INTRODUCTION

Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue. They are not present in smooth muscle tissue. With the proteins actin and tropomyosin, they are part of the thin filaments within the myofibrils and are essential for the calcium-mediated regulation of muscle contraction. The troponin complex consists of 3 interacting and functionally distinct proteins (troponin I, T, and C) ***(Manoj et al., 2013).***

Both cardiac and skeletal muscle express troponin C; whereas troponin T and I are generally thought to be cardiac-specific. However, one study had challenged that troponin T is exclusively cardiac-specific ***(Jaffe et al., 2011).***

The total mass of the troponin complex is minuscule when compared with the protein mass of other myofibrillar proteins like actin and myosin. However, both troponin T and I are ideally suited for the detection of myocardial damage as they are expressed as cardio-specific isoforms ***(Thygesen et al., 2010).***

There is a distinct release kinetics following MI showing a first peak resulting from the loosely bound troponin pool and a second prolonged elevation due to degradation of the contractile apparatus ***(Thygesen et al., 2010).***

Blood from healthy individuals with no evidence of cardiac disease contains very low amounts of cardiac troponin ***(Venge et al., 2009).***

Elevated troponin value may be encountered in 1% of a healthy reference population. A cardiac troponin (cTn) elevation reflects acute or chronic myocardial damage but is not exclusive for acute coronary syndrome (ACS) thus causing some problems with interpretation of results ***(Hamm et al., 2002).***

Sometimes the term false-positive is being used to describe a patient with suspected ACS and elevated troponin but subsequently absence of significant coronary disease on coronary angiogram. In this setting several differential diagnosis have to be considered where troponin elevation may be related to underlying cardiac but non-coronary pathology or extracardiac disease, such as severe renal dysfunction ***(Hamm et al., 2002).***

Rarely, elevated troponin concentrations cannot be explained despite thorough clinical examination. These rare instances are referred to as truly false-positives, and are most frequently related to heterophilic antibodies or other analytical issues ***(Panteghini, 2009).***

Haemolysis may interfere with cTn and cause measurement of higher or lower cTn concentrations ***(Giannitsis et al., 2010).***

This issue is not relevant with mild haemolysis or high cTn concentrations but may be relevant with more severe haemolysis more than (100 mg/dL), particularly at concentrations near the 99^{th} percentile value, and in clinical settings where haemolysis is more prevalent like in emergency departments ***(Bais, 2010).***

AIM OF THE ESSAY

The aim of this essay is to discuss causes that can elevate cardiac troponin concentration in the absence of an acute coronary syndrome.

Chapter 1

CARDIAC TROPONIN PHYSIOLOGICAL OVERVIEW

1) Physiology of Troponin:

Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue. They are not present in smooth muscle tissue. With the proteins actin and tropomyosin, they are part of the thin filaments within the myofibrils and are essential for the calcium-mediated regulation of muscle contraction. The troponin complex consists of 3 interacting and functionally distinct proteins (troponin I, T, and C) ***(Manoj et al., 2013).***

Tissue-specific isoforms exist for each type of troponin ***(Filatov et al., 1999).***

Troponin is itself a complex of 3 protein subunits: troponin T, troponin I, and troponin C. Troponin T binds the troponin complex to tropomyosin. Troponin I inhibits actomyosin ATPase in relation to the calcium concentration. Troponin C, with its 4 binding sites for calcium, mediates calcium dependency ***(Jaffe, 1999).***

In the cytosol, troponin T is found in both free and protein-bound forms. The unbound (free) pool of troponin T is the source of the troponin T released in the early stages of

myocardial damage. Bound troponin T is released from the structural elements at a later stage, corresponding with the degradation of myofibrils that occurs in irreversible myocardial damage. The most common cause of cardiac injury is myocardial ischemia, it mean acute myocardial infarction. Troponin T becomes elevated 2 to 4 hours after the onset of myocardial necrosis, and can remain elevated for up to 14 days ***(Jaffe, 1999).***

Within the thin filament, tropomyosin dimmers form a continuous chain along the groove of the actin helix. The troponin complex lies at regular intervals along the filament. Tropomyosin acts to block the myosin binding sites on actin. Each troponin protein has specific functions that regulate muscle contraction. Troponin C binds calcium to initiate muscle contraction. Multiple isoforms of troponin T (TnT) exist in skeletal muscle. Cardiac troponin T (cTnT) has a molecular weight of 37,000 Dalton (Da). Troponin C (TnC) is present in 2 isoforms. One isoform is present in fast-twitch muscle fibers and the other is present in both cardiac and slow-twitch muscle fibers. Homology between the cardiac isoform and one of the skeletal muscle isoforms reduces the cardiac specificity of TnC and therefore limits its diagnostic usefulness in heart disease ***(Scott and Meg, 2008).***

Troponin T attaches the troponin complex to tropomyosin and actin. In human cardiac tissue 4 isoforms

exist, but only one is characteristic of the adult heart. The other three cardiac isoforms are expressed in fetal tissue. The fetal isoforms may be re-expressed during heart failure or in damaged skeletal muscle ***(Filatov et al., 1999).***

Troponin I inhibits actomyosin ATPase and prevents the structural interaction of myosin with actin-binding sites. The binding of calcium to troponin C displaces troponin I and causes a conformational change in tropomyosin so that it no longer interferes with myosin/actin binding and muscle contraction can occur. Mutations in the genes encoding for cTnT and cTnI cause hypertrophic cardiomyopathy in humans ***(Maass and Leinwand, 2003).***

Three isoforms exist for troponin I (TnI). Two are present in skeletal muscle and the other is present only in cardiac muscle. The cardiac isoform (cTnI), with a molecular weight of 24,000 Da, is larger than the other isoforms as it contains an additional 32 amino acid N terminal peptide. The rest of the protein has greater than 40% dissimilarity in its amino-acid sequence compared with skeletal muscle TnI ***(Babuin and Jaffe, 2005).***

2) Troponin Release:

The troponin protein exists in two populations within the cells. The majority of troponin is structurally bound within the thin filaments of the contractile apparatus. A small percentage

of protein remains free in the cytosol. This percentage is approximately 2–4% for cTnI and 6–8% for cTnT ***(Scott and Meg, 2008).***

Troponins are considered leakage markers. Damage to cardiac myocytes resulting in loss of membrane integrity causes the release of cTn into the circulation. Apoptosis, a genetically programmed form of cell death, does not result in loss of cell membrane integrity and therefore will not cause leakage of troponins ***(Kostin et al., 2003).***

Troponin release kinetics are consistent with two separate intracellular populations. After acute cardiac injury, the cytosolic pool is released resulting in an early rise in blood levels. This is followed by the slower release of structurally bound troponin that results in a sustained elevation (see Figure 1) ***(Jaffe et al., 2001).***

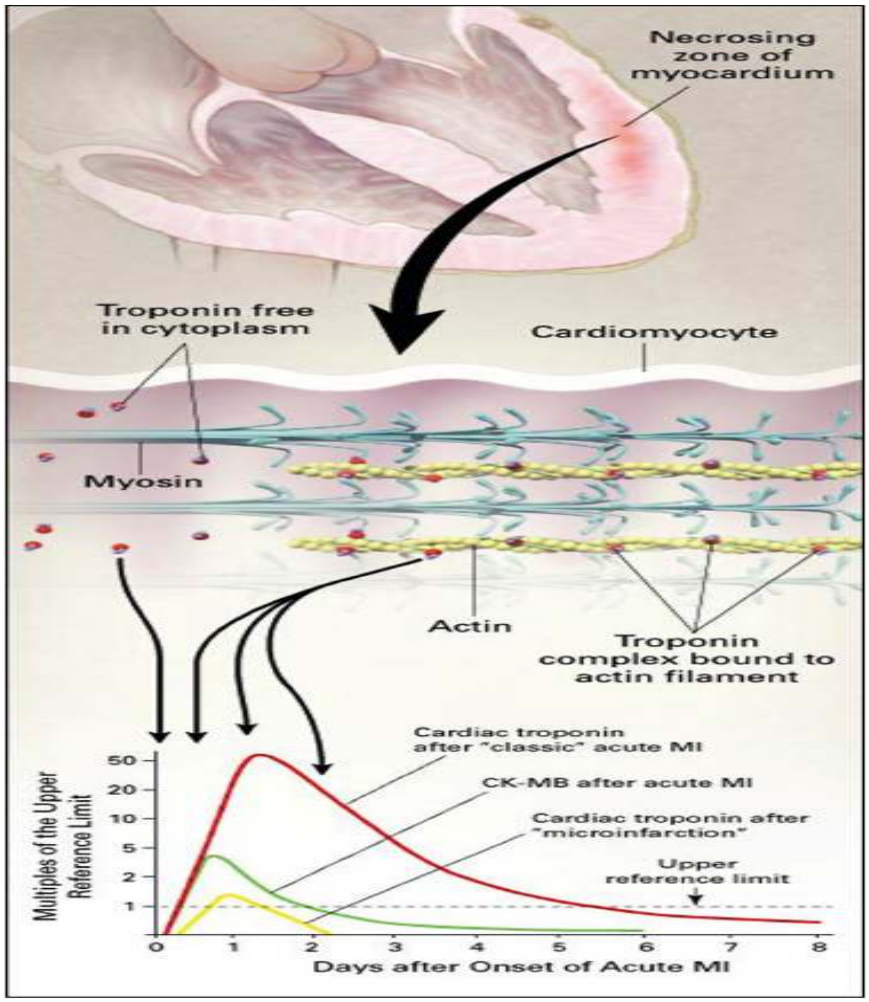

Figure (1): Release of cardiac troponins during acute myocardial infarction. After injury resulting in loss of sarcolemmal membrane integrity, the free cytoplasmic troponins are released first followed by a more prolonged release of the structurally bound troponin proteins ***(Antman, 2002)***.

The half-life of troponin and its complex in the circulation is about 2 hours ***(Scott and Meg, 2008).***

In humans with acute myocardial infarction (AMI), cTn levels begin to rise 4–12 hours after the infarction and reach peak values at 12–48 hours. The levels remain elevated for 7–10 days (cTnI) and 10–14 days (cTnT) ***(Babuin and Jaffe, 2005).***

This earlier peak was hypothesized to be due to more rapid development of necrosis in the experimental situation. The sustained elevation of cTn for several days after AMI is likely due to ongoing release from damaged myocytes rather than impaired elimination ***(Fishbein et al., 2003).***

The exact mechanism for elimination of troponins is unknown but it is thought to involve clearance by the reticuloendothelial system ***(Freda et al., 2002).***

There is also some evidence that troponins may be broken down into small fragments that could be renally excreted ***(Diris et al., 2004).***

3) Assays:

Troponin levels are determined using enzyme-linked immunosorbent assays (ELISA). The difference in amino- acid sequences from skeletal muscle and cardiac troponin I and T has allowed production of antibodies specific for cardiac troponins in these assays ***(Muller-Bardorff et al., 2002).***

The first assays for detection of cTn were developed in the late 1980s. These assays have evolved dramatically since their introduction with greater sensitivity and improved precision. The turnaround time for results has also decreased from several hours to a few minutes. Point-of-care assays now exist that may be run bedside or in the field ***(Apple, 1999).***

There are multiple assays available from a variety of manufacturers for cTnI. This has led to some confusion regarding interpretation of results. The assays are not standardized so manufacturers may design the tests using proprietary antibodies that target varying amino-acid sequences on the cTnI molecule. In the bloodstream, cTnI can be modified or complexed to other proteins, such as cTnC, and the antibodies used in the assays may have differing specificity for each circulating form of cTnI ***(Panteghini et al., 2004).***

There exists no gold standard assay for cTnI at this time. Comparison studies using a number of analyzers have concluded with the recommendation that, until assays are standardized, reference ranges should be established for each individual assay. Also, absolute values obtained from different assays cannot be compared ***(Adin et al., 2006).***

The first generation cTnT assays used an antibody that cross-reacted with skeletal muscle troponin T, thereby decreasing its specificity for cardiac injury ***(Scott and Meg, 2008).***

A Subsequent generations of cTnT assays have replaced this antibody with one more specific for cTnT, thereby eliminated the false positives related to skeletal muscle leakage ***(Muller-Bardorff et al., 2002).***

Although sensitivity of the assays for troponins has improved over the years, concern remains about their precision at low levels ***(Apple et al., 2002).***

The occurrence of false positive troponin results due to interfering substances in the blood has also been reported. Rheumatoid factor, excess fibrin, heterophile antibodies, hemolysis, lipemia, elevated alkaline phosphatase, and immune complex formation have all been implicated as causes of false positive troponin results ***(Lum et al., 2006).***

If a false positive result is suspected and instrument malfunction is ruled out, then repeating the test on a recentrifuged or new blood sample is indicated. The use of blocking reagents for substances such as rheumatoid factor and heterophile antibodies may decrease false results due to these interferences. Alternatively, because the problem of interference is assay dependent, the sample may be tested using a different manufacturer's assay ***(Lum et al., 2006).***

4) Role of Troponin in Muscle Contraction:

The contractile apparatus of myocytes is the myofibril, a cylindrical structure composed of thick and thin filaments

arranged longitudinally parallel within the myofibril. The thin filament is composed of two strands of F-actin wound around each other. Each strand of F-actin is composed of globular G-actin monomers, polymerized in a linear pattern. The thick filament is composed of myosin, a molecule with a long "tail" section made up of two helices twisted around one another, each attached to a roughly globular "head." There is a hinge point between the tail and the head that allows the head segment of the molecule to fold back upon itself. The thick filament, then, is an association of all the tail portions of many myosin molecules with the head portions protruding toward the adjacent thin filaments ***(Bers, 2000).***

The sarcomere is the functional unit of the myofibril. Each sarcomere is about 2μm in length, and there are many copies throughout the length of the myofibril. The sarcomere is bounded at each end by the Z-bands, which run perpendicular to the thick and thin filaments and to which the thin filaments are attached. In the relaxed state, the thin filaments extend from the Z-band toward the center of the sarcomere. The gap in the center between the thin filaments is the H-zone. The thick filaments are interspersed between the thin filaments (parallel to them) in the center of the sarcomere, crossing the H-zone. During contraction, the thick filaments "pull" the thin filaments and the Z-bands toward the center of the sarcomere. Both the H-zone and the sarcomere itself shorten in length during contraction ***(Bers, 2000).***

a) Mechanism of Muscle Contraction

Muscular contraction is the shortening of the myofibrils that occurs when the thick and thin filaments slide past one another. The sliding of the thick and thin filaments is thought to occur by repetitive binding of the myosin heads to actin, flexion of the myosin molecules at their hinge points, release of the binding between the molecules, and relaxation of the myosin molecule prior to binding the actin again. This repetitive motion is referred to as cross-bridge cycling ***(Ferrier and Howlett, 2001).***

In this way, the thick filament is thought to "ratchet" itself past the thin filament. In the resting state, the interaction of the thick and thin filaments is physically blocked by the presence of the protein tropomyosin, which lies in the groove of the thin filament formed by the two F-actin strands. Tropomyosin is closely associated with three other proteins, troponin I, troponin T, and troponin C ***(Ferrier and Howlett, 2001).***

It is the troponin-tropomyosin complex that regulates the interaction of the thick and thin filaments and therefore muscular contraction. Troponin C has a calcium binding site that, when occupied by a calcium ion, causes a conformational change in the troponin-tropomyosin complex (see Figure 2). This change moves the tropomyosin molecule from its resting position, which makes the myosin binding site accessible to the

myosin head. Cross-bridge cycling then begins and the myofibrils forcefully shorten until calcium is removed from troponin C and tropomyosin returns to its resting position, blocking actin and myosin binding ***(Bers, 2000).***

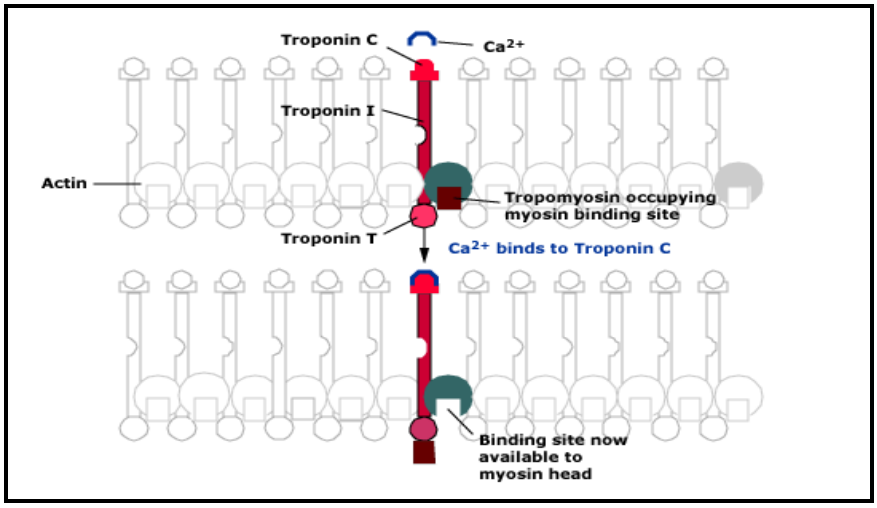

Figure (2): Interaction of the troponin-tropomyosin complex with the myosin binding site in the actin strands. Top panel: Interaction of the myosin with actin is physically blocked by the presence of the protein tropomyosin, which lies in the groove of the thin filament formed by the two F-actin strands, occupying the myosin binding site. Bottom panel: Calcium (Ca2+) binds to troponin C, leading to a conformational change which moves the tropomyosin molecule and makes the myosin binding site accessible to the myosin head. The other troponins also contribute to this process ***(Bers, 2000)***.

Troponin I is an inhibitory protein that inhibits the ATPase of actomyosin and also modulates calcium binding to troponin C. Troponin T serves to attach the troponin complex to actin and tropomyosin; in the relaxed state, it blocks the actin-myosin binding site ***(Ferrier and Howlett, 2001).***