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Short Communication

Falsely Elevated Cardiac Troponin I Levels

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Summary: The measurement of cardiac troponins (cTn) is of considerable usefulness in the diagnosis of acute coronary syndrome. Abnormal levels of serum cTn are occasionally found in patients who are not suffering a myocardial infarction. This may be observed in several well-known situations including pulmonary embolism, pericarditis, myocarditis, coronary vasospasm, sepsis, congestive heart failure, supraventricular tachycardia with hemodynamic compromise, renal insufficiency, and prolonged strenuous endurance exercise. Endogenous antibodies such as heterophile antibodies, rheumatoid factor, and other autoantibodies are known to interfere with the immunoassay measurements of many different analytes, including the widely used Abbot AxSYM™ cTnI analyzer. Other sources of circulating antibodies include immunotherapies, vaccinations, or blood transfusions that may interfere with these immunoassays as well. We examine the case of a 48-year-old man with a history of hypercholesterolemia and obesity who presented with chest pain and was found to have elevated Tn I levels on two separate occasions. Further work-up revealed that the Tn I levels were spuriously elevated because the patient's blood revealed a normal cTnI level when mixed with polyethylene glycol to inactivate any antibodies interfering with the cTnI assay.

Key words: troponin, acute coronary syndrome, laboratory testing

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Introduction

In the diagnosis of acute coronary syndrome, measurement of cardiac troponin (cTn) is of considerable usefulness. Abnormal levels of serum cTn are occasionally found in patients who are not suffering a myocardial infarction (MI). In these situations, several well-known cases include pulmonary embolism, pericarditis, myocarditis, coronary vasospasm, sepsis, congestive heart failure, supraventricular tachycardia, renal insufficiency, and prolonged strenuous endurance exercise.[1–4]

Endogenous antibodies such as heterophile antibodies, rheumatoid factor, and other autoantibodies are known to interfere with the immunoassay measurements of many different analytes of Tn. Other sources of circulating antibodies include immunotherapies, vaccinations, or blood transfusions that may interfere with these immunoassays as well.[5–8]

Although a very rare finding, it is important to consider the diagnosis of a falsely elevated Tn level due to interference with the Tn assay secondary to antibodies once the initial workup is found to be nondiagnostic for coronary disease. This will avoid further unnecessary testing and procedures, and will alleviate patient worries. Clinicians must remain aware of the fact that if the work-up and clinical scenario do not support the diagnosis, then the possibility of spurious immunoassay results must be considered.

Case Summary

We report the case of a 48-year-old man with a history of hypercholesterolemia and obesity who was admitted to our hospital 4 years prior to the admission described herein, with increasing frequency of chest pressure located in the middle of his chest, that lasted for seconds at a time and had persisted for weeks, but had increased in frequency the day prior to that admission. Chest pressure was localized without radiation to his jaw or arms. He denied associated diaphoresis, shortness of breath, nausea, or vomiting. Cardiac enzymes revealed an elevated cTnI level of 264 ng/ml. The patient underwent cardiac catheterization, which revealed normal coronary arteries and normal left ventricular ejection fraction. He was discharged home on atorvastatin and aspirin.

Four years later, he presented with similar symptoms. On physical examination, vital signs included blood pressure 158/66 mmHg, pulse of 66/min, respirations 20/min, and temperature of 37°C. Cardiac examination was significant for distal heart sounds with a regular rate and rhythm without murmurs, rubs, or gallops. Lung examination was clear to auscultation bilaterally, and the extremities had no clubbing or edema with 2+ pulses bilaterally. Laboratory work revealed a leukocyte count of 11,300/μl with a normal differential cell count, hemoglobin of 16.2 gm/dl, hematocrit of 42.8%, and platelets of 228 k/ml. Cardiac enzymes revealed a creatinine kinase (CK) of 430 u/l, CK-MB fraction of 11.3%, and Tn of 107 ng/ml. The patient's electrocardiogram (ECG) revealed normal sinus rhythm and a ventricular rate of 68/min, with no acute ST-segment changes. The patient underwent exercise myocardial perfusion single-photon emission computed tomography imaging, which showed no evidence of ischemia, with normal wall motion and a calculated ejection fraction of 58%.

Outpatient evaluation 1 month later showed no symptoms of chest pain or pressure with repeat cardiac cTnI at 108 ng/ml. Because of persistent elevations in the cTnI level, further work-up was undertaken. Laboratory analysis was performed by mixing the patient's blood with polyethylene glycol to precipitate any antibodies that might be interfering with the cTnI assay. Polyethylene glycol was mixed at a 1:1 ratio with our patient's plasma. The sample was cooled in an ice bath, centrifuged, and the supernatant was removed for analysis. The normalization of the cTnI level in our patient from 40 to 0.6 ng/mL, which was within the normal range in our laboratory, confirmed the presence of antibodies that were interfering with the Tn assay. The new Abbott AxSYM™ (Abbott Laboratories, Abbott Park, Ill.) troponin I ADV assay, also available at our institution, revealed undetectable levels of Tn I. This new assay has a preincubation step as well as three separate monoclonal antibodies to increase specificity of the assay and has now been adopted at our institution as the standard assay. The patient's plasma was sent to an affiliated hospital laboratory for measurement with the Roche Elecsys™ system for Tn T (Roche Diagnostics, Indianapolis, Ind.), which was also undetectable. Simple dilution was also utilized and it also was unsuccessful in identifying interference. The patient's plasma was diluted with normal saline at a ratio of 1:5, which revealed a Tn level of 60 (up from 40 ng/ml). The patient was tested for rheumatoid factor and heterophile antibodies with negative results. Presumably, the patient has an unidentified antibody leading to interference with the cTnI assay, and further analysis is being undertaken.

Discussion

Cardiac biomarkers have now become integral players in the diagnosis of an acute coronary syndrome. They have allowed clinicians a greater degree of confidence in diagnosing or excluding MI in a patient who presents with signs and symptoms of coronary ischemia and damage. Troponin I and Tn T are not normally detectable in the circulation, and different genes encode these proteins in skeletal muscle and myocardium. Therefore, serum elevations are abnormal, and cardiac and skeletal muscle forms can be distinguished by specific antibodies that also permit quantitative immunologic assays. Cardiac troponins have been shown to be an important tool in the diagnosis of MI. Elevation levels of Tn are also useful in assessing prognosis in these patients with respect to 30-day morbidity and mortality.[1, 7, 9]

Abnormal levels of serum cardiac troponins are occasionally found in patients not suffering an MI. This may be observed in pulmonary embolism, pericarditis, myocarditis, coronary vasospasm, congestive heart failure, renal insufficiency, prolonged strenuous exercise, and several arrhythmias.[1–4, 11]

Spuriously elevated Tn levels appear to occur at many institutions, often adversely influencing the triage and treatment of patients. Many of these false-positive levels occur at the lower ranges of analytic sensitivity where it is most difficult to separate clinically important myocardial cell damage from spurious Tn I elevation. There are instances in which endogenous antibodies such as heterophile antibodies, rheumatoid factor, and other autoantibodies are known to interfere with the immunoassay measurements of many different analytes, including the widely used Abbott AxSYM analyzer.[6] Other sources of circulating antibodies including immunotherapies, vaccinations, or blood transfusions may also interfere with these immunoassays. After obtaining a thorough history, we were able to confirm that our patient had recently received no immunotherapies or blood transfusions that might have spuriously elevated the cTnI results obtained by the AxSYM analyzer.

Analytic and clinical performance characteristic variations in the various available commercial assays can result in up to a 20-fold variation in serum cTnI concentration for a given patient sample.[12] Different strategies are available to inactivate antibodies and prevent interference with the analyzer. These include immobilized protein A column, dilution, heterophile blocking tubes, immunoglobulin inhibiting reagent, or polyethylene glycol precipitation. Immunofixation electrophoresis, in which the interfering substances can potentially be removed, can also allow for an accurate assessment of true Tn level.[5–8, 12]

The widely used AxSYM analyzer uses reagents that contain mouse and goat antibodies, as well as mouse, goat, bovine, and fish protein stabilizers, and porcine gelatin. These proteins are apparently placed in the reagents with the intent of blocking the most common human antianimal antibodies in patient samples. However, the above-mentioned methods are not always successful in removing the potential interference, and thus can result in a false elevation of the troponin level and an inaccurate picture.[6]

One method used to reduce the interference is a process of dilution. Dilution of a patient's blood with different ratios of animal serum, such as 1:2 to 1:16 with mouse serum, goat serum, or a mouse/goat serum combination, can potentially reduce the cTnI levels by 80–120%. The dilution process along with the antibodies present in the reagents may have a synergistic effect that blocks the interfering substance and

allows the true Tn value to be measured. Another frequently used method is passage of a patient's plasma through an immobilized protein A column. This removes the interfering substance and reduces cTnI levels by 3–44% to true levels. Another simple but effective method is a process of using polyethylene glycol precipitation to remove potential interference from the serum. In our patient, polyethylene glycol and serum were mixed in a 1:1 ratio. Using this method, cTnI was reduced by 98.5%, resulting in a level which was within the normal range in our laboratory.[13]

Newer assays incorporate these methods of reducing spuriously elevated levels. The new Abbott AxSYM troponin I ADV assay used to test our patient's serum is one of these; it incorporates a preincubation step as well as three separate monoclonal antibodies to increase specificity. Although these new assays can be successful in removing most of the interference present in plasma, in rare instances the blocking agent itself can cause a falsely elevated cTnI level through a process not well understood and requiring further study.[6, 13]

Conclusion

Once the initial workup is found to be non-diagnostic for coronary artery disease, it is important to consider the diagnosis—although rare—of falsely elevated troponin due to antibody interference with the Tn assay. Such a diagnosis makes further testing and procedures unnecessary, and can alleviate patient worries. Use of a focused algorithm with frequent testing of multiple cardiac biomarkers, using a panel of biochemical markers that includes myoglobin, creatinine kinase, and Tn can help distinguish between a true elevation of cTnI and one that is spurious. Thus, in patients presenting with possible acute coronary syndromes, MI can be ruled out in the emergency department and diagnosis can be facilitated. Multiple strategies from simple polyethylene glycol precipitation, as used in our patient, to more complex methods, are readily available to block potential interference. For clinicians to utilize the many strategies available for correct diagnosis in patients presenting with possible acute coronary syndrome, they—and their laboratories—must first be aware of potential interference.

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