Cardiac Troponins: Bench to Bedside Interpretation in Cardiac Disease

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Abstract: Cardiac troponins are the preferred biomarkers for the determination of acute myocardial necrosis. The high sensitivity of the available assays has significantly increased the detection of microscopic amounts of myocardial damage. Although compelling evidence indicates that elevated cardiac troponins are markers of poor prognosis and increased mortality, irrespective of the clinical scenario, small elevations can be seen in protean conditions and may confound the diagnosis of acute coronary syndromes. Emerging evidence suggests multiple different cellular mechanisms leading to cardiac troponin release, which challenge long held paradigms such as equivalency between troponin release into the circulation and irreversible cell death. Hence, knowledge of the physiology and pathophysiology of these cardiac biomarkers is essential for their accurate interpretation and consequent correct clinical diagnosis. Herein, the current relevant information about cardiac troponins is discussed, with special emphasis on pathophysiology and clinical correlates.

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Over the last decade, a clear consensus has emerged to indicate that circulating cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are the preferred biomarkers for detecting myocardial necrosis. The high sensitivity (hs) of the available assays has significantly increased the detection of microscopic amounts of myocardial damage, which were hitherto undetectable. Presently, the cutoff for myocardial necrosis is defined as the 99th percentile of the values of a normal reference population measured with an imprecision limit or coefficient of variation (CV) of less than or equal to 10%.[1] However, implicit in this definition is the fact that 1% of the normal population will have an elevated plasma cTn. Moreover, an ever increasing number of nonischemic cardiac events can lead to troponin "leaks," which confound the diagnosis and treatment of cardiac disease, in particular, acute coronary syndrome (ACS).

Thus, these "positive troponins" must be put in the appropriate clinical context to reach the correct diagnosis and to ensure optimal treatment. To this end, knowledge of the pathophysiology of the available cardiac biomarkers and the assays used for their detection is necessary.

The magnitude of the problem in interpreting circulating troponins is exemplified from the data of a recent study[2] that examined 69,299 emergency room patients, 48% of whom had their cTn measured. Of these, 7% had an elevated cTn concentration. Approximately 43% of these did not have an ACS as determined by invasive and noninvasive studies. In this regard, it is important to point out that while compelling evidence indicates that elevated troponins are a marker of poor prognosis and increased mortality, irrespective of the clinical scenario,[2–4] it is less clear whether troponins are released into the circulation only with irreversible cardiomyocyte damage as has long been believed.[5–10] Evidence in animal models and *in vitro* cell studies suggests that not only may troponins be released in the absence of irreversible injury, but there may be more than 1 mechanism behind their release.[5–7]

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STRUCTURE, PHYSIOLOGY AND MEASUREMENT OF CARDIAC TROPONINS

The sarcomere is the fundamental unit of a myofibril and consists of 7 actin monomers, a double-stranded tropomyosin and a troponin complex. The troponin complex is tadpole-shaped and is composed of 3 subunits. Troponin T (tropomyosin binding) is a 37-kDa protein that anchors the troponin complex to the tropomyosin strand of the thin filament. Troponin C (calcium binding) is an 18-kDa protein that binds calcium ions released from the sarcoplasmic reticulum. Troponin I (inhibitory) is a 24-kDa protein that inhibits the enzymatic hydrolysis of adenosine triphosphate that powers muscle contraction. More than 90% of the cTn is bound to tropomyosin on the thin filament of the myofibril, and the remainder exists as a small unbound cytosolic pool. The function of this pool is not fully understood, but it may serve as a reservoir for the repair and regeneration of tropomyosin-bound troponin. Only 7% of cTnT and 3.5% of cTnI exists freely in the cardiac myocyte cytoplasm with the rest being bound to the sarcomere.[11,12]

Using unique amino acid sequences, specific monoclonal antibodies have been developed against human cTnT and cTnI. These antibodies are the basis of the highly cardiac-specific immunoassay methods available to detect cTn.[13,14]

Cellular Mechanisms of Troponin Release

Historically, it is a long-held belief that troponin release is a marker of irreversible myocardial injury, and it has been equated to cardiac cell necrosis. Recent evidence suggests that this may not be the case and that troponins may be released into the circulation in conditions of reversible injury or even normal physiological processes.[5–10] Studies in normal individuals demonstrating that cTn is released into the circulation after prolonged exercise support this concept.[8,9] In these investigations, patients with positive circulating troponins had an unremarkable cardiac workup, including a normal echocardiogram, magnetic resonance imaging (MRI) scans and coronary angiograms. Case reports of patients with supraventricular tachycardia with positive cTnI, normal echocardiographic examination and normal coronary angiography are also in agreement with this concept.[10]

Relevant studies in normal rats have demonstrated that low-dose isoproterenol infusion resulted in reversible cardiac myocyte lesions and slight cTnT elevations, whereas higher doses of isoproterenol led to irreversible myocyte lesions with interstial fibrosis and more significant increases in serum cTnT.[5] In cultured myocytes, integrin stimulation promoted increased release of cTnI from viable myocytes, indicating that intact cTnI can be released from viable cardiomyocytes by stimulation of stretch-responsive integrins in the cell membrane.[7]

In view of the considerations mentioned above, a clear understanding of the physiological and pathophysiological mechanisms involved in the cellular release of cTn is of great value for the proper interpretation of a "positive troponin" in any given clinical context. These cellular mechanisms include:

1. Apoptosis—Programmed cell death, which results in rapid uptake by scavenging macrophages before the spillage of significant cellular contents. With normal cellular turnover and apoptosis in healthy individuals only low levels of troponin would be present in the serum. With the old assays, these low levels would not be within detectable limits, however, with newer high-sensitivity assays, these will probably be detectable and a new definition of the upper limit of "normal" will be necessary.[15]
2. Infarction or ischemic myocyte necrosis—This cause of troponin release is of critical interest to the clinician. Early detection of ACS results in greater myocardial salvage and better patient outcomes. Defined by a classic kinetic pattern of troponin release timed from the initial ischemic insult, it is crucial to distinguish ischemic necrosis from other causes of troponin release as discussed in detail in the subsequent sections.
3. Nonischemic myocyte necrosis—This is promoted by oxidative stress, reactive oxygen species, inflammatory cytokines, neurohormonal activation, altered calcium handling and acid base disturbances.[16] Altered calcium handling as a result of increased preload results in activation of intracellular proteolytic enzymes that degrade cTn, releasing cTn fragments into the circulation, which may have epitopes with an affinity for the cTnI immunoassays.
4. Reversible myocyte injury—Myocardial stretch by means of stretch responsive integrins with increased membrane permeability leading to leakage of cytoplasmic troponin.[7]

CARDIAC TROPONIN ASSAYS

Immunoassays for the quantification of serum cTn isoforms (both T and I) were developed in the late 1980s and became commercially available in the United States and Europe in the mid to late 1990s. The 1st-generation cTnT immunoassay used bovine cTnT as the reference material, which cross-reacted with human skeletal TnT. Thus, early assays could not reliably differentiate TnT of myocardial and skeletal muscle origin. The development of 2nd- and 3rd-generation cTnT assays, with recombinant human cTnT as the reference, eliminated this problem. More recently, ultra-high-sensitive assays have been developed and are undergoing clinical evaluation and application.[15,17]

Factors Affecting the Reliability and Variability of cTn Assays

The understanding of certain basic characteristics of the particular assay offered in a practice is of great value for clinicians.

1. Assays differ in their ability to measure accurately and reliably in the range of the 99th percentile of troponin values in normal individuals.[14]
2. Cardiac troponin values are not currently standardized; hence, troponin values vary from assay to assay and can have different values for the 99th percentile of normal.[14] Although there are many assays for troponin I, there is currently only 1 assay for troponin T in the United States (Roche Diagnostics, Indianapolis, IN).
3. Intermethod variability exists, especially with assays of cTnI. This troponin is released into the circulation as binary or tertiary complexes. This may result in differing levels of measurement at the same absolute concentration because of specificity of the assay for different molecular locations on the fragments.[18]
4. The terms "ultra," "fourth generation," "high-sensitivity" and "contemporary," for troponin assays, are often used rather loosely without any well-defined criteria and may not necessarily represent superior performance.
5. Infrequently, auto antibodies to cTnI and cTnT may result in false negatives or falsely lower concentrations of cTn.[19] This is much less frequent with cTnT.[19]
6. Hemolysis may interfere with cTn measurement.[20,21]

PATHOPHYSIOLOGY OF CARDIAC BIOMARKERS IN UNCOMPLICATED ACUTE CORONARY SYNDROME

Release Kinetics of cTn in Acute Coronary Syndrome

The release kinetics of troponin follow a classical pattern during an ACS, which is not seen with other cardiovascular conditions that may lead to a positive biomarker. Troponin T has a biphasic pattern of release in ACS, a smaller initial peak secondary to cytosolic release followed by a 2nd larger and sustained release secondary to degradation of the contractile apparatus to which it is bound, peaking at 12 to 24 hours. On the other hand, troponin I has a monophasic release pattern. The reason for this difference is poorly understood but may be secondary to dissimilar molecular weights of cTnT and cTnI and the higher unbound fraction of cTnT as detailed below.[19] Although debated, the initial release of cytosolic cTn with ischemia might be because of changes in myocardial membrane permeability, whereas the release of bound cTn requires proteolytic degradation and cellular necrosis.[11,18] The half-life of cTnT in the circulation is 120 minutes; the half-life of cTnI is presently not known.[11,18,22]

The reasons for the difference in cTn T and I release kinetics may be related to the higher molecular weight and higher unbound fraction of cTnT and less degradation. Cardiac troponin I is more frequently found as binary or tertiary complexes in blood, which may also affect its measurement by a specific assay. Release kinetics are obviously affected by the size of the infarct and the speed and effectiveness of revascularization procedures. There is evidence to suggest that the initial release may be a reflection of the quality of microvascular reperfusion.[19] The infarct size is typically determined by the value of cTnT on days 3 to 4.[19]

Because the rising/falling temporal pattern for troponin is a fundamental concept of the universal definition of myocardial infarction (MI), the concepts of "delta" (change in troponin level over time) and troponin velocity are of importance.[19] The magnitude of delta that fulfills the criterion for rise and fall is a topic of debate. The delta should