

False-Positive Causes in Serum Cardiac Troponin Levels

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Abstract

Cardiac troponins (cTns) are the most valuable and specific markers of cardiovascular diseases, including acute myocardial infarction. These biomarkers can also be used to assess the degree of myocardial damage in non-cardiac diseases that can negatively affect the cells of cardiac muscle tissue. However, in everyday clinical practice, doctors often encounter with false-positive cases of increased cTns. False-positive cases of increased cTns can contribute to incorrect diagnosis and subsequent inadequate treatment, which causes significant harm to the patient. This review discusses some common causes of a false-positive increase in the level of cTns in the blood serum. Such causes are fibrin clots, heterophilic antibodies, alkaline phosphatase, rheumatoid factor, and cross-reactions of diagnostic (anti-cTn) antibodies with skeletal troponins. Detailed attention is focused on the mechanisms of false-positive increase, and ways to identify and combat these false-positive causes of increased cTns. This has an important practical significance in modern clinical practice.

Keywords: Cardiovascular diseases; Acute myocardial infarction; Biomarkers; Troponin T; Troponin I; False-positive

Introduction

Cardiac troponins (troponin T (cTnT) and troponin I (cTnI)) are the main and most specific biomarkers for the early diagnosis of acute myocardial infarction (AMI) [1-4]. In accordance with the main guidance document (Fourth Universal Definition of Myocardial Infarction), the main criteria for AMI are the following: 1) myocardial damage detected using cTns; 2) symptoms of myocardial ischemia; 3) ischemic changes on an electrocardiogram and in particular the appearance of a pathological Q wave; 4) identification of areas of non-viable myocardium using imaging methods; and 5) detection of a blood clot in the coronary arteries using coronary angiography or autopsy [1].

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Due to modern ultra-sensitive tests, medical practitioners got the opportunity to early diagnose AMI (within the first 2 h from admission of the patient) through the evaluation of dynamic changes of cardiac troponins. The changes (increase) of the concentration of cTn molecules within the first 2 h are very small (may amount to as little as several ng/L) and cannot be detected by moderately sensitive test systems. It should be noted that due to a number of multicenter studies, there have been validated algorithms of early diagnostics (0 → 1 h and 0 → 2 h) of non-ST-segment elevation AMI (NSTEMI) for ultra-sensitive test systems of various manufacturers (Tables 1 and 2) [5]. These diagnostic algorithms of AMI are recommended by the European Society of Cardiology (ESC) for clinical practice [6].

In addition, cTns can be used to assess the prognosis of patients suffering from many non-cardiac pathologies that damage cardiac myocytes (Fig. 1) [7-15].

However, in some cases, elevated (positive) troponin concentrations cannot be explained, even after careful clinical examination and exclusion of all possible pathologies that may cause cardiomyocyte damage. Such cases are called false-positive and are most often associated with the following reasons: fibrin clots, heterophile antibodies, alkaline phosphatase, rheumatoid factor, and cross-reactions of diagnostic (anti-cTn) antibodies with troponin molecules released from skeletal muscle [16-28]. Knowledge of the main causes and mechanisms of a false-positive increase in cTn concentrations is important in clinical practice, since many physicians may make incorrect diagnoses and prescribe unnecessary treatment based on laboratory results, which can be harmful to the patient and lead to unnecessary economic costs. The main causes and mechanisms of false-positive troponin elevations, as well as ways to combat these types of interference, are sequentially discussed below.

Fibrin Clots

Fibrin clots are one of the most important factors causing interferences in laboratory studies of blood serum. Fibrin clots are formed due to incomplete blood clotting under the action of coagulants added to the test blood to obtain serum. Standard biochemical test tubes (vacuum tubes with a red lid) use dry clot activator (silica) applied to the inner surface of the test tube wall as a coagulant. The main reason for the formation of fibrin clots is incomplete clotting of blood prior to centrifugation. Most often this occurs in patients with coagulopathies or against the background of anticoagulant therapy [16, 29-31]. In addition, extra-laboratory errors (violation of blood collection technique) and intra-laboratory violations (reduction of recommended time from blood receipt to centrifugation) leading to formation of fi-

Table 1. Current Diagnostic Algorithms for Confirmation/Exclusion of NSTEMI Approved by the ESC: One-Hour NSTEMI Diagnostic Algorithm

Troponin immunoassay (company; manufacturer)	Biomarker concentration that indicates an extremely low probability of an NSTEMI diagnosis, ng/L	Biomarker concentration that indicates a low probability of an NSTEMI diagnosis, ng/L	Changes in biomarker concentration after 1 h at which a diagnosis of NSTEMI should be excluded, ng/L	Biomarker concentration that indicates a high probability of an NSTEMI diagnosis, ng/L	Changes in biomarker concentration after 1 h at which a diagnosis of NSTEMI should be confirmed, ng/L
High-sensitivity cardiac troponin T (Elecsys; Roche)	< 5	< 12	< 3	≥ 52	≥ 5
High-sensitivity cardiac troponin I (Architect; Abbott)	< 4	< 5	< 2	≥ 64	≥ 6
High-sensitivity cardiac troponin I (Centaur; Siemens)	< 3	< 6	< 3	≥ 120	≥ 12
High-sensitivity cardiac troponin I (Access; Beckman Coulter)	< 4	< 5	< 4	≥ 50	≥ 15
High-sensitivity cardiac troponin T (Clarity; Singulex)	< 1	< 2	< 1	≥ 30	≥ 6
High-sensitivity cardiac troponin T (Vitros; Clinical Diagnostics)	< 1	< 2	< 1	≥ 40	≥ 4
High-sensitivity cardiac troponin T (Pathfast; LSI Medience)	< 3	< 4	< 3	≥ 90	≥ 20

Table 2. Current Diagnostic Algorithms for Confirmation/Exclusion of NSTEMI Approved by the ESC: Two-Hour NSTEMI Diagnostic Algorithm

Troponin immunoassay (company; manufacturer)	Biomarker concentration that indicates an extremely low probability of an NSTEMI diagnosis, ng/L	Biomarker concentration that indicates a low probability of an NSTEMI diagnosis, ng/L	Changes in biomarker concentration after 2 h at which a diagnosis of NSTEMI should be excluded, ng/L	Biomarker concentration that indicates a high probability of an NSTEMI diagnosis, ng/L	Changes in biomarker concentration after 2 h at which a diagnosis of NSTEMI should be confirmed, ng/L
High-sensitivity cardiac troponin T (Elecsys; Roche)	< 5	< 14	< 4	≥ 52	≥ 10
High-sensitivity cardiac troponin T (Architect; Abbott)	< 4	< 6	< 2	≥ 64	≥ 15
High-sensitivity cardiac troponin T (Centaur; Siemens)	< 3	< 8	< 7	≥ 120	≥ 20
High-sensitivity cardiac troponin T (Access; Beckman Coulter)	< 4	< 5	< 5	≥ 50	≥ 20
High-sensitivity cardiac troponin T (Clarity; Singulex)	< 1	To be determined	To be determined	≥ 30	To be determined
High-sensitivity cardiac troponin T (Vitros; Clinical Diagnostics)	< 1	To be determined	To be determined	≥ 40	To be determined
High-sensitivity cardiac troponin T (Pathfast; LSI Medience)	< 3	To be determined	To be determined	≥ 90	To be determined

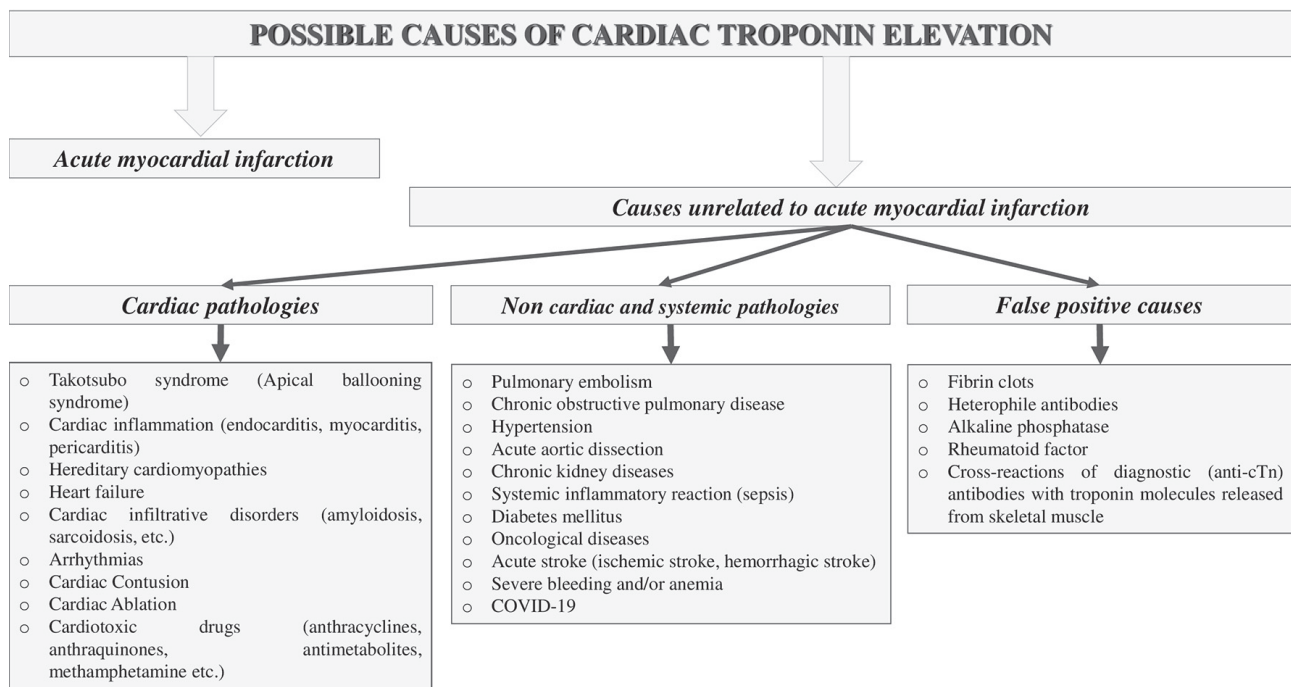


Figure 1. Possible causes of cardiac troponin elevation.

brin clots are also possible. The optimal time for the complete clotting of the blood sample is approximately 30 - 60 min from the time of blood collection. However, in some cases, laboratory staff, under pressure from clinicians, are forced to reduce the time allotted for clotting a blood sample. This increases the likelihood of fibrin clots and filaments formation in the tubes after centrifugation. Additional factors increasing the risk of fibrin clots are hypocoagulant states (e.g., in patients taking anticoagulant drugs). Cases of false-positive increases in cTn concentrations in sera with fibrin clots on Abbott AxSYM [17] and Dade Stratus II immunoanalyzers [18] can be found in the literature. A presumed mechanism underlying false-positive troponin tests is the competitive interaction of fibrin clots with diagnostic antibodies (anti-cTn). The prevalence of false-positive increases in cTn levels differs significantly according to different studies. Thus, Nosanchuck et al (1999) found false-positive results due to fibrin clots in all serum samples (n = 8) [17]. Roberts et al (1997) in their study identified 2.2% of false-positive results from > 900 patient specimens due to fibrin clots [18]. Ways to combat fibrin clots are: adherence to blood collection and sample preparation guidelines (paying particular attention to the clotting time guidelines), careful visual inspection of the blood sample after centrifugation, and switching to the routine use of whole blood or plasma as biomaterial instead of serum. The latter condition is the most optimal for laboratories involved in the diagnosis of acute conditions, including AMI.

Heterophile Antibodies

Heterophile antibodies are immunoglobulins (antibodies) formed by B lymphocytes against poorly recognized antigens

(such as foreign animal proteins). Heterophilic antibodies have a weak but polyvalent activity (avidity) to antigens. The main reasons for the formation of heterophile antibodies in humans are: the use of mouse monoclonal sera (antibodies) or incompletely humanized (human) antibodies for the treatment of a number of diseases (e.g., systemic connective tissue diseases or oncopathology); frequent contact with microbial antigens, animal antigens (e.g., when keeping pets), foreign proteins (e.g., in food workers, veterinarians, farmers); vaccination; blood transfusion and long-term persistence of viral agents in the body [19, 20, 32-35]. According to various estimates, the prevalence of heterophile antibodies in the population ranges from < 1% to 80%. However, not all patients with heterophile antibodies in the blood have false-positive reactions [19, 20]. Unlike a number of pre-laboratory factors (hemolysis, lipemia, and fibrin clots), heterophile antibodies cannot be detected by visual inspection of the specimen under examination.

The mechanism of false-positive elevation of cTn concentrations lies in the cross-interaction of heterophile antibodies with anti-cTn included in the diagnostic test system. Lum et al (2006) described an interesting clinical case of a false-positive increase in cTnI concentration in a patient without myocardial infarction. A 57-year-old patient admitted to the emergency department had complaints and symptoms similar to AMI. The cTnI concentration measured on admission with the Beckman Coulter immunoassay was 41.0 ng/mL, significantly higher than normal (0 - 0.5 ng/mL). However, the levels of total creatine kinase and creatine kinase-MB (CK-MB) isoform were within the normal range and the electrocardiogram data also did not indicate AMI. After careful examination, other possible causes of elevated troponin I concentration were also excluded. Based on these data, cardiologists suggested the

presence of a false-positive result. When troponin I concentrations were repeatedly tested with diagnostic test systems from different manufacturers (Beckman Coulter, Abbot, Bayer, Roche), troponin I levels were positive only with the Beckman Coulter immunoassay, whereas all other immunoassays were negative. Serial dilution of the patient's plasma samples with control plasma (with normal troponin I levels) revealed nonlinear results and led to the assumption of heterophilic antibody interference. To finally confirm this assumption, blood plasma samples were transferred to the research laboratory of Beckman Coulter, where after adding heterophilic antibody blockers to the patient's original blood sample, the troponin I concentration decreased from 41.0 to 1.04 ng/mL [19].

Researchers Zaidi et al (2010) described a clinical case of a false-positive increase in cTnI concentration in a 53-year-old female patient admitted to the emergency department with complaints of chest pain. The medical history revealed that the patient had been admitted with similar symptoms three times during the current year. The cTnI concentration at the time of admission (0.37 ng/mL) was five times the upper reference limit (0.00 - 0.069 ng/mL). However, electrocardiography (ECG) and coronarography data did not reveal signs of ischemia and obstruction of the coronary arteries, so physicians suspected a false-positive cTnI increase. The blood sample was sent to another laboratory, where troponin T was measured and was negative. Further analysis revealed the presence of heterophile antibodies in the patient's blood, which led to a false-positive increase in cTnI [36].

The largest systematic literature review by Lippi et al (2012) summarized 16 studies and clinical cases demonstrating the effect of heterophile antibodies on cTn concentrations. On average, the rate of false-positive increases in cTn levels ranged from 0.1% to 3.0%, and in some studies, it was significantly higher, up to 50%. The effect of heterophile antibodies is an unpredictable phenomenon and can affect both cTnI and cTnT test systems of any manufacturer. According to a systematic literature review, the best way to detect false-positive troponin levels caused by heterophilic antibodies is to pretreat the blood sample with heterophilic antibody blockers. According to most studies, the addition of a blocking reagent led to a dramatic decrease in cTn concentrations in patients' blood [20]. Some researchers believe that the prevalence of false-positive results due to the influence of heterophile antibodies may increase significantly in the future due to the widespread use of immunotherapy for the treatment of many diseases, as well as the use of antibodies in diagnostic immunoscintigraphic studies [37].

Fast detection of false-positive elevation of cTn levels is important in the emergency diagnosis of AMI. It is possible only with the coordinated interaction of clinicians and laboratory diagnostics specialists. This is due to the fact that laboratory diagnosticians only have access to laboratory results and therefore cannot compare troponin levels with data from other test methods. Clinical laboratory diagnosticians may suspect incorrect (false-positive) results if, in addition to cTns, a patient has been prescribed study of other myocardial damage biomarkers (total creatine kinase and its MB isoform, aspartate aminotransferase, myoglobin, lactate dehydrogenase and others). Thus, normal levels of these biomarkers with sharply elevated levels of cTns should alert laboratory diagnosticians. An equally important

role in identifying a possible false-positive result of troponin immunoassay is played by clinicians, who have maximum access to the results of all diagnostic methods used in relation to a particular patient. If the laboratory results are inconsistent with the clinical and instrumental data, clinicians should notify the diagnostic laboratory and initiate further investigation. Possible ways to detect false-positive troponin immunoassay results in the laboratory are: 1) testing the sample on another analyzer (if available), or measuring another cardiac marker (another cardiac isoform of troponin, CK-MB, myoglobin, and others); 2) serial dilution of biomaterial with control samples or saline several times and assessment of linearity of the values obtained; and 3) pretreating samples with special reagents that block heterophile antibodies (if available) or sending samples to specialized laboratories for these manipulations.

Alkaline Phosphatase

Alkaline phosphatase is a hydrolase enzyme which is widely used to diagnose liver and biliary tract diseases. In addition to its diagnostic value, this enzyme is also used in some immunoassays, including troponin immunoassays, for signal amplification. Some immunoassays using alkaline phosphatase as a component of the immunochemical reaction have been reported to be affected by endogenous (serum) alkaline phosphatase interference [21, 22, 38, 39]. Butch et al (1989) first established that alkaline phosphatase can have a significant effect on the concentration of a cardiac-specific enzyme CK-MB, measured on a Stratus immunochemical analyzer. The researchers found that in 12 of 23 patients with elevated serum alkaline phosphatase activity, CK-MB levels were falsely elevated [37]. Subsequently, Dasgupta et al (2001) reported the effect of alkaline phosphatase on cTnI concentration. With alkaline phosphatase activity of 46 U/L, the serum troponin I concentration in sample was 0.5 ng/mL. Researchers then added alkaline phosphatase solutions to this serum to increase the activity of this enzyme and evaluate its effect on the troponin concentration. With alkaline phosphatase activity of 129 U/L, the troponin I concentration increased to 4.3 ng/mL. A further increase in alkaline phosphatase activity to 222 and 913 U/L also proportionally increased the troponin I concentration to 9.4 and 40.1 ng/mL, respectively. Other test systems not using alkaline phosphatase as an immunochemical reaction component are unresponsive to such influence [22].

In a recent research, Marinheiro et al (2018) also proved that alkaline phosphatase was the cause of the false-positive troponin I result in a patient [38]. According to some authors, immunoassays that do not use this enzyme should be used for serum testing in patients with increased alkaline phosphatase activity [39]. In the absence of such a possibility, the results of patients who have elevated serum/plasma alkaline phosphatase activity should be interpreted with care.

Rheumatoid Factor

Rheumatoid factors are autoantibodies (immunoglobulins) that

are directed against their own IgG. Elevated levels of rheumatoid factor are not only of diagnostic value, but can also have a significant impact on the results of laboratory tests performed on immunochemical analyzers [23, 40-45]. In patients with autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, etc.), the main cause of falsely elevated troponins is rheumatoid factor [42-44]. According to Al-Awadhi et al (2007), five of 50 patients with seropositive rheumatoid arthritis had troponin I concentrations > 0.1 ng/mL (diagnostic threshold for AMI), while none of patients with seronegative rheumatoid arthritis had troponin I concentrations above the reference limit. One-factor regression analysis showed a positive correlation between troponin I and rheumatoid factor concentrations ($r = 0.35$; $P < 0.02$) [44]. Dasgupta et al (1999) in their research found false-positive troponin I concentrations in four of 12 patients with elevated rheumatoid factor levels. To eliminate the interference, the researchers used polyclonal antisera against rheumatoid factor, which resulted in a normalization of cTnI levels [42].

A large multi-center study of analytical interferences on laboratory results concentration examined the prevalence of false-positives. This study included patients with autoimmune diseases associated with elevated rheumatoid factor concentrations. In general, about 8.7% of the 3,445 results were false-positive. However, only a small fraction (21% of all false-positives) of the results were corrected with a blocking reagent, whereas 49% of the false-positives were not corrected with blocking reagents and would potentially mislead clinicians in making the diagnosis [45]. Thus, clinicians should be very careful when interpreting laboratory immunochemical studies in patients with autoimmune diseases and elevated serum rheumatoid factor levels.

Cross-Reactions of Diagnostic (Anti-cTn) Antibodies With Troponin Molecules Released From Skeletal Muscle

Damage to striated skeletal muscle in congenital and acquired diseases (myopathies, rhabdomyolysis) can lead to a false-positive increase in cTn levels due to the cross-reactions of anti-cTnI and anti-cTnT antibodies with skeletal troponin molecules. Most often, such false-positive reactions occurred with first and second generation troponin immunoassays with weakly specific antibodies that could interact with skeletal troponin molecules [46-49]. However, subsequently, a considerable number of cases of false-positive increases in cTns were registered when using more specific third and fourth generation troponin immunoassays [24-27]. The specific cause and mechanism of increase in cTns in patients with skeletal myopathies has not been unraveled yet, and cases of false-positive increases in cTns have been described even with the use of modern highly sensitive immunoassays [49]. There are two possible mechanisms for increase in the levels of cTns in diseases and injuries of skeletal muscles: 1) re-expression of cTn molecules in skeletal muscles after injury and the release of these molecules into the bloodstream from skeletal muscle fibers [50-52]; and 2) cross-reactions of diagnostic antibodies

(anti-cTnI and anti-cTnT) with skeletal troponin molecules released into the bloodstream during skeletal muscle injury [47-49, 53]. Discussions about these mechanisms are still ongoing [54, 55].

A number of studies have reported elevated serum cTn levels in many patients with skeletal myopathies even in the absence of ischemia and myocardial injury. Puntukollu et al (2004) reported elevated serum cTnT concentrations in 19 of 91 patients with rhabdomyolysis with no signs of coronary artery damage [25]. Egholm et al (2015) described a clinical case of a significant increase in high-sensitivity troponin T (hs-TnT) (471 ng/L, 99th percentile < 14 ng/L) in a 48-year-old patient with drug-induced rhabdomyolysis. The concentrations of myoglobin (29,120 μ g/L), total creatine kinase (30,750 U/L) and its MB isoform (162 μ g/L) were also significantly increased [26].

Rheumatologists revealed elevated cTnT concentrations in many patients with idiopathic inflammatory myopathies (polymyositis, dermatomyositis, and myositis associated with systemic connective tissue disease). Eighteen of 23 patients with myopathies had elevated levels of creatine kinase and cTnT, while the remaining five patients had normal creatine kinase and cTnT levels. Only one patient with myopathy had elevated cTnI level. Researchers also noted that creatine kinase levels correlated closely with cTnT levels ($r = 0.62$; $P = 0.001$) [46]. The most likely mechanism for the elevation of troponin T in this study is the cross-reaction of anti-cTnT with skeletal troponin T molecules. This is evidenced by the close correlation of cTnT with another skeletal muscle damage marker (creatine kinase) and the absence of a significant increase in cTnI. Thus, cTnT and cTnI have almost the same diagnostic value, and in case of cardiomyocyte damage, the concentration of cTnT and cTnI in serum would increase proportionally. A significant increase in only one cardiac troponin isoform (cTnT or cTnI), however, would be more indicative of analytical problems, particularly, cross-reactivity of the diagnostic antibodies included in the corresponding troponin immunoassay.

In another study, cTnT or cTnI levels were measured in 78 patients with skeletal myopathies including muscular dystrophies, myotonic dystrophies, inflammatory myopathies, myotonia, and neurogenic muscle pathologies. The cTnT was increased in 56 patients (72.8%) and cTnI in only two (2.6%). When grouping patients with elevated troponin T levels by nosology, it turned out that cTnT was elevated in all patients (100%) with neurogenic muscle pathologies, in 87% of patients with muscular dystrophy, in 75% of patients with inflammatory myopathies, in 72% of patients with myotonic dystrophy and in none of the patients with myotonia (0%). Studies of skeletal muscle biopsy specimen using western blotting and mass spectrometry revealed no cardiac troponins [53], which indicates the absence of cTns expression in skeletal muscle. Based on these results, the most likely mechanism for the troponins elevation in this study is the cross-reactions (false-positive) of diagnostic antibodies with skeletal troponin molecules that are released from damaged muscle fibers.

Schmid et al (2018) used highly sensitive assays to measure troponin I and troponin T during the examination of 74 patients with hereditary and acquired skeletal myopathies. Hs-TnT levels were elevated in a much larger number of patients

Table 3. False-Positive Causes in Serum Cardiac Troponin Levels

Major factors	Reason of interference	References
Fibrin clots	Competitive interaction of fibrin clots with diagnostic antibodies	[17-19, 30, 31]
Heterophile antibodies	Cross interaction of heterophile antibodies with anti-cTn included in the diagnostic test system	[19, 20, 32-37]
Alkaline phosphatase	Endogenous alkaline phosphatase can catalyze the enzymatic reaction in immunoassay and thereby amplify the signal, which is proportional to the concentration of cardiac troponins in the sample	[3, 21, 22, 38, 39]
Rheumatoid factor	Nonspecific interaction of rheumatoid factor (autoantibodies) with diagnostic antibodies	[3, 27, 33, 40-45]
Cross-reactions of diagnostic (anti-cTn) antibodies with troponin molecules released from skeletal muscle	Cross-reactions of diagnostic antibodies with skeletal troponin molecules released into the bloodstream during skeletal muscle injury Re-expression of cardiac troponin molecules in skeletal muscles after injury and the release of these molecules into the bloodstream from skeletal muscle fibers	[47-49, 53]

(> 14 ng/L; 68.9%) compared with hs-cTnI levels (> 26 ng/L; 4.1%). There was a close correlation of hs-cTnT with creatine kinase ($r = 0.679$) and even closer one with myoglobin ($r = 0.786$). Serial measurements of hs-TnT concentrations revealed a chronic elevation of hs-cTnT in most patients. The study of skeletal muscle biopsy specimens showed no expression of cTn isoforms in them, leading the researchers to a conclusion that there is no re-expression of cTn molecules in skeletal muscle. According to the researchers, the most likely reason for the increase in serum hs-cTnT and hs-cTnI levels was the false-positive (cross) reactions of anti-hs-cTnT and anti-hs-cTnI with skeletal troponin isoforms [49, 56, 57]. However, several other studies have revealed the expression of cTn molecules in skeletal muscle in skeletal myopathies, which may indicate the possibility of increasing the serum cTns concentration through the release of cTn molecules from skeletal muscle fibers into the bloodstream [50, 51]. A recent cohort study has also detected messenger RNA and peptide fragments of cTnT using mass spectrometry in patients with Pompe disease, an inherited glycogen storage disease predominantly damaging nerve and muscle cells throughout the body [52]. Thus, the data regarding the source and mechanism of positive troponin tests results in skeletal myopathies are inconsistent and need further clarification.

False-positive causes of increased cTn levels are summarized in Table 3 [3, 17-22, 27, 30-45, 47-49, 53].

Conclusions

Physicians and researchers should also keep in mind that there are a significant number of factors (fibrin clots, heterophilic antibodies, rheumatoid factor, alkaline phosphatase, and cross-reactions of diagnostic antibodies (anti-cTn) with skeletal troponin molecules) that cause false-positive elevations in cTns, as well as ways to detect false-positive results and counteract them. Understanding these causes and mechanisms of a false-positive increase in cTns in blood serum will help practitioners and researchers improve the diagnosis of cardiovascular diseases, in particular myocardial infarction, and reduce the risk of misdiagnoses.

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Financial Disclosure

None to declare.

Conflict of Interest

None to declare.

Data Availability

The author declares that data supporting the findings of this study are available within the article.

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