Chapter 10
Cardiac Muscle and the Troponins

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Abstract The cardiac troponins (cTns) are structural proteins of the cardiac myocyte contractile apparatus. When measured in circulating blood, the cardiac troponins are sensitive and specific indicators of cardiac myocyte necrosis. The elevation of cardiac troponins without necrosis remains controversial. Understanding of the troponins now includes recognition of the role of posttranslational modifications of troponin T and troponin I in the modulation of cardiac muscle contraction and overall physiology. The assays currently available for detection of troponins in the circulation are immunologically based, using antibodies to a number of epitopes of troponin I and T. Posttranslational modifications, proteolysis, and single nucleotide polymorphisms may affect the ability of the assay antibodies to recognize epitopes on the circulating troponins. The focus of this chapter is an overview of the biology of the cTns, their role in cardiac muscle contraction, and the detection of cardiac troponins with commercially available assays.

Keywords Cardiac troponin • Troponin • Troponin I • Troponin T • Cardiac biomarker • Biomarkers of myocardial damage

10.1 Introduction

The cardiac troponins (cTns) are a complex of three protein subunits, troponin C (TnC, the Ca^{2+}-binding subunit), troponin I (TnI, the inhibitory subunit), and troponin T (TnT, the tropomyosin-binding subunit). The complex as a whole provides physiologic modulation of calcium sensitivity and cardiac myocyte contraction.

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Circulating cardiac troponins are the gold standard clinical chemistry test for diagnosing myocardial infarction as recommended by the European and American Cardiology Societies in 2000 and again in 2007 and 2012 [1–3].

Although an elevated cTn is sensitive and specific for cardiac myonecrosis, it is not specific for myocardial infarction (MI) (i.e., cellular injury related to myocardial ischemia) [4]. Cardiac troponin can also be elevated in other conditions such as heart failure, pulmonary embolus, hypertrophic cardiomyopathy, hypertension with or without left ventricular hypertrophy, renal failure, severe anemia, strenuous exercise, and sepsis [3]. In general, cTn elevation, regardless of the etiology, conveys a worse prognosis.

The focus of the chapter is an overview of the biology of the cardiac troponins (cTns) and their role in cardiac muscle contraction. In addition, this chapter also discusses the evolution of cTn assays and factors that may affect detection.

10.2 Comparison of Cardiac and Skeletal Muscle

Muscle is classified into three categories: smooth (nonstriated), striated skeletal, and striated cardiac muscle. Striated cardiac muscle can be further categorized into atrial muscle, ventricular muscle, and specialized muscle of the cardiac conduction system. The specialized muscles of the conduction system have few contractile fibers and, therefore, limited contractility. The contraction of the heart muscle is influenced by numerous factors, including the intrinsic properties of the different cardiac muscle types, the isoforms, and the variations and modifications in any and all of the muscle proteins [5].

The skeletal and cardiac muscle have similarities of structure, function, and microscopic appearance. Both skeletal and cardiac muscle cells have myofibrils, arranged in parallel fashion. The cardiac muscle fibers have extensively branching networks, whereas the skeletal muscle fibers primarily run in parallel throughout the length of the muscle. Both muscle types have interdigitating thick and thin filaments arranged in the repeating unit of the sarcomere. Striated skeletal muscle is multinucleate, while cardiac muscle cells are mononucleate. Both muscle types contain sarcoplasm (cytoplasm), sarcoplasmic reticulum, and a T-tubule system formed by invaginations of the sarcolemma.

Because the heart muscle beats continuously, there is a constant demand for energy. The cytoplasm surrounding the cardiac myofibrils contains a high density of mitochondria, comprising 25 % of the cellular volume. Unlike skeletal muscle, the heart contracts involuntarily in a rhythmic fashion and cannot draw upon different types and groups of muscle fibers to meet metabolic demands. Cardiac muscle contraction is brief, and relaxation must occur after each contraction.

Other unique properties of cardiac muscle include automaticity and intercalated disks. Automaticity is the ability of cardiac myocytes to beat spontaneously even when denervated. Intercalated disks are a connection and communication system containing three intercellular adhesion structures: fascia adherens junctions,

desmosomes, and gap junctions. The intercalated discs allow for the rapid transmission of the action potential. This rapid communication system facilitates coordinated contraction of the heart, permits diffusion of ions and small molecules, and allows for coupling of electrical and metabolic activity [6, 7].

10.3 The Sarcomere

The sarcomere, illustrated in Fig. 10.1, is the functional unit of striated muscle. These units are arranged in series, creating the myofibrils. The striated microscopic appearance is due to the alternating A bands (thick filaments) and I bands (thin filaments). Thick filaments are composed of two myosin heavy chains (MHC) complexed with two molecules each of myosin light chains (MLC-1, essential light

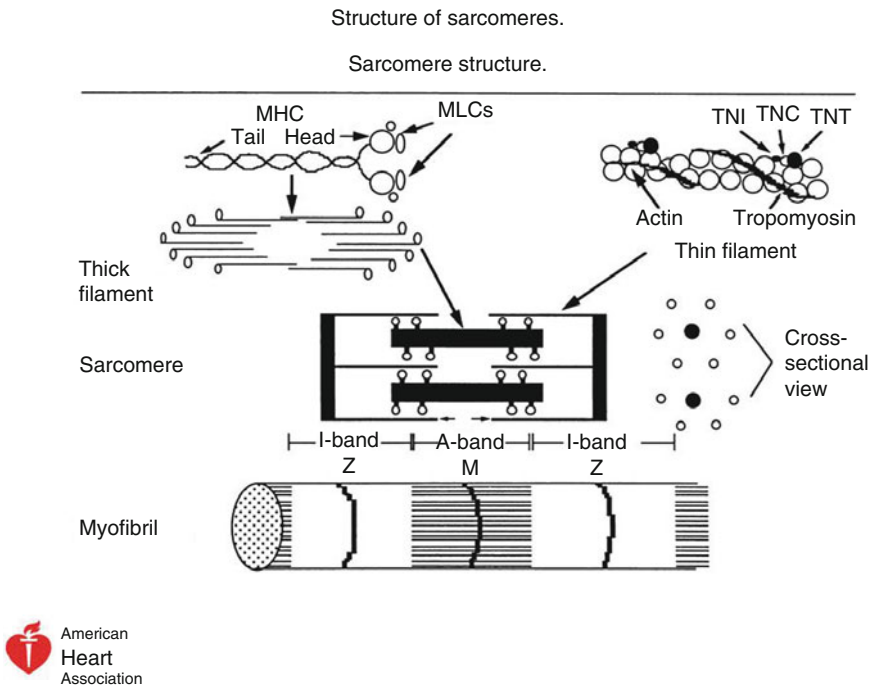


Fig. 10.1 The schematic illustrates the main structural components of a striated muscle cell sarcomere. Thin filaments (I bands) are composed of actin monomers and tropomyosin. The thick filaments (A bands) are composed of two myosin chains complexed with myosin light chains. The striated microscopic appearance of the sarcomere is due to alternating A bands and I bands. The Z lines, forming the lateral boundaries of the sarcomere, are comprised primarily of α-actinin and other proteins. The M-band in the middle of the sarcomere is formed of cross-connecting elements of the cytoskeleton. Myosin filaments are cross-linked by myomesin (This figure which originally appeared in Marian and Roberts [8] is used here with permission)

chain, and MLC-2, regulatory light chain). Thin filaments are composed of Tm and actin. The actin monomers polymerize into a double-helical structure longitudinally oriented around myosin. The tropomyosin molecule is arranged end to end to form a continuous strand along the actin filament. The cardiac troponin complex is bound via tropomyosin to every seven actin monomers in a 7:1:1:1 actin-tropomyosin-troponin ratio [9]. Tropomyosin binds to seven actin monomers in the thin filament and overlaps adjacent Tm molecules. The Z lines are the lateral boundaries of the sarcomere, comprised of a backbone of layers of α-actinin and other associated proteins, that facilitate interactions of actin filaments, titin molecules, and other elements. The T-tubule system communicates with the sarcoplasmic reticulum, assists in transmitting the action potential across the cell, and interdigitates with the Z lines. Costameres link the Z band to the sarcolemma [10]. The M-band, located in the middle of the sarcomere, is formed of cross-connecting elements of the cytoskeleton. The myosin filaments interact with the M-band protein, myomesin [11]. A three-filament model of muscle contraction has been proposed that includes the giant protein titin in addition to the thin and thick filaments. Titin, sometimes referred to as the third filament system, spans half the sarcomere from the Z-disk to the M-band as a springlike filament and is thought to provide the elastic properties of the sarcomere, among other possible functions [12].

10.4 Biology of the Cardiac Troponins

The cTns are a complex of three protein subunits, troponin C (TnC, the calcium-binding subunit), troponin I (TnI, the inhibitory subunit), and troponin T (TnT, the tropomyosin-binding subunit), arranged in a 1:1:1 stoichiometric ratio. This complex plays a role in the physiologic modulation of calcium sensitivity and cardiac myocyte contraction.

As structural components of the cardiac myocyte, cTns are well conserved across species, making them a “translational biomarker” in both veterinary and human medicine and a useful indicator of drug-related cardiac damage in biomedical research. Different isoforms, alternative splicing, mutations, and posttranslational modifications to the troponins have been associated with the function and health of the heart as well as various pathologic conditions such as cardiomyopathies (Chap. 1).

Troponin C Cardiac TnC is an 18 kDa EF-hand calcium-binding protein with two lobular domains, each of which has two Ca^{2+}-binding sites. Each of the four EF-hands has two α-helices between which is a Ca^{2+}-binding loop. There is an additional α-helix at the N-terminal (N-helix). The N-terminal binding sites control the regulatory function of the subunit. The same isoform of TnC is expressed in cardiac and slow skeletal muscle (ssTnC) and is not specific for the heart (c/ssTnC). Nonconservative amino acid substitutions in the first EF-hand of c/ss TnC impede calcium binding [13–15, 16, 18].

Troponin I Cardiac TnI, a 24 kDa protein, was named for its inhibition of actin-activated myosin ATPase activity [22]. In humans, cardiac troponin I is produced from the TNNI3 gene on chromosome 19. When intracellular Ca^{2+} is low, the inhibitory region of cTnI binds to actin, inhibiting muscle contraction. Binding of Ca^{2+} to TnC increases as the intracellular concentration of Ca^{2+} increases. This induces a conformational change that increases the affinity of TnC for cTnI. Conformational changes to cTnI allow the molecule to alternate binding between TnC and actin in response to intracellular calcium concentrations [19]. Both cTnI and ssTnI are detectable throughout fetal development, but the skeletal isoform is predominant during that time period. There is a transition to expression of cTnI in the heart during the first nine postnatal months. Some data suggest that this isoform shift from fetal to adult may have functional implications for the heart. Hearts with congenital malformations may have a different time frame of isoform transition [20, 21].

Cardiac TnI has six distinct functional regions including: (1) an N-terminal extension, $cTnI_{(1–30)}$, unique to the cardiac isoform, containing phosphorylation sites Ser-23 and Ser-24; (2) the N-terminal, $cTnI_{(34–71)}$, a region that provides contact with troponin C; (3) $cTnI_{(80–136)}$ that binds to C-terminal regions of cTnT; (4) the overlapping inhibitory region, $cTnI_{(128–147)}$, that binds cTnC and actin-tropomyosin; (5) switch or triggering region, $cTnI_{(147–163)}$; and (6) the C-terminal domain that binds actin-tropomyosin, $cTnI_{164\text{-}210}$ [17]. Residues 42–136 are sometimes referred to as part of the IT arm [18].

In addition to regulating the actin-myosin interaction, regions of the cTnI subunits as well as specific modifications to those regions have been shown to have additional regulatory control over cardiac function. The inhibitory region of cTnI contains Thr-143, a major phosphorylation site, which may affect velocity of shortening and is also implicated in the posttranslational modifications of cardiac troponins associated with hypertrophy and subsequent heart failure [22]. The phosphorylation sites in the NH_2-terminal extension are targets for a number of protein kinases (PK), including protein kinase A (PKA). Under ß-adrenergic stimulation, the phosphorylation of Ser-23/Ser-24 decreases the Ca^{2+} affinity of the cTn complex and increases the rate of myocardial relaxation [23, 24]. Protein kinase C may phosphorylate cTnI at Ser-23/Ser-24, Ser-43/Ser-45, and Thr-144. Phosphorylation at Thr-144 appears to depress cooperative activation of the thin filament [25]. The process of dephosphorylation is little understood at this time. The N-terminal extension of cTnI may be removed, posttranslationally, due to hemodynamic stress or ß-adrenergic deficiency. This truncation removes the Ser 23/Ser-24 phosphorylation sites; however, the core structure of cTnI remains functional. Genetically modified mice with this alteration are viable through adulthood. The truncation of the N-terminal seems to increase the rate of relaxation of the ventricular muscle, increasing tolerance to decreased preload, an effect similar to phosphorylation of Ser 23/Ser-24 [24, 26–28].

Troponin T Cardiac TnT, a 37 kDa protein, the largest of the three subunits, has diverse intermolecular interactions. Cardiac TnT binds to cTnI and cTnC to form the overall troponin complex and also binds to tropomyosin (Tm) and actin. In

humans, three TnT genes have been described. These separate genes encode the isoforms found in slow skeletal muscle, fast skeletal muscle, and cardiac (cTnT) muscle. Each isoform is subject to alternative RNA splicing, producing multiple tissue-specific isoforms [19, 29]. The cardiac-specific TnT is encoded by the TNNT2 gene, located on chromosome 1. A number of isoforms have been identified, four of which have been described for the human heart. Cardiac TnT_1 and TnT_2 are expressed primarily in the fetal heart. Cardiac TnT_3 is the primary isoform in the healthy adult heart, and $cTnT_4$ is expressed in the fetal heart and is reexpressed in the adult failing heart. Although most of cTnT is highly conserved, there is a unique, hypervariable N-terminal region (residues 1–69) which is variable across isoforms [16, 19, 30].

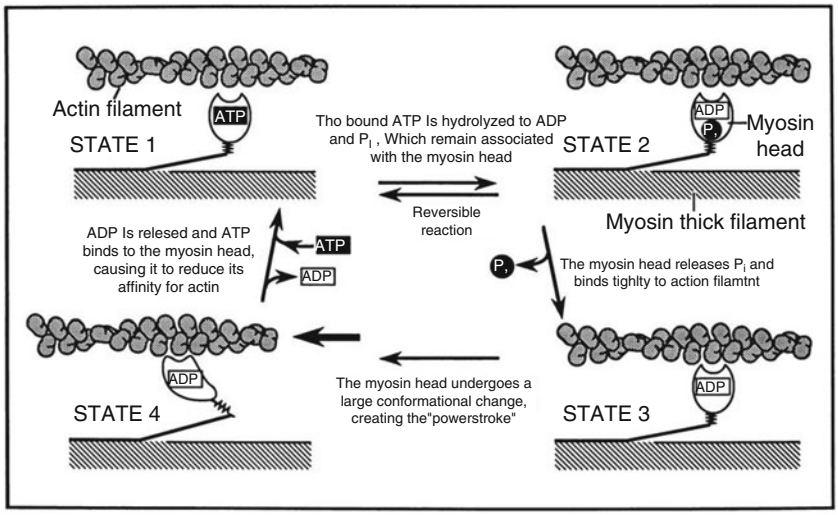
Similar to cTnI, the N-terminal region of cTnT contains several phosphorylation sites, primarily Ser-2, Thr-294, Thr-204, Thr-213, and Ser-208. The role of cTnT phosphorylation and cardiac function is unclear, but data suggest that phosphorylation of cTnT decreases the binding affinity of cTnT for Tm and reduces the ability of troponin to expose blocked myosin binding sites. The net result is a decrease in Ca^{2+}-dependent actomyosin ATPase activity [31]. A number of mutations associated with hypertrophic and dilated cardiomyopathies have been identified in human cTnT [32].

10.5 Cardiac Muscle Contraction

The sliding filament theory of muscle contraction was proposed in 1954 by Huxley and Hanson, years before it was recognized that calcium and the cardiac troponin complex played a key role in this process. The sliding of the interdigitated thin and thick filaments causes a shortening of the distance between the Z lines, as observed by microscopy. Later work demonstrated that contraction involves a cycle of myosin "reaching forward" to bind to actin and form the crossbridge, contraction, release of actin, and then the forward movement of the hinged globular region of the myosin S1 segment to begin the cycle again, as shown in Fig. 10.2.

Hydrolysis of ATP provides the energy for myosin to pull the actin filaments. The myosin crossbridges rotate toward the center of the sarcomere in what is referred to as the "power stroke." Subsequently, an ATP molecule binds to the myosin head, breaking the crossbridge and allowing myosin to reattach to the actin at a site further along the actin filament. After decades of rigorous scientific examination, the sliding filament model still stands, albeit refined by new data. A plethora of new proteins and lipids have been demonstrated to be involved in contraction, restoration of relaxation, and maintenance of structural integrity under conditions of consistent deformation (contraction-relaxation).

The cycle of a heartbeat is the process that leads to and includes contraction, ejection, subsequent relaxation, and filling of the cardiac chambers with blood. The cardiac cycle begins with an action potential and membrane depolarization.



American Heart Association

Fig. 10.2 Cardiac muscle contraction through one cycle of cardiac contraction and relaxation (This figure which originally appeared in Marian and Roberts [8] is used here with permission). In State 1, ATP is bound to the globular head of myosin. The myosin ATPase hydrolyzes ATP, generating ADP and Pi (State 2). The Pi is released and the globular head binds to the actin filament (State 3). Flexion of the hinge region of myosin displaces the globular head over the actin filament, causing the power stroke and muscle contraction (cardiac systole (State 3 to State 4)). ADP is released, with ATP again taken into the binding site on the globular head, releasing myosin from actin (return to State 1)

The concentration of intracellular Ca^{2+} rises, followed by Ca^{2+} binding to cTnC, changes in the thin filament, and force generation of crossbridges. The cycling of muscle fibers between contraction and relaxation must occur within the heartbeat and be adaptable to a wide range of heart rates and contractility. It has been proposed that the rise of intracellular Ca^{2+} and the Ca^{2+}-induced troponin switch occurs within approximately 20 ms, the first 20–30 % of the isovolumetric pressure rise. This suggests that the rate of myocardial contraction is limited by processes other than Ca^{2+}-regulated troponin switching [33] (Chap. 4).

In diastole, the N-terminal tail of cTnT, the inhibitory peptide of cTnI, and an actin-binding region of cTnI hold Tm in a blocking position that prevents the development of crossbridges (steric blocking model) [34]. The electrical events of the action potential and membrane depolarization are linked to the mechanical result of cardiac contraction. This excitation-contraction coupling begins with the release of calcium from the sarcoplasmic reticulum. Calcium enters the cytoplasm primarily via the L-type channels and triggers the release of more calcium from the sarco-

plasmic reticulum via the ryanodine receptor (i.e., Ca^{2+}-induced Ca^{2+} release). The increased level of cytosolic Ca^{2+} interacts with the regulatory N-domain of cTnC. The Ca^{2+} binding to cTnC induces exposure of a hydrophobic patch that draws the switch peptide region of cTnI to cTnC. The movement of the switch peptide toward the hydrophobic area of cTnC drags the adjoining inhibitory region of cTnI, causing release of the thin filament and an actin-binding site. The calcium-binding signal also induces release of the cTnT to tropomyosin interaction [5, 35]. The C-terminus of cTnI is carried along with this motion. The sequence is sometimes referred to as the "drag and release" mechanism [17]. This conformational change in the troponins allows actin crossbridge formation and sarcomeric shortening or contraction.

Return to the diastolic conformation of the cardiac troponin complex is an energy-requiring process that involves dissociation of calcium from cTnC, reversion of the conformation of the different subunits, and reduction of crossbridge formation. Four pathways modulate the removal of Ca^{2+} from the cytosol during the active process of relaxation, including the sarcoplasmic reticulum Ca^{2+} ATPase, sarcolemmal Na^{+}/Ca^{2+} exchanger, mitochondrial Ca^{2+} uniporter, and sarcolemmal Ca^{2+} ATPase [35]. Factors such as phosphorylation of the subunits, mutations, and isoform switching of the troponin subunits as well as the assay system used (e.g., isolated subunit versus troponin complex, reconstituted into thin filament or cardiac myocyte) can alter the kinetics of Ca^{2+} association and dissociation with cTnC [23]. Liu evaluated the effect of cTnI and cTnT modifications associated with cardiac disease on the rates of calcium association and dissociation both in isolated troponin complex and when these modifications were reconstituted into the thin filament [36]. Effects on the kinetics of Ca^{2+} association and dissociation from the reconstituted thin filament were observed when almost no effects were detected in the isolated cTn complex. The majority of disease-related protein modifications did not alter the Ca^{2+}-binding properties of the isolated troponin complex. However, when reconstituted into the thin filament, the mutations associated with dilated cardiomyopathy decreased the Ca^{2+} sensitivity of the thin filament. The mutations associated with restrictive and hypertrophic cardiomyopathy, as well as the ischemia-induced truncation of cTnI, increased the Ca^{2+} sensitivity of the thin filament. The various protein modifications altered the steady-state Ca^{2+} binding to TnC by influencing both the Ca^{2+} association and dissociation rates on the thin filament.

10.6 Increases in Circulating Cardiac Troponins

The circulating forms of cTns (i.e., those proteins identified in the systemic circulation after a myocardial infarction) include cTnT, cTnI, the cTnI-TnC complex, and a ternary complex of cTnT-cTnI-TnC [37, 47]. Most cTns are present in the myocyte as part of the structural elements. An estimated 3–8 % of the total cellular troponin is free in the cytoplasmic pool. It is hypothesized that free troponin in the

cytoplasmic pool contributes to initial increases in circulating cTn following myocardial injury. Subsequent increases are thought to be due to degradation of structural proteins [38]. The probability of proteins the size of the troponins leaking from a viable cell is uncertain. The leakage from viable cells of fragments detectable by assays is possible but raises additional questions.

There are six proposed mechanisms for cTn release: (1) myocyte necrosis, (2) apoptosis, (3) normal myocyte cell turnover, (4) cellular release of proteolytic troponin degradation products, (5) increased cellular wall permeability, and (6) formation and release of membranous blebs. Cardiac myocyte death can be further categorized to include pathways of extrinsic death receptor pathway, intrinsic apoptosis, necrosis, necroptosis, and possibly others [39, 40]. How the mechanism of damage influences the form in which the troponins are released is not completely characterized.

Some baseline level of circulating cTns might be expected due to normal maintenance and turnover of cardiac myocytes. Replacement of cTns includes the synthesis, assembly, and degradation of the individual components of the cardiac sarcomere. The mechanism by which the individual components of this multiprotein complex are removed and replaced while maintaining the functionality of the contractile unit is incompletely understood. In rats, the rate of incorporation of radiolabeled amino acids into the troponin complex in situ demonstrated that cTnI and cTnT had similar half-lives (i.e., similar turnover rate) of 3.2 and 3.5 days, respectively. These half-lives were significantly different from cTnC's half-life of 5.3 days [41].

It is generally accepted that circulating cTn is highly susceptible to phosphorylation and proteolysis. Regardless of the mechanism by which troponins are released into circulation, some sections of the complex and fragments are more susceptible than others to degradation and modification. Modifications may include acetylation, protein sequence variants such as mutants, alternatively spliced isoforms, amino acid polymorphisms, and protein complexes. Each of these posttranslational modifications may have variable effects on the ability of a given antibody to detect cTnI [42]. Degradation of cTnT after release into the circulation is not as well understood. At least in hemodialysis patients, circulating cTnT molecules are degraded into smaller fragments that may be detectable by assays [43]. The process and overall kinetics of clearance are incompletely understood.

10.7 Assays

In 1987, Cummins et al. [58] described a radioimmunoassay for cardiac troponin I. Two years later, Katus et al. [59] announced an enzyme-linked immunosorbent assay (ELISA) for cardiac troponin T. Currently, there is one commercially available cTnT assay and numerous commercially available cTnI assays.

In this chapter, assays will be discussed in terms of sensitivity, specificity, and factors that may influence detection. The diagnostic interpretation of cardiac troponin increases and the clinical application are discussed in the next Chapter.

The oldest category of commercial assays, no longer in use, was less sensitive in detecting increases in cTn, compared to newer assays. A 10 % coefficient of variation was achieved at approximately 1 μg/l, and, therefore, pathologic elevations were detected almost exclusively. The current assays have third- and fourth-generation antibodies and incorporate newer technology to increase the analytical sensitivity. Assays currently available are informally referred to as high-sensitive, ultrasensitive, or sensitive-contemporary assays. These assays reliably detect to greater than the 99th percentile value but only quantitate cTn in a fraction of clinically healthy individuals. The IFCC recommends describing an assay as "high sensitivity" only if the cTn is measurable in more than 50 % of healthy subjects (and preferably in more than 95 %) below the 99th percentile of the assay and above the assay's limit of detection. The total imprecision (coefficient of variation) at the 99th percentile should be ≤10 % [45, 46]. Conrad and Jarolim suggest reserving the term "ultrasensitive" for assays capable of quantitating cTn at levels less than the lowest cTn concentrations seen in healthy individuals [46].

The use of antibodies to a variety of troponin epitopes has several significant consequences. First, the available assays have different sensitivities to detect circulating troponins. Second, results generated with one assay may not be comparable to results produced with another assay in another facility, making analysis or meta-analysis of results difficult. The interaction of the assay antibodies with the targeted epitope of the cTn fragment may be influenced by the posttranslational or post-release modification of the measured molecule. That is, the epitope in the circulating troponin species may be masked, destroyed, or otherwise unavailable for antibody recognition. Posttranslational modifications, complexes with other proteins (e.g., using heparin for anticoagulation), heterophile or human anti-mouse antibodies, and cTnI autoantibodies may affect the immunochemical measurement of cTnI. The chelating agent EDTA has been demonstrated to split the calcium-dependent I-T-C and I-C troponin complexes, affecting measured concentrations in assays that preferentially measure these forms [19].

When a serum sample is analyzed for circulating cTns, it is unlikely that a single chemical entity is detected. Several investigators have demonstrated progressive cTn degradation following acute myocardial infarction, contributing to a profile of cardiac troponin fragments and modified fragments and variants, some of which provide the necessary epitope for recognition by the assay antibody. This profile may vary among patient populations, and fragments may not necessarily be detected in a 1:1 ratio [37, 47, 48]. In addition to degradants and posttranslational modifications, natural variants, such as single nucleotide polymorphisms (SNPs), can also impair the ability of the commercial antibodies to recognize the targeted epitopes. Investigators have used the UniProt database to identify the SNPs in the TNNI3 and TNNT2 genes that might involve antibody-binding domains of either cTnI or cTnT. The number of polymorphisms identified in TNNI3 and TNNT2 are 19 and 4, respectively. The investigators also noted that 12 out of 17 commercial cTnI

assays and the cTnT immunoassay contained antibodies targeting SNP-containing domains. Although two of the SNPs are considered to be clinically silent thus far, the majority of these SNPs are associated with inherited cardiac disorders. Due to incomplete penetrance and variable clinical expression of the disorders, carriers of the identified SNPs may experience myocardial infarctions before they exhibit signs of cardiomyopathy. In this patient population, cTn values may be false negative because of their variant forms [49]. Mutations in cTnI and cTnT subunits have been shown to be associated with dilated, hypertrophic, and restrictive cardiomyopathies. Mutations in cTnC have also been associated with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) [50].

Other isoforms of troponins may cross-react with the antibodies in the cTnT assay, leading to a false-positive result. Several recent publications describe persistent elevations in circulating cTnT without concomitant elevations in circulating cTnI in patients with various neuromuscular disorders. This finding is suggestive of re-expression of isoforms in diseased skeletal muscle and release into circulation [52–54].

Although there are standardized clinical chemistry measurands (e.g., glucose), there is no standardization of the troponin I assays for reasons that include the heterogeneity of the analyte(s) as described above. The IFCC Working Group on Standardization of Troponin I has described the challenges for achieving metrological traceability for troponin I assays [55]. The current recommendations include the ability of each assay to accurately and reliably report troponin values in the upper reference limit of a one-tailed 99th percentile of a reference population. The 99th percentile was chosen as the cut point of normality by a consensus that an acceptable false-positive rate would be approximately 1 % [4]. Clearly, the reference population is a significant factor in determining the diagnostic criteria for any given assay. There is no universal or consensus definition on what characteristics constitute a reference population or how many individuals are needed. Both the age and sex of the reference population as well as the baseline characteristics in general have been suggested to affect the values obtained and, therefore, the upper reference limit (URL) for the cut point. Three contemporary sensitive assays were used to measure cTnI in 2,404 individuals and a subgroup with more stringent inclusion criteria of 908 individuals. One assay showed significantly higher values in men than in women. Age dependency was not demonstrated in this study although others have suggested that increasing age of the population may correlate with higher 99th percentile values [45, 56, 57].

10.8 Concluding Remarks

The cardiac troponins are structural proteins in the contractile apparatus of the cardiac myocyte. The role of posttranslational modification has been recognized to affect both the function of the troponins and the overall regulation of cardiac muscle contraction. The cardiac troponins in the systemic circulation are sensitive and specific biomarkers for myocardial necrosis. Currently available assays

for the cardiac troponins are based upon antibody recognition of different epitopes of the cardiac troponin molecules. Posttranslational modification, proteolysis, and single nucleotide polymorphisms of the troponins may affect the ability of the antibodies to access or recognize the critical epitopes. This may have implications for the diagnostic accuracy of troponins in some situations or patient populations. Considerations for the clinical use of troponins and the role in diagnostic criteria are discussed in the following Chapter.

References

1. Alpert JS, Thygesen K, Antman E, et al. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol. 2000;36:959–69.
2. Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. J Am Coll Cardiol. 2007;50:2173–95.
3. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. Third universal definition of myocardial infarction. J Am Coll Cardiol. 2012;60:1581–98.
4. Newby LK, Jesse RL, Babbb JD, Christenson RH, De Fer TM, Diamond GA, Fesmire FM, Geraci SA, Gersh BJ, Larsen GC, Kaul S, McKay CR, Philippides GJ, Weintraub WS. ACCF 2012 expert consensus document on practical clinical considerations in the interpretation of troponin elevations. J Am Coll Cardiol. 2012;60(23):2427–63.
5. Solaro RJ. Sarcomere control mechanisms and the dynamics of the cardiac cycle. J Biomed Biotech. 2010;2010:105648. Published online 2010 May 10. doi: 10.1155/2010/105648. PMCID:PMC2866969.
6. Balse E, Steele DF, Abriel H, Coulombe A, Fedida D, Hatem SN. Dynamic of ion channel expression at the plasma membrane of cardiomyocytes. Physiol Rev. 2011;92:1317–58.
7. Brashers VL, McCance KL. Structure and function of the cardiovascular and lymphatic systems. In: McCance KL, Huether SE, Brashers V, Rote NS eds. Pathophysiology: the biological basis for disease in adults and children. 6th ed. Maryland Heights: Mosby, Elsevier; 2010.
8. Marian A, Roberts R. Recent advances in the molecular genetics of hypertrophic cardiomyopathy. Circulation. 1995;92:1336–47.
9. Parmacek MS, Solaro RJ. Biology of the troponin complex in cardiac myocytes. Prog Cardiovasc Dis. 2004;47(3):159–76.
10. Frank D, Frey N. Cardiac Z-disc signaling network. J Biol Chem. 2011;286(12):9897–904.
11. Auerbach D, Bantle S, Keller S, Hinderling V, Leu M, Ehler E, Perriard J-C. Different domains of the M-band protein myomesin are involved in myosin binding and M-band targeting. Mol Biol Cell. 1999;10:1297–308.
12. Krüger M, Linke WA. The giant protein titan: a regulatory node that integrates myocyte signaling pathways. J Biol Chem. 2011;286(12):9905–12.
13. Herzberg O, James MN. Structure of the calcium regulatory muscle protein troponin-C at 2.8A resolution. Nature. 1985;313(6004):653–9.
14. Sundaralingam M, Bergstrom R, Strasburg G, Rao ST, Roychowdhury P, et al. Molecular structure of troponin C from chicken skeletal muscle at 3-angstrom resolution. Science. 1985;227:945–8.
15. Houdusse A, Love ML, Dominguez R, Grabarek Z, Cohen C. Structures of four Ca2+ -bound troponin C at 2.0 Å resolution: further insights into the Ca2+ switch in the calmodulin superfamily. Structure. 1997;5:1695–711.
16. Jin J-P, Huang Q-Q, Ogut O, Chen A, Wang J. Troponin T isoform regulation and structure-function relationships. Basic Appl Myol. 2000;10(1&2):17–26.

17. Li MX, Wang X, Sykes BD. Structural based insights into the role of troponin in cardiac muscle pathophysiology. J Muscle Res Cell Motil. 2004;25:559–79.
18. Katrukha IA. Human cardiac troponin complex. Structure and functions. Biochemistry (Moscow). 2013;78(13):1447–65.
19. Lippi G, Targher G, Franchi M, Plebani M. Genetic and biochemical heterogeneity of cardiac troponins: clinical and laboratory implications. Clin Chem Lab Med. 2009;47(10):1183–94.
20. Saggin L, Gorza L, Ausoni S, Schiaffino S. Troponin I switching in the developing heart. J Biol Chem. 1989;264:16299–302.
21. Sasse S, Brand NJ, Kyprianou P, Dhoot GK, Wade R, Arai M, Periasamy M, Yacoub MH, Barton PJ. Troponin I gene expression during human cardiac development and in end-stage heart failure. Circ Res. 1993;72:932–8.
22. Kobayashi T, Solaro RJ. Calcium, thin filaments, and the integrative biology of cardiac contractility. Annu Rev Physiol. 2005;67:39–67.
23. Davis JP, Tikunova SB. Ca2+ exchange with troponin C and cardiac muscle dynamics. Cardiovasc Res. 2008;77:619–26.
24. Akhter S, Zhang Z, Jin J-P. The heart specific NH2-terminal extension regulates the molecular conformation and function of cardiac troponin I. Am J Physiol Heart Circ Physiol. 2012;302:H923–33.
25. Lu QW, Hinken AC, Patrick SE, et al. Phosphorylation of cardiac troponin I at protein kinase C site threonine 144 depresses cooperative activation of thin filaments. J Biol Chem. 2010;285: 11810–7.
26. Feng HZ, Chen M, Weinstein LS, Jin JP. Removal of the N-terminal extension of cardiac troponin I as a functional compensation for impaired myocardial beta-adrenergic signaling. J Biol Chem. 2008;283:33384–93.
27. Yu ZB, Zhang LF, Jin JP. A proteolytic NH2-terminal truncation of cardiac troponin I that is up-regulated in simulated microgravity. J Biol Chem. 2001;276:15753–60.
28. McConnell BK, Popovic Z, Mal N, Lee K, Bautista J, Forudi F, Schwartzman R, Jin JP, Penn M, Bond M. Disruption of protein kinase A interaction with A-kinase anchoring proteins in the heart in vivo: effects on cardiac contractility, protein kinase A phosphorylation, and troponin I proteolysis. J Biol Chem. 2009;284:1583–92.
29. Jin JP. Alternative RNA, splicing-generated cardiac troponin T isoform switching: a non-heart-restricted genetic programming synchronized in developing cardiac and skeletal muscles. Biochem Biophys Res Commun. 1996;225:883–9.
30. Anderson PA, Greig A, Mark TM, Malouf NN, Oakeley AE, Ungerleider RM, et al. Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing heart. Circ Res. 1995;76:681–6.
31. Streng AS, de Boer D, van der Velden J, van Diejen-Visser MP, Wodzig WKWH. Post-translational modifications of cardiac troponin T: an overview. J Mol Cell Cardiol. 2013;63:47–56.
32. Willott RH, Gomes AV, Chang AN, Parvatiyar MS, Pinto JR, Potter JD. Mutations in troponin that cause HCM, DCM and RCM: what can we learn about thin filament function? J Mol Cell Cardiol. 2010;48:882–92.
33. Stehle R, Iorga B. Kinetics of cardiac sarcomeric processes and rate-limiting steps in contraction and relaxation. J Mol Cell Cardiol. 2010;48:843–50.
34. Solaro RJ, Henze M, Kobayashi T. Integration of troponin I phosphorylation with cardiac regulatory networks. Circ Res. 2013;18(112):355–66.
35. Bers DM, Despa S. Cardiac myocytes Ca^{2+} and Na^{+} regulation in normal and failing hearts. J Pharmacol Sci. 2006;100:315–22.
36. Liu B, Tikunova SB, Kline KP, Siddiqui JK, Davis JP. Disease-related cardiac troponins alter thin filament Ca^{2+} association and dissociation rates. PLoS One. 2012;7(6e38259):1–11.
37. Bates KJ, Hall EM, Fahie-Wilson MN, et al. Circulating immunoreactive cardiac troponin forms determined by gel filtration chromatography after acute myocardial infarction. Clin Chem. 2010;56(6):952–8.
38. 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(16):1360–7.

39. Orogo AM, Gustafsson AB. Cell death in the myocardium: my heart won't go on. Int Union Biochem Mol Biol. 2013;65(8):651–6.
40. White HD. Pathobiology of troponin elevations. Do elevations occur with myocardial ischemia as well as necrosis? J Am Coll Cardiol. 2011;57(24):2406–8.
41. Martin AF. Turnover of cardiac troponin subunits: kinetic evidence for a precursor pool of troponin-I. J Biol Chem. 1981;256(2):964–8.
42. Guy MJ, Chen Y-C, Clinton L, Zhang H, Zhang J, Dong X, Xu Q, Ayaz-Guner S, Ge Y. The impact of antibody selection on the detection of cardiac troponin I. Clin Chim Acta. 2013;420:82–8.
43. 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;109:23–5.
44. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) website. http://www.ifcc.org/media/245272/IFCC%20Troponin%20I%20and%20T%20(ug_L%20units)%20_update%20December%202013.pdf
45. Apple FS, Collinson PO. Analytical characteristics of high-sensitivity cardiac troponin assays. Clin Chem. 2012;58(1):54–61.
46. Conrad MJ, Jarolim P. Cardiac troponins and high sensitivity cardiac troponin assays. Clin Lab Med. 2014;34:59–73.
47. Madsen LH, Christensen G, Lund T, Serebruany VL, Granger CB, Hoen I, Grieg Z, Alexander JH, Jaffee AS, Van Eyk JE, Atar D. Time course of degradation of cardiac troponin I in patients with acute ST-elevation myocardial infarction: the ASSENT-2 troponin substudy. Circ Res. 2006;99:1141–7.
48. Cardinaels EPM, Mingels AMA, van Rooij T, Collinson PO, Prinzen FW, van Diejen-Visser MP. Time-dependent degradation pattern of cardiac troponin T following myocardial infarction. Clin Chem. 2013;59(7):1083–90.
49. Lippi G, Cervellin G. Genetic polymorphisms of human cardiac troponins as an unrecognized challenge for diagnosing myocardial injury. Int J Cardiol. 2014;171(3):467–70.
50. Lu Q-W, Wu X-Y, Morimoto S. Inherited cardiomyopathies caused by troponin mutations. J Geriatr Cardiol. 2013;10:91–101.
51. Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich KW, Vinar G, Noe A, Matern G, Kuebler W. Diagnostic efficiency of troponin T measurements in acute myocardial infarction. Circulation. 1991;83:902–12.
52. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS. Diseased skeletal muscle: a noncardiac source of increased circulating concentrations of cardiac troponin T. J Am Coll Cardiol. 2011;18(58):1819–24.
53. Dhir T, Jiang N. Misleading elevation of troponin T caused by polymyositis. Int J Biomed Sci. 2013;9(2):107–11.
54. Rittoo D, Jones A, Lecky B, Neithercut D. Elevation of cardiac troponin T but not cardiac troponin I in patients with neuromuscular diseases: implications for the diagnosis of myocardial infarction. J Am Coll Cardiol. 2014;03(027).
55. Christenson RH, Bunk DM, Schimmel H, Tate JR, IFCC Working Group on Standardization of Troponin I. Put simply, standardization of cardiac troponin I is complicated. Clin Chem. 2012;58(1):165–8.
56. Gore MO, Seliger SL, deFilippi CR, Nambi V, Christenson RH, Hashim IA, Hoogeveen RC, Ayers CR, Sun W, McGuire DK, Ballantyne CM, deLemos JA. Age and sex-dependent upper reference limits for the high sensitivity cardiac troponin T assay. J Am Coll Cardiol. 2014;63(14):1441–8.
57. Petersmann A, Ittermann T, Fries C, Lubenow N, Kohlmann T, Kallner A, Greinacher A, Nauck M. Comparison of the 99th percentiles of three troponin I assays in a large reference population. Clin Chem Lab Med. 2013;51(11):2181–6.
58. Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J. 1987;113:1333–44.
59. Katus HA, Remppis A, Looser S, Hallermeier K, Scheffold T, Kubler W. Enzyme linked immuno assay of cardiac troponin T for the detection of acute myocardial infarction in patients. J Mol Cell Cardiol. 1989;21:1349–53.