False Increase of Cardiac Troponin I with Heterophilic Antibodies, Thomas F. Fitzmaurice,1 Charles Brown,1 Nader Rifai,2 Alan H.B. Wu,3 and Kiang-Teck J. Yeo1*

1 Department of Pathology, Dartmouth-Hitchcock Medical Center and Dartmouth Medical School, 1 Medical Center Drive, Lebanon, NH 03756; 2 Department of Laboratory Medicine, Children’s Hospital & Harvard Medical School, Boston, MA 02115; and 3 Clinical Chemistry Laboratory, Hartford Hospital, Hartford, CT 06102; * author for correspondence: fax 603-650-6120, e-mail Kiang-Teck.J.Yeo@dartmouth.edu

The effects on immunoassays of heterophilic antibodies (HAs) in the plasma and serum have been well documented (1–5). The use of monoclonal mouse antibodies for radioimaging of tumors as well as in the treatment of some cancers often gives rise to human anti-mouse antibodies. HAs can also arise as a result of incidental or occupational exposure to foreign proteins, as in the case of veterinarians, farm workers, and food preparers, or by the presence of domestic animals in the home environment. The prevalence of HAs in the general population has been estimated to be as high as 40% (1). Typically, these antibodies are of the IgG class and recognize epitopes on the Fc portion of the foreign immunoglobulin, although instances where the epitope is located in the Fab region have been reported (6,7). Additionally, the binding of these antibodies is not always species-specific, making it possible that antigens from two different species can be cross-linked. Although usually not clinically significant, the presence of high titers of these antibodies can lead to analytical errors in commonly used “sandwich” immunoassays by cross-linking the capture and label antibodies in the absence of specific analyte. Such cross-linking by HAs has been shown to lead to falsely increased CK-MB and CA125 (6–8).

Most modern immunoassays contain nonspecific, “blocker” immunoglobulins originating from the same species as the analyte-specific antibodies in an attempt to limit the effect of HAs; however, the problem persists. We report here a 69-year-old man whose plasma and serum cardiac troponin I (cTnI) concentrations were falsely increased when measured using the Abbott AxSYM® cTnI assay. We demonstrate that HAs accounted for the falsely increased cTnI, and we suggest how to resolve such situations.

A 69-year-old Caucasian man was found to have an asymptomatic 4.8-cm infrarenal aortic aneurysm on a computer-assisted tomograph scan. One month before admission, the tumor had grown to 6 cm, and surgical repair was complicated by left lower extremity ischemia and left femoral, anterior tibial, peroneal, and posterior tibial artery thrombi that required embolectomy. He was extubated after surgery; however, he required prompt reintubation during which brief cardiopulmonary resuscitation was performed. Shortly after the surgical procedure, his cTnI was 106 μg/L, suggesting that he had experienced an intraoperative myocardial infarction. At 5.5 h after surgery, his cTnI was 146 μg/L, but the electrocardiographic changes expected with markedly increased cTnI were not seen. The cTnI was 105 μg/L at 11 h and 103 μg/L at 21 h after surgery. His total creatine kinase (CK), CK-MB, and CK-MB index were 175 U/L, 2.9 μg/L, and 1.7% at 0 h; 1925 U/L, 20.2 μg/L, and 1.7% at 5.5 h; and 4397 U/L, 28.5 μg/L, and 0.6% at 11.0 h, respectively. He improved steadily and was discharged to home on day 9 after his initial admission.

cTnI and CK-MB were measured on an AxSYM analyzer (Abbott Diagnostics) by microparticle enzyme immunoassay with a capture monoclonal antibody and an alkaline phosphatase-conjugated polyclonal goat second antibody. Total CK was measured using a Hitachi 917 analyzer (Roche/Boehringer Mannheim Corp.). Treatment of samples with heterophilic blocking reagent (HBR) was performed according to the manufacturer’s instructions (Scantibodies Laboratory, Inc.). Briefly, 500 μL of either serum or plasma was added to a lyophilized pellet consisting of a mixture of animal immunoglobulins, mixed, and incubated at room temperature for 1 h before assay on the AxSYM analyzer. Plasma pools from either the patient with suspected HAs (HA sample) or patients with confirmed acute myocardial infarctions (positive control) were prepared and subjected to HBR treatment as described above. Treated and untreated HAs and positive-control samples were also analyzed using the Behring Opus cTnI assay (Dade Behring Corp.), the Immuno-1 cTnI assay (Bayer Corp.), the Dade Dimension cTnI assay (Dade Behring Corp.), and the Roche/BMC cTnT assay (Roche/Boehringer Mannheim Corp.) according to each manufacturers’ instructions.

Pretreatment of samples with AxSYM CK-MB diluent containing goat serum was performed by adding 0, 10, 20, 30, 75, and 150 μL of CK-MB diluent to the appropriate volume of either patient or positive-control sample to a final volume of 300 μL. Samples were incubated for 1 h at room temperature before duplicate cTnI assay on the AxSYM.

The case presented here illustrates how HAs continue to pose problems for modern sandwich immunoassays. The patient was a retired carpenter and had no obvious history of exposure to animal proteins either therapeutically or in a social setting. The increased plasma cTnI that did not correlate with the patient’s clinical picture initiated an investigation to determine if HAs were present. A plasma pool from this patient and a positive-control pool from patients with confirmed acute myocardial infarctions were treated with HBR. Both treated and untreated samples were assayed using the AxSYM cTnI assay as well as four other cardiac troponin platforms. The results of these assays (Table 1) indicate that only the untreated patient sample pool (HA sample) measured by the AxSYM cTnI assay gave an increased result (84.0 μg/L), which decreased to 1.5 μg/L on addition of HBR. The other four troponin assays gave results essentially below reference values for this sample. The sample from the positive-control individual showed a small decrease on HBR treatment, which has been noted previously and attributed to the blocking agent (8). When samples from
this patient were assayed for total CK, CK-MB, and cTnI in the presence or absence of HBR, only the AxSYM cTnI assay was completely blocked by the HBR. In addition, there was no clear peak observed for serial cTnI, suggesting an absence of an evolving myocardial injury (Fig. 1, panels A–C). The increase in total CK and CK-MB is consistent with skeletal muscle regeneration after surgery. The results of these studies suggested to us that HAs were present in the patient’s plasma.

If HAs are the cause of the falsely increased cTnI concentration, we questioned why the AxSYM CK-MB assay, which is also a sandwich assay based on a similar microparticle enzyme immunoassay format, was not affected. Both assays utilize a mouse monoclonal antibody as the primary, or “capture”, antibody and a goat polyclonal antibody as the secondary, or “labeled”, antibody. On further examination of reagent composition, it was noted that, although the mouse monoclonal anti-troponin I reagent contains mouse and goat proteins, the reagent containing the goat anti-troponin I conjugate antibody contains bovine and fish stabilizers. In the AxSYM cTnI procedure, the sample is first incubated with the monoclonal antibody reagent in the reaction vessel before being transferred to the matrix cell and washed. The conjugate antibody reagent is then added, and a second incubation takes place.

Knowing this sequence of events, we propose the following: Multispecific mouse/goat antibodies present in the patient’s serum at sufficient titer to overcome the effect of the “blocking” proteins bind to the mouse monoclonal anti-cTnI during the first incubation. An aliquot of this is added to the matrix cell and washed, removing all unbound proteins, including the blocking proteins. The conjugate antibody is then added, in the absence of any proteins capable of blocking the binding of the HAs to the goat immunoglobulin. The mouse anti-cTnI and goat anti-cTnI cross-link, producing a false increase of the measured cTnI concentration.

In contrast, the AxSYM CK-MB assay utilizes an assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>HA sample</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>AxSYM cTnI, μg/L</td>
<td>Untreated Treated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Untreated Treated&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Opus cTnI, μg/L</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Immuno-1 cTnI, μg/L</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Dade Dimension cTnI, μg/L</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>Roche/BMC cTnT, μg/L</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Refers to treatment with heterophilic blocking agent.

---

Fig. 1. Effects of HBR and CK-MB diluent on patient cardiac marker profiles.

Serial plasma samples from an HA patient were collected, and an aliquot of each sample was left untreated (▲) or was treated with HBR (●). These samples were assayed for total CK (A), for CK-MB in the AxSYM assay (B), and for cTnI in the AxSYM assay (C). (D) Various amounts of AxSYM CK-MB diluent (mL/L) were added to an HA sample (●) and a positive-control sample (●) and incubated for 1 h at room temperature before the cTnI assay.

Table 1. Comparison of cTnI values for a plasma pool from a patient with suspected HA and a positive-control pool from a patient with confirmed acute myocardial infarction.
diluent that contains goat serum. This diluent is added to the reaction vessel along with sample and the mouse monoclonal antibody and is also added to the matrix cell after the wash step but before the conjugated goat antibody is added. We hypothesized that the AxSYM CK-MB diluent acts as a better blocking reagent than the reagents used in the AxSYM cTnI assay. To test this theory, we diluted samples from both the patient and a positive-control individual with increasing amounts of AxSYM CK-MB assay diluent before performing the AxSYM cTnI procedure. The results in Fig. 1D show that increasing amounts of CK-MB diluent containing goat serum reduced the patient’s measured cTnI to 1.2% of initial untreated value (100 μg/L) when the diluent comprised 75% (by volume) of the total mixture. In contrast, a small decrease of ∼15% of the untreated value was observed in the positive-control sample (with no HAs).

These results strongly suggest that HAs present in this patient’s plasma are the cause of the falsely increased cTnI values. Although in this instance it appears that only the AxSYM troponin method was sensitive to the interference caused by the HAs, instances of false-positive results attributable to the presence of HAs have also been noted when other cTnI assays are used (personal observation by N. Rifai). Thus, it is important that the laboratory maintains a close liaison with the physician and that “discrepant” cTnI results are investigated by the use of other markers (such as CK-MB and total CK), HA blockers, and alternative cardiac troponin assays.

We thank Kelly Quinn-Hall, Jason Linscott, and Kim Laramie for expert technical assistance in performing these cardiac marker assays.

References