the Ca(2+)-saturated form, Nature 424 (2003) 35–41.
[21] Z. Mahmud, S. Zahran, P.B. Liu, B. Reiz, B.Y.H. Chan, A. Roczkowsky, C.E. McCartney, P.L. Davies, L. Li, R. Schulz, P.M. Hwang, Structure and proteolytic susceptibility of the inhibitory C-terminal tail of cardiac troponin I, Biochim. Biophys. Acta Gen. Subj. 1863 (2019) 661–671.
[22] I.A. Katrukha, A.E. Kogan, A.V. Vylegzhanina, A.V. Kharitonov, N.N. Tamm, V.L. Filatov, A.V. Bereznikova, E.V. Koshkina, A.G. Katrukha, Full-size cardiac troponin I and its proteolytic fragments in blood of patients with acute myocardial infarction: antibody selection for assay development, Clin. Chem. 64 (2018) 1104–1112.
[23] S. Zahran, V.P. Figueiredo, M.M. Graham, R. Schulz, P.M. Hwang, Proteolytic digestion of serum cardiac troponin I as marker of ischemic severity, J. Appl. Lab. Med. 3 (2018) 450–455.
[24] J.R. Tate, D.M. Bunk, R.H. Christenson, J.H. Barth, A. Katrukha, J.E. Noble, H. Schimmel, L. Wang, M. Panteghini, Evaluation of standardization capability of current cardiac troponin I assays by a correlation study: results of an IFCC pilot project, Clin. Chem. Lab. Med. 53 (2015) 677–690.
[25] P.A. Kavsak, J.T. Neumann, L. Cullen, M. Than, C. Shortt, J.H. Greenslade, J.W. Pickering, F. Ojeda, J. Ma, N. Clayton, J. Sherbino, S.A. Hill, M. McQueen, D. Westermann, N.A. Sörensen, W.A. Parsonage, L. Griffith, S.R. Mehta, P.J. Devereaux, M. Richards, R. Troughton, C. Pemberton, S. Aldous, S. Blankenberg, A. Worster, Clinical chemistry score versus high-sensitivity cardiac troponin I and T tests alone to identify patients at low or high risk for myocardial infarction or death at presentation to the emergency department, CMAJ 190 (33) (2018, Aug 20) E974–E984.
[26] J.T. Neumann, R. Twerenbold, F. Ojeda, N.A. Sörensen, A.R. Chapman, A.S.V. Shah, A. Anand, J. Boeddinghaus, T. Nestelberger, P. Badertscher, A. Mokhtari, J.W. Pickering, R.W. Troughton, J. Greenslade, W. Parsonage, M. Mueller-Hennessen, T. Gori, T. Jernberg, N. Morris, C. Liebetrau, C. Hamm, H.A. Katus, T. Münzel, U. Landmesser, V. Salomaa, L. Iacoviello, M.M. Ferrario, S. Giampaoli, F. Kee, B. Thorand, A. Peters, R. Borchini, T. Jørgensen, S. Söderberg, S. Sans, H. Tunstall-Pedoe, K. Kuulasmaa, T. Renné, K.J. Lackner, A. Worster, R. Body, U. Ekelund, P.A. Kavsak, T. Keller, B. Lindahl, P. Wild, E. Giannitsis, M. Than, L.A. Cullen, N.L. Mills, C. Mueller, T. Zeller, D. Westermann, S. Blankenberg, Application of high-sensitivity troponin in suspected myocardial infarction, N. Engl. J. Med. 380 (26) (2019, Jun 27) 2529–2540.

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