Interference by Human Anti-mouse Antibodies in Immunoassays: Falsely Elevated Cardiac Troponins Leading to Negative Coronary Angiograms

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Authors’ contributions

This work was carried out in collaboration between all authors. Author MBA designed the study, wrote the protocol and interpreted the data. Author SK anchored the field study, gathered the initial data and performed preliminary data analysis. Authors while IS, KMAH, MA and ANK managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

ABSTRACT

Cardiac Troponins are an extremely important indicator of myocardial injury and American College of Cardiology (ACC) and American Heart Association (AHA) guidelines include Cardiac Troponins as one of the major diagnostic criteria for myocardial damage. However in some instances the presence of heterophilic antibodies like Human Anti-Mouse Antibodies (HAMA) may interfere with the sandwich assays used for the detection and quantification of cardiac troponins giving false results which can have a major impact on the management of an individual suspected to have myocardial injury.

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We present a case of a 56 year old Caucasian male who had negative coronary angiogram 2 years ago presenting with acute chest pain and significantly elevated levels of Cardiac Troponin I (cTnI). He underwent cardiac catheterization which again turned out to be completely normal. His cTnI levels continued to be consistently elevated for months after cardiac catheterization. The reason for elevated cTnI levels was due to high levels of HAMA antibodies that caused heterophilic antibody interference in the assay of cTnI, resulting in falsely elevated levels of cTnI.

Keywords: Cardiac troponin I (cTnI); cardiac troponin T (cTnT); human anti-mouse antibodies (HAMA); heterophilic antibodies.

1. INTRODUCTION

Analytical interference in immunoassay can produce serious errors in clinical laboratory results. Clinicians should be aware of these limitations, since immunoassay results are used for both disease diagnosis and for monitoring response to treatment [1]. This paper reports an extremely rare case of a 56 year old man who presented with chest pain on two occasions with elevated plasma Troponin I levels (cTnI). Patient underwent cardiac catheterization only to reveal normal coronary arteries. We demonstrated that HAMA accounted for the falsely increased cTnI and how to manage such conditions. Our review of biomedical literature demonstrates previously known cases of HAMA and Troponins interaction leading to misdiagnosis or improper treatment.

2. CASE

A 56 year old non-smoker Caucasian male without risk factors of coronary artery disease and with a history of hereditary coproporphyria presented to the hospital with complaint of non-exertional, sharp, intermittent chest pain for the past four weeks. He described the pain as of moderate intensity, retro-sternal in origin without radiation or concomitant symptoms. He has had previous cardiac catheterization done for similar symptoms 2 years ago which showed no evidence of coronary artery disease. Our patient denied exposure to animals or animal based medications including any recent vaccinations. He denied history of blood transfusions in past.

On physical examination patient was found to be comfortable without acute distress. His vitals included temperature of 96.9°F, pulse 79/min, respiratory rate 18/min and blood pressure 148/82 mmHg. Cardiovascular examination showed normal pulses in all four extremities. He did not have heaves or thrills on palpation. S1 and S2 were normally auscultated and no murmurs were present. JVD was not raised. Rest of the physical examination was also unremarkable.

His basic lab workup did not show any abnormalities except for elevated Troponin I (cTnI).

EKG at presentation showed sinus rhythm at 84/min with normal axis. Chest x-ray did not reveal active pulmonary disease.

The cardiac marker notably cTnI was 16.90 ng/ml that remained elevated at 17.36 ng/ml at 8 hrs and 19.72 ng/ml at 16 hrs following admission in hospital. Total CK and CKMB were in normal range (Table 1).

After initial medical management, the patient underwent cardiac catheterization which showed coronary arteries to be angiographically normal with no impairment of ventricular function. Later the transthoracic echocardiogram failed to reveal any abnormalities. After the uneventful short course of stay in hospital, patient was discharged home in a stable condition.

Since this was the second time our patient underwent cardiac catheterization for elevated chest pain and cTnI only to reveal normal findings, a suspicion of HAMA related heterophilic antibody interference in the assay was made. This lead to measure cardiac troponins and Human Anti-mouse Antibody (HAMA) levels one month after discharge. The cTnI level continued to remain elevated at 17.99 ng/ml (normal range: 0-0.02 ng/ml), cTnT level was normal at <0.01 ng/ml (<0.01 ng/ml) and HAMA level was elevated at > 600 ng/ml (0-188 ng/ml).

Serial cTnI levels were obtained in months to follow which showed consistently elevated levels (Table 2).
3. DISCUSSION

Troponin-I (TnI) along with troponin-C and troponin-T subunits form the troponin complex, which is associated with the actin thin filament within muscle cells [2]. Troponin complex plays an integral part in regulating muscle contraction.

Cardiac troponin is the preferred biomarker for the detection of myocardial injury based on improved sensitivity and superior tissue specificity compared to other available biomarkers of necrosis, including CK-MB, myoglobin and lactate dehydrogenase [3].

The World Health Organization (WHO) criteria for describing myocardial infarction are the presence of two of the following three features: EKG changes, serum cardiac enzyme changes, and prolonged chest pain [4]. More recently, a Global Task Force with joint leadership among the European Society of Cardiology (ESC), the American College of Cardiology Foundation (ACCF), the American Heart Association (AHA), and the World Heart Federation (WHF) refined previous criteria with a universal definition of myocardial infarction that also supports use of cTnI as a preferred biomarker for myocardial injury [5].

At Conemaugh Memorial hospital we use the Abbott’s Architect stat kit to measure cTnI in serum. The ARCHITECT STAT Troponin-I assay is a two-step immunoassay to determine the presence of cTnI in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample, assay diluent and anti-troponin-I antibody-coated paramagnetic microparticles are combined. Troponin-I present in the sample binds to the anti-troponin-I coated microparticles. After incubation and wash, anti-troponin-I acridinium-labeled conjugate is added in the second step. Following another incubation and wash, pre-trigger and trigger solutions are then added to the reaction mixture. The resulting chemiluminescence is measured as relative light units (RLUs). A direct relationship exists between the amount of troponin-I in the sample and the RLUs detected by the ARCHITECT immunoassay system optics. The concentration of troponin-I is read relative to a standard curve established with calibrators of known troponin-I concentrations.

Our case highlights a very rare but known problem, in which the heterophilic antibodies interacts with the reagent giving false positive results. Human antibodies in circulation reactive with animal proteins i.e. anti-animal antibodies are an often under acknowledged and unanticipated source of interference in immunological assays, in particular non-competitive immunoassays [6].

Heterophilic antibodies, also known as anti-animal antibodies, can develop as a result of human body exposure to a known antigen e.g. therapy with mouse monoclonal antibody, but in most cases the antigens that gave rise to the anti-animal antibodies remains unclear [3]. One of such heterophilic antibodies are formed against mouse immunoglobulins, are known as human anti-mouse antibodies (HAMAs) [7]. HAMA is the most common kind of human anti-animal antibody. HAMA develop from iatrogenic and noniatrogenic sources. Iatrogenic being

<table>
<thead>
<tr>
<th>Cardiac markers (Ref.)</th>
<th>At presentation</th>
<th>After 8 hrs</th>
<th>After 16 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKMB (0-6.6 ng/ml)</td>
<td>0.9</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>CK total (30-280 IU/L)</td>
<td>72</td>
<td>65</td>
<td>71</td>
</tr>
<tr>
<td>Cardiac troponin I (cTnI) (0-0.02 ng/ml)</td>
<td>16.90</td>
<td>17.36</td>
<td>19.72</td>
</tr>
</tbody>
</table>

| Cardiac Troponin T-I and HAMA levels, 1 month after catheterization | |
|---------------------------------------------------------------|
| Cardiac troponin I (cTnI) level - ng/ml | 17.99 (0-0.02) |
| Cardiac troponin T (cTnT) level - ng/ml | <0.01 (<0.01) |
| Human antimouse antibody (HAMA) level - ng/ml | > 600 (0-188 ) |

| Serial troponin I (cTnI) levels ng/ml (Post Catheterization) | |
|---------------------------------------------------------------|
| 2 months           | 19.82       |
| 4 months           | 17.61       |
| 7 months           | 16.65       |

Table 1. Cardiac markers within 16 hours of hospitalization

Table 2. Post catheterization cTnI, cTnT, and HAMA levels
normal response of the human immune system to an administered foreign protein antigen including vaccination, antibody-targeted imaging reagents [8] and blood transfusions [9]. Non-iatrogenic causes of heterophilic antibodies include maternal transfer across the placenta to the unborn child [10], animal husbandry or the keeping of animals as pets [11], and the transfer of dietary antigens across the gut wall in conditions such as inflammatory bowel disease and celiac disease [12]. The main cause and reason for the increase in the incidence of HAMA is the use of mouse monoclonal antibodies for therapeutic and imaging purposes [13-15].

Heterophilic antibodies in human serum can react with assay immunoglobulins causing assay interference [16]. When they interfere, it is difficult to predict direction and magnitude of the interference. There are several possible mechanisms for assay interference. Either falsely high or falsely low immunoassay results may be produced by HAMA, depending on the configuration of the immunoassay and the specificity of the HAMA. Falsely elevated values typically occur when nonspecific HAMAs cross-link the capture and signal antibodies of “sandwich” immunoassays by binding to isotypic determinants expressed on the Fc portions of both antibodies. They may block the binding of the diagnostic antibodies, thereby producing a false-negative signal. Falsely low results occur with sandwich assays if the HAMA binds in a position that sterically inhibits the binding of the diagnostic antibodies to their antigens. Finally, anti-anti-idiotypic HAMAs can interfere with immunoassays by mimicking the role of the diagnostic antibodies and competing with them in binding with the target antigens [17,18].

4. HAMA AND CARDIAC TROPONINS

Although interference secondary to anti-animal antibodies is well documented in many assay like CA-125, CEA, CK-MB, FSH, hCG and TSH etc [19] but limited data is available when it comes to HAMA interference with cardiac troponins. Our review of literature reveals that Fitzmaurice in 1998 described the first case of HAMA interference with cTnI in an asymptomatic Caucasian male [20]. In 2002 multiple cases were reported. Cassim M in an Italian article described 2 cases of falsely elevated cTnI due to heterophilic antibodies interaction[21]. The same year E.N Ringdabl described a 43 year old woman with chest pain and elevated cTnI but the author did not establish elevated cTnI and HAMA relationship [22]. In 2007 Bionda C. described HAMA interaction in a 51 yr old male who unnecessarily underwent coronary angiography due to false positive CTnI [23]. Makaryus AN in 2007 described a case of 48-year-old man with elevated troponins attributed to heterophilic antibody interactions [24]. More recently in 2013, Giuseppe Lippi described a 76 year old female case of HAMA interaction with troponins leading excessive testing [25].

5. CONCLUSION

This presented case with falsely elevated cTnI confirms the limitations of immunoassays in the routine clinical setting. Although the risk of diagnostic errors cannot be avoided, it could be reduced through a closer and better communication between the physicians and the clinical laboratory on unexpected immunoassay test results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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