Persistent Increase of Cardiac Troponin I in Plasma without Evidence of Cardiac Injury

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CASE

A 69-year-old man with diabetes mellitus type II, hypertension, dyslipidemia, and prior ischemic strokes presented to the emergency department with complaints of balance difficulties and inability to stand unassisted of 2 weeks’ duration. The patient’s home medication regimen included atenolol, lisinopril, amiodipine, metformin, and glipizide. He is a retired chef and a former smoker (20 pack-years). He has 2 brothers, both of whom had myocardial infarctions in their 50s. The patient’s physical examination was remarkable for frequent premature contractions, left lower extremity weakness, and impaired coordination. His electrocardiogram revealed sinus rhythm with frequent premature ventricular contractions and diffuse nonspecific T-wave abnormalities.

Results of a comprehensive metabolic chemistry panel were within the reference intervals except for increases in glucose (158 mg/dL; reference interval, 74–99 mg/dL) and creatinine (1.5 mg/dL; reference interval, 0.7–1.3 mg/dL). The hemoglobin A1c value was 7.4% (reference interval, 6.0%). Cardiac troponin I (cTnI)3 concentrations were increased at 0.27, 0.22, and 0.25 μg/L (Abbott Architect assay; 99th percentile, 0.03 μg/L) over a span of approximately 8 h. The patient was admitted to the cardiology service on the basis of these abnormal results.

A transthoracic echocardiogram revealed a preserved left ventricular systolic function with evidence of impaired diastolic filling. A cardiac catheterization evaluation revealed an ulcerated plaque in the left anterior descending artery with >70% stenosis, which was treated with a bare-metal stent. The presenting symptom of balance difficulty failed to resolve after the coronary intervention, and the patient remained unable to stand unassisted. A neurologic evaluation included magnetic resonance imaging, which found no evidence of an acute stroke. He was discharged with a diagnosis of orthostatic hypotension.

Three months later, the patient presented to the emergency department with several days of balance difficulty. The initial cTnI value was increased at 0.10 μg/L, prompting admission to the cardiology service. Over the subsequent 12 h, cTnI values for 2 additional samples remained stable at 0.10 μg/L. Other laboratory tests included a comprehensive metabolic panel and a complete blood count, with results within reference intervals except for a hemoglobin concentration of 12.5 g/dL (reference interval, 14.0–18.0 g/dL) and a hematocrit of 35.5% (reference interval, 40.0%–52.0%). The patient’s symptoms were not consistent with a cardiac etiology, and no further cardiac evaluation was pursued. A neurologic evaluation again found no evidence of acute stroke, and his symptoms were believed to reflect a combination of orthostatic hypotension, pontine gliosis, and cerebellar atrophy. The unexplained abnormal troponin results prompted the attending cardiologist to contact the director of clinical chemistry.

DISCUSSION

Cardiac troponins play a central role in diagnosis and risk stratification in acute coronary syndromes (1), but troponins are recognized as markers of cardiac myocyte injury, not of the etiology of injury. A wide range of clinical conditions has been associated with increased

QUESTIONS TO CONSIDER

1. Describe common pathologic reasons for increased plasma concentrations of cardiac troponins in the absence of an acute coronary syndrome.

2. What analytical interferences increase measured cTnI concentrations?

3. How would you investigate the abnormal cTnI results in this patient?
troponin values (1), most or all of which have been shown to entail cardiac injury. Thus, it is necessary to consider these conditions when investigating unexpected increases in cardiac troponins.

In the patient described above, the rise and fall in troponin concentrations that are characteristic of acute myocardial infarction was not seen on either admission. This finding pointed toward other etiologies for the increased troponin values (1). The clinical findings and other laboratory information excluded most if not all of these other causes of increased troponin (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Noncoronary causes of increased cardiac troponin. a</th>
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<tbody>
<tr>
<td>Cardiac contusion</td>
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<td>Heart failure</td>
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<td>Aortic dissection</td>
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<td>Aortic valve disease</td>
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<td>Hypertrophic cardiomyopathy</td>
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<td>Tachy- or bradyarrhythmias</td>
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<td>Heart block</td>
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<td>Apical ballooning syndrome</td>
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<td>Rhabdomyolysis</td>
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<td>Infiltrative diseases (e.g., amyloidosis, sarcoidosis)</td>
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| a Modified from Thygesen et al. (1). |

Laboratory Investigation of Increased cTnI

Two important interferences reported to produce false-positive results in cardiac troponin immunoassays are fibrin clots and heterophile antibodies, such as human antimouse antibodies (HAMAs) or rheumatoid factor (2). No fibrin clots were seen in the patient’s sample and increased cTnI values were seen in multiple samples and upon their reanalysis, providing presumptive evidence that fibrin clots were not involved. This patient’s plasma was evaluated for HAMAs by measuring cTnI before and after treatment with a heterophile blocking tube from Scantibodies Laboratory. The plasma cTnI concentration remained unchanged, suggesting that HAMAs were not a source of analytical interference in the immunoassay.

Investigation of Possible Immunoglobulin-cTnI Complex

We suspected the presence of a macrocomplex of cTnI with an immunoglobulin molecule in the patient’s plasma. We reasoned that such a complex would delay the clearance of cTnI from the circulation and thus produce persistently increased troponin concentrations (3). We therefore treated the patient’s plasma either with protein A bound to Sepharose to deplete the sample of IgG, along with any cTnI complexed with IgG, or with an equal volume of buffer. Protein A decreased the cTnI concentration from 0.10 µg/L to undetectable (<0.02 µg/L), whereas buffer produced only the decrease expected from dilution, to 0.06 µg/L (the between-day total imprecision of the assay at 0.04 – 0.06 µg/L is <10%). In samples from 5 other patients, the cTnI concentrations obtained for the protein A–treated sample matched those of the dilutional control. Unfortunately, no plasma from the index patient’s initial hospitalization was available for a similar analysis.

Macrocomplexes of Troponin: Chemistry, Prevalence, and Clinical Findings

Naturally occurring immunoglobulin–cardiac troponin complexes in plasma were first recognized only recently. In 1996, Bohner et al. suspected the presence of such a complex in a patient after elective coronary artery bypass graft surgery. The patient had undetectable cTnI despite increased concentrations of creatine kinase isoenzyme MB (CK-MB) and cTnT (4). The authors added increasing amounts of cTnI to the sample, up to 38.5 µg/L, and found that cTnI continued to be undetectable. The authors concluded that an antibody that binds cTnI was complexing cTnI, rendering it unable to bind to the antibodies in the immunoassay reagent.

In 2002, an immunoglobulin–cTnI complex was identified as the source of increased cTnI concentrations (3). The patient described in the report had increasing cTnI concentrations over several months without clinical evidence of myocardial infarction. Treatment of a sample with antihuman IgG antiserum removed the cTnI. The authors hypothesized that the complex extended the normally brief half-life of the cTnI and allowed accumulation of cTnI in the blood to measurable concentrations.

Recent reports indicate that IgGs that bind cardiac troponins are common in healthy blood donors. IgG autoantibodies against cTnI, cTnT, or both cTnI and cTnT were found in 12.7% (n = 750), 9.9% (n = 467), and 1.7% (n = 345) of the donors, respectively (5–7). In a study of patients with cardiomyopathy (8), antibodies to cTnT (or cTnI) were present in 1.7% (or 7.7%) of patients with dilated cardiomyopathy (n = 272) and in 0.5% (or 9.2%) of 185 patients with ischemic cardiomyopathy. The lower prevalence than in
the healthy blood donors is thought to reflect a difference in the types of assays used to identify autoantibodies (6).

Pettersson et al. (9) showed that the presence of antibodies to cTnI slowed the apparent clearance of cTnI from the circulation (Fig. 1). The plasma cTnI concentrations in the patient described here are indicated by the asterisks and dashed line added to the figure of Pettersson et al. (Fig. 1). As can be seen, the rate of clearance of cTnI from the circulation of this patient is like that of the patients of Pettersson et al., who had anti-cTnI antibodies, and is much slower than that seen in the patients without antibodies to cTnI (shaded area).

Circulating immunoglobulins complexed with enzymes and other proteins that are measured in the diagnostic laboratory have been recognized for many years, back at least to the recognition of macroamylase (10). None of the other macrocomplexes are likely to have a more serious immediate clinical impact than an immunoglobulin–cardiac troponin complex (or macrotroponin). Even small increases in cardiac troponin in plasma or serum will be identified as abnormal by the sensitive cardiac troponin assays currently available (and even more sensitive assays are forthcoming). Such results carry the potential to lead to unnecessary interventions, as seen in this case. Results of increased cardiac troponin concentrations are expected with any cardiac troponin assay because the increased cardiac troponin concentration measured does not represent an interference, but rather an analytically correct result that is nonetheless misleading.

In light of the high prevalence of immunoglobulin–cardiac troponin complexes and the critical importance of cardiac troponin testing, it is necessary to be aware of the possibility of these antibodies in patients whose clinical presentations do not match their increased cardiac troponin concentrations. A second blood sample obtained a few hours later should provide clarification. With acute myocardial injury, cardiac troponin will continue to increase, whereas it will be stable with an immunoglobulin–cardiac troponin complex, as it was on both admissions of this patient. Another approach is parallel analysis of CK-MB. A rise and fall in the CK-MB concentration suggests an acute myocardial infarction, but the test is less diagnostically sensitive and specific than troponin assays.

One approach to identify an immunoglobulin–cardiac troponin complex is to measure cardiac troponin before and after treatment of the plasma or serum with protein A, which removes IgG and thus the complex containing the cardiac troponin and IgG. Heterophile blocking tubes, as were used in this case, provide a simple approach to exclude the possibility that protein A is removing an interfering HAMA. Other approaches for identifying the presence of macrocomplexes include electrophoresis and immunofixation (10).

CONCLUSION

We conclude that this patient’s cTnI most likely circulated as a complex with IgG. Recognition of such complexes is critical to avoid erroneous diagnosis of cardiac injury.
In the last decade, sensitive and specific immunoassays for cardiac troponins have revolutionized the diagnosis and management of cardiac disease. The absolute specificity of cardiac troponins for cardiac tissue and the sensitivity of the immunoassays have redefined risk stratification and treatments. The required sensitivity of these assays, however, has made the occurrence of false-positive cardiac troponin results an infrequent but very real laboratory problem. Any immunoassay has nonspecific background “noize,” and the signal-to-noise ratio defines the sensitivity of the assay. In this regard cardiac troponin assays are particularly challenged because they must distinguish very small, but clinically significant, concentrations of cardiac troponin from “noize.” Few immunoassays are asked to distinguish between “any antigen” and “none” (drug screens and human chorionic gonadotropin come to mind). Thus, unlike most immunoassays, additional “noize” in a cardiac troponin assay will be considered clinically important.
serially, any small shift in the calibration of a cardiac troponin assay or lot-to-lot variation may cause an increase in the number of low but important cardiac troponin results. Indeed, multiple manufacturers have had to recall their cardiac troponin assays over the years because of false “low positive” values. Such small changes in the noise or baseline would not be clinically important or detected in most clinical immunoassays. For instance, a thyroid-stimulating hormone result of 1.2 μIU/mL vs a true concentration of 1.0 μIU/mL or a prostate-specific antigen result of 3.4 μg/L vs 3.1 μg/L would never be questioned. The authors mention clots as a cause of false-positive cardiac troponin values. We have found that the most common cause of false-positive cardiac troponin values in our laboratory is “dirty” samples (e.g., microclots, cells). False-positive results from these types of samples often are not repeatable, or the false signal can be eliminated by recentrifugation of the sample. When clinical decisions are made at the technical limits of an assay, the laboratory must be aware of the possibility of false-positive results and communicate with clinicians about their infrequent, but inevitable, occurrence. As ultrasensitive cardiac troponin assays debut in the next few years, this challenge is unlikely to go away.

Commentary

Robert H. Christenson

Serial quantitative measurements of cardiac troponin I or T have become the cornerstone for diagnosis of myocardial infarction (MI) and are useful for stratifying risk and guiding management of patients with suspected acute coronary syndrome (1). This clinical case reminds us that in addition to documenting values exceeding a specified cutpoint, a rise and/or fall in the biomarker is essential for establishing the diagnosis. Cardiac troponin is a marker of cardiac injury, not etiology. Many non-MI conditions may cause troponin increases, and analytical false positives are also possible. Although cardiac troponin measurements are central for assessment of patients with suspected acute coronary syndrome, MI is a clinical diagnosis, and other nonlaboratory data are essential.

The presence of antibodies that affect cardiac biomarker results was documented in the 1970s and 1980s, when antibodies to CK-BB were identified as an interference with some CK-MB methods. Antibodies against other species, e.g., HAMA, can cause a positive interference with immunoassay reagents for cardiac markers. Autoantibodies that react with cardiac troponin can cause either negative interference (2) or positive interference, as was seen in this case. An important point made in this case was that autoantibodies against cardiac troponin can occur in about 10% of healthy individuals. The common occurrence of such autoantibodies in healthy individuals compels us to be very thoughtful when establishing 99th percentile cutpoints for MI and risk assessment in referral control populations, as recommended in MI redefinition and guideline documents (1, 3).

Coronary heart disease remains the most common cause of death in the Western world, and approximately 6–9 million patients with suspected acute coronary syndrome present annually to emergency departments in the US alone. Given the central role of cardiac troponin measurements, laboratories should be prepared to investigate potential interferences by having on hand heterophile blocking tubes, stacking protein A columns, and/or arranging access to alternative cardiac troponin methods.

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