The biological basis of troponin in heart disease: possible uses for troponin fragmentology

Vlad C. Vasile and Allan S. Jaffe
Department of Internal Medicine, Division of Cardiovascular Diseases and Department of Laboratory Medicine and Pathology, Mayo Clinic and Mayo Medical School, Rochester, Minnesota, USA

Correspondence: Dr Allan S. Jaffe, Mayo Clinic, 200 First St SW, Division of Cardiovascular Diseases, Gonda 5, Rochester, Minnesota 55905, USA.
Tel: +1 507 284 3680; fax: +1 507 266 0228; e-mail: Jaffe.Allan@mayo.edu

Conflict of interest: Dr Vasile, None; Dr Jaffe is a consultant and has received research support from Siemens and Beckman-Coulter. He is currently a consultant, or has consulted over time, for most of the major diagnostic companies.

Abstract

This article describes the molecular biology of troponin in heart disease. Prospective uses for this biomarker are mentioned, with a particular emphasis on cardiac troponin fragments that could be associated with distinct clinical entities. The article explores the topic of specific cardiac troponin fragments resulting from modification or degradation, linked with different pathological processes, as indications of novel potential uses for this biomarker in heart disease. In addition, it addresses some implications that could have an impact on several circumstances that confront physicians within clinical practice.
■ *Heart Metab.* 2009;43:5–8.

Keywords: Troponin fragments, phosphorylation, dephosphorylation

Introduction

Detection of myocardial injury relies on the highly sensitive and specific determination of cardiac troponin (cTn). Thus increased cTn is essential for the diagnosis of acute myocardial infarction (AMI) [1]. However, there may be other specific applications for cTn fragments, and increases in cTn concentrations are observed in conditions other than AMI.

The troponin complex consists of three subunits: troponin C (cTnC), troponin I (cTnI) and troponin T (cTnT); it interacts with components of the thin tropomyosin and actin filament to ensure correct contraction coupling [2]. Several isoforms of cTns C, I, and T assist in this task [3].

Troponin C (TnC) lacks cardiac specificity. In contrast, it is believed that all isoforms of cTnI are expressed exclusively in cardiomyocytes, thus its detection in the blood is synonymous with myocardial injury. The cTnI isoforms are characterized by a 32 amino acid posttranslational tail at the N-terminus [4]. The junction between this sequence and the stable central part of the molecule is the target for monoclonal antibodies that recognize cTnI [5]. Expression of troponin T or I is controlled by three genes [6]. By alternative splicing, several isoforms of cTnT are generated, with sequence variability regions located at the C- or the N-terminus, or both [6,7].

Conditions other than AMI in which cTn concentrations are increased

Physical exercise

Transient increases in cTnT occur after severe physical exercise, early during exertion [8]. Initially, it was

Basic article

Vlad C. Vasile and Allan S. Jaffe

believed that these increases resolved in 24 h, but recent data from sensitive assays do not support this [9]. It is difficult to distinguish whether cTn is released by reversible or irreversible injury. Similarly, whether there are reparative processes is unclear. Either way, different fragments might be elaborated in this situation.

Chronic renal failure

Concentrations of cTnT are often abnormal in patients with chronic renal failure [10]. The etiology of these increases is obscure, but probably includes any of: endothelial dysfunction, acute cardiac stretch, intradialysis hypotension and hypertension, left ventricular hypertrophy, and coronary artery disease, occult or overt. Some authors report small cTn fragments [11] but others dispute that [12].

Myocardial stunning

Myocardial stunning is a reversible myocardial dysfunction seen after ischemia. It also can be induced by arrhythmias or open heart surgery [13]. A specific degradation product occurs in this circumstance from the loss of 17 residues at the C-terminus of cTnI [14]. Transgenic animals overexpressing this truncated form, $cTnI_{1-193}$, exhibit myocardial dysfunction with properties similar to those of myocyte stunning. A comparable fragment was found after bypass surgery [15]. In in-vitro experiments, transfection of truncated $cTnI_{1-193}$ produces a diastolic impairment in healthy human cardiomyocytes [16]. This suggests that the degradation of cTnI contributes to diastolic dysfunction in vivo. If these cTn fragments were causative, they could be monitored to identify which patients with impaired function might improve.

Transient left ventricular apical ballooning syndrome

This syndrome can mimic AMI clinically [17]. It is characterized by more extensive ventricular dysfunction than would be expected from the modest increases observed in cTn values, which are good surrogates for infarct size [18,19]. Phosphorylated or truncated cTn may be the basis for this dysfunction.

Reperfusion in acute myocardial infarction

Reperfusion in AMI can be induced by thrombolytic therapy or percutaneous coronary angioplasty. It may increase the extent of cardiac injury [20]. Under hypoxia, modifications of cTn may be specifically associated with this ''ischemia-reperfusion'' process [21]. Patients with larger myocardial infarctions display greater amounts of degraded cTn fragments. It is possible that the proteases involved in clot dissolution or those implicated in induction of inflammation in response to injury could degrade cTns. The biochemical environment of the myocardium might be different than that of blood, leading to different cTn changes. Detectable changes might be useful in distinguishing more from less robust recanalization.

Acute myocarditis

Acute myocarditis can present similarly to AMI [22]. Increases in cTnI occur in mouse models of autoimmune myocarditis [23] and in patients with heart failure secondary to chronic myocarditis. Presumably, inflammation leads to myocardial injury. It may be that fragments produced secondary to inflammation are different from those triggered by ischemia. Recognition of dissimilarities might permit differentiation of distinct disease states in clinical practice. Moreover, cTn may play a part in inflammation itself [24].

Basic molecular biology on which fragmentology is based

The regulation of contraction physiology is complex and includes abundant phosphorylation and dephosphorylation steps within troponin and actin-tropomyosin. cTnI is phosphorylated at specific serine/threonine residues by protein kinase A (PKA) [25]. The extent of phosphorylation of cTnI and cTnT depends on these kinases and phosphatases, but also on their spatial conformation, which confers accessibility to phosphorylation sites. Posttranslational phosphorylation of cTnI/cTnT by processes that block or reveal specific sites may also change the status of cTn. Physiologic degradation occurs at the N-terminus; residues 27, 28 or 31 seem to possess key significance.

During ischemia, protein kinase C (PKC) and p21-activated kinase induce cTn phosphorylation [26]. Proteolysis occurs initially at the C-terminus, at amino acid 192, followed by additional cleavages. Truncation of cTnI at amino acid residue 192 is associated with human disease [13,14]. The alteration of cTnT/cTnI by proteolysis, phosphorylation, or both, generates an assortment of fragments (*Table I*).

The majority of cTnI interacts with TnC, and cTn subunits or serum proteins [27]. In patients with AMI, a proportion of the cTnI circulates bound to cTnC along with complexes of cTnsT, I and C. During ischemia, truncation of the N-terminus eliminates PKA phosphorylation sites and sites involved in cTnI–TnC interaction. Thus degradation and phosphorylation also influence circulating fragments. The detection of these isoforms could elucidate specific etiologies for cardiac disease.

One pool of cTn corresponds to a ''cytosolic'' localization. Such localization is based on solubility

Table I. Modified forms of cTnI.

Condition	Type of modification	Amino-acid position	Enzyme
Physiological	Phosphorylation	N-terminus	Increased concentrations of protein kinase A
Physiological	Proteolysis	N-terminus (starting at position 27, 28, or 31)	Proteases
Pathological	Phosphorylation	C-terminus	Reduced concentrations of protein kinase A
Pathological	Phosphorylation	C-terminus	Protein kinase C
Pathological	Phosphorylation	C-terminus	P21-activated kinase
Pathological	Proteolysis	C-terminus (ends at 192)	Proteases
Pathological	Proteolysis	N-terminus (ends at 63)	Proteases
Pathological	Proteolysis	N-terminus (ends at 73)	Proteases

studies; thus a better term might be "early-releasable pool." The other pool is believed to be structurally bound. Only 4% of cTnI and 5% of cTnT are found in the cytosolic compartment [28]. Presumably, the initial release of troponin derives from the cytosolic pool, whereas the persistent increases are from degradation of the structural compartment. Patients with renal failure demonstrate the same cTn clearance curves [29], suggesting that the persistence of cTn is not as a result of delayed elimination. During reperfusion, the early peak in cTnT occurs at 14 h after the onset of pain [22], probably from the cytosolic pool. This peak is absent in patients reperfused later than 5.5 h after the onset of pain and in patients with AMI who do not undergo reperfusion [28]. The cytosolic compartment of the unbound cTnT and cTnI is responsible for this initial peak in patients with early reperfused AMI, and seems to be composed mostly of free chains [30]. Persistent increases in cTn concentration are attributed to lysosomal degradation of the "structural" pool of cTn during infarct remodeling and collagen deposition [31]. Increases in cTn in pulmonary embolism resolve by 40 h in the absence of current emboli. The short duration of cTn in the bloodstream after acute exertion and pulmonary embolism might be explained by the cTn released from the cytosolic compartment rather than from the structural pool; the latter would be affected only if necrosis occurs, if it is extensive enough for detection, or by changes in the fragments released.

Conclusion

The complex biology of cTns should allow for the development of novel assays able to discriminate various fragments that may be elaborated selectively within distinct pathological processes. These fragments resulting from metabolism or phosphorylation have the potential for both diagnostic and therapeutic importance. ■

REFERENCES

1. Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *J Am Coll Cardiol.* 2007;50:2173–2195.
2. Takeda S, Yamashita A, Maeda K, Maéda Y. Structure of the core domain of human cardiac troponin in the Ca(2+)-saturated form. *Nature.* 2003;424:35–41.
3. Dhoot GK, Perry SV. Distribution of polymorphic forms of troponin components and tropomyosin in skeletal muscle. *Nature.* 1979;278:714–718.
4. Perry SV. Troponin I: inhibitor or facilitator. *Mol Cell Biochem.* 1999;190:9–32.
5. Katrukha A. Antibody selection strategies in cardiac troponin assay. In: *Cardiac Markers,* 2nd ed. Edited by Wu H. Totowa, NJ: Humana Press; 2003. pp. 173–185.
6. Perry SV. Troponin T: genetics, properties and function. *J Muscle Res Cell Motil.* 1998;19:575–602.
7. Gahlmann R, Troutt AB, Wade RP, Gunning P, Kedes L. Alternative splicing generates variants in important functional domains of human slow skeletal troponin T. *J Biol Chem.* 1987;262:16122–16126.
8. Middleton N, George K, Whyte G, Gaze D, Collinson P, Shave R. Cardiac troponin T release is stimulated by endurance exercise in healthy humans. *J Am Coll Cardiol.* 2008;52:1813–1814.
9. Mingels A, Jacobs L, Michielsen E, Swaanenburg J, Wodzig W, van Dieijen-Visser M. Reference population and marathon runner sera assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and I assays. *Clin Chem.* 2009;55:101–108.
10. Freda BJ, Tang WH, Van Lente F, Peacock WF, Francis GS. Cardiac troponins in renal insufficiency: review and clinical implications. *J Am Coll Cardiol.* 2002;40:2065–2071.
11. Diris JH, Hackeng CM, Kooman JP, Pinto YM, Hermens WT, van Dieijen-Visser MP. Impaired renal clearance explains elevated troponin T fragments in hemodialysis patients. *Circulation.* 2004;109:23–25.
12. Fahie-Wilson MN, Carmichael DJ, Delaney MP, Stevens PE, Hall EM, Lamb EJ. Cardiac troponin T circulates in the free, intact form in patients with kidney failure. *Clin Chem.* 2006;52:414–420.
13. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev.* 1999;79:609–634.

14. Van Eyk JE, Powers F, Law W, Larue C, Hodges RS, Solaro RJ. Breakdown and release of myofilament proteins during ischemia and ischemia/reperfusion in rat hearts: identification of degradation products and effects on the pCa-force relation. *Circ Res*. 1998;82:261–271.

15. McDonough JL, Labugger R, Pickett W, et al. Cardiac troponin I is modified in the myocardium of bypass patients. *Circulation*. 2001;103:58–64.

16. Narolska NA, Piroddi N, Belus A, et al. Impaired diastolic function after exchange of endogenous troponin I with C-terminal truncated troponin I in human cardiac muscle. *Circ Res*. 2006;99:1012–1020.

17. Tsuchihashi K, Ueshima K, Uchida T, et al. Transient left ventricular apical ballooning without coronary artery stenosis: a novel heart syndrome mimicking acute myocardial infarction. Angina Pectoris-Myocardial Infarction Investigations in Japan. *J Am Coll Cardiol*. 2001;38:11–18.

18. Steen H, Giannitsis E, Futterer S, Merten C, Juenger C, Katus HA. Cardiac troponin T at 96 hours after acute myocardial infarction correlates with infarct size and cardiac function. *J Am Coll Cardiol*. 2006;48:2192–2194.

19. Vasile VC, Babuin L, Giannitsis E, Katus HA, Jaffe AS. Relationship of MRI-determined infarct size and cTnI measurements in patients with ST-elevation myocardial infarction. *Clin Chem*. 2008;54:617–619.

20. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest*. 1985;76:1713–1719.

21. Li G, Martin AF, Solaro JR. Localization of regions of troponin I important in deactivation of cardiac myofilaments by acidic pH. *J Mol Cell Cardiol*. 2001;33:1309–1320.

22. Assomull RG, Lyne JC, Keenan N, et al. The role of cardiovascular magnetic resonance in patients presenting with chest pain, raised troponin, and unobstructed coronary arteries. *Eur Heart J*. 2007;28:1242–1249.

23. Smith SC, Ladenson JH, Mason JW, Jaffe AS. Elevations of cardiac troponin I associated with myocarditis. Experimental and clinical correlates. *Circulation*. 1997;95:163–168.

24. Goser S, Andrassy M, Buss SJ, et al. Cardiac troponin I but not cardiac troponin T induces severe autoimmune inflammation in the myocardium. *Circulation*. 2006;114:1693–1702.

25. Mittmann K, Jaquet K, Heilmeyer LM Jr. Ordered phosphorylation of a duplicated minimal recognition motif for cAMP-dependent protein kinase present in cardiac troponin I. *FEBS Lett*. 1992;302:133–137.

26. Buscemi N, Foster DB, Neverova I, Van Eyk JE. p21-activated kinase increases the calcium sensitivity of rat triton-skinned cardiac muscle fiber bundles via a mechanism potentially involving novel phosphorylation of troponin I. *Circ Res*. 2002;91:509–516.

27. 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;44:1198–1208.

28. Katus HA, Remppis A, Scheffold T, Diederich KW, Kuebler W. Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. *Am J Cardiol*. 1991;67:1360–1367.

29. Ellis K, Dreisbach AW, Lertora JL. Plasma elimination of cardiac troponin I in end-stage renal disease. *South Med J*. 2001;94:993–996.

30. Atar D, Madsen LH, Labugger R, VanEyk JE. Molecular modifications of troponin I and T detected in serum from patients with acute myocardial infarction. *Ugeskr Laeger*. 2003;165:116–120.

31. Jugdutt BI. Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? *Circulation*. 2003;108:1395–1403.