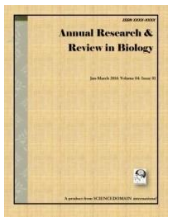

Annual Research & Review in Biology
6(5): 323-327, 2015, Article no.ARRB.2015.091
ISSN: 2347-565X

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

Mian Bilal Alam[1*], Surachit Kumar[2], Ibrahim Sbietan[2], K. M. Anwar Hussain[2], Mehwish Asad[2] and Asif N. Khan[3]

[1]Department of Internal Medicine, University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, United States of America.
[2]Department of Internal Medicine, Conemaugh Memorial Medical Center, Johnstown, PA, United States of America.
[3]Department of Internal Medicine, Charleston Area Medical Center, Charleston, WV, United States of America.

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.

Article Information

DOI: 10.9734/ARRB/2015/11941
Editor(s):
(1) George Perry, University of Texas at San Antonio, USA.
Reviewers:
(1) Hiroyuki Tamemoto, Department of Biochemistry, Jichi Medical University, Japan.
(2) Krystyna Sztefko, Clinical Biochemistry Department, P-A PI, Jagiellonian University College of Medicine, Poland.
(3) Anonymous, China.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=864&id=32&aid=8178

Case Report

Received 10th June 2014
Accepted 29th January 2015
Published 19th February 2015

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.

**Corresponding author: E-mail: bilalalam1@yahoo.com;*

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).

Table 1. Cardiac markers within 16 hours of hospitalization

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

Table 2. Post catheterization cTnI, cTnT, and HAMA levels

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

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

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

1. Bonetti A, Monica C. Interference by heterophilic antibodies in immunoassays: Wrong increase of myoglobin values. Acta Biomed. 2008;79(2):140-3.
2. Mair J, Wagner I, Puschendorf B, et al. Cardiac troponin I to diagnose myocardial injury. Lancet. 1993;341:838-9.
3. National Academy of Clinical Biochemistry Committee. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Clinical Characteristics and Utilization of Biochemical Markers in Acute Coronary Syndromes. Clin Chem. 2007;53:552‑574.
4. The Joint International Society and Federation of Cardiology/World Health Organization Task Force on Standardization of Clinical Nomenclature. Nomenclature and criteria for diagnosis of ischemic heart disease. Circulation. 1979;59:607-9.
5. Jneid H, Anderson JL, Wright R, et al. 2012 ACCF/AHA Focused Update of the Guideline for the Management of Patients With Unstable Angina/Non–ST-Elevation Myocardial Infarction (Updating the 2007 Guideline and Replacing the 2011

Focused Update): A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2012;60(7):645-681. DOI:10.1016/j.jacc.2012.06.004.

6. Ammann AJ, Hong R. Anti-antiserum antibody as a cause of double precipitin rings in immunoglobulin quantitation and its relation to milk precipitins. J Immunol. 1971;106:567–9.
7. Boscato LM, Stuart MC. Incidence and specificity of interference in two-site immunoassays. Clin Chem. 1986;32:1491–5.
8. Grossman HB. Clinical applications of monoclonal antibody technology. Urol Clin N Am. 1986;13:465–74.
9. Hawkins BR, Saueracker GC, Dawkins RL, Davey MG, O'Connor KJ. Population study of heterophile antibodies. Vox Sang. 1980;39:339–42.
10. Czernichow P, Van dalem JL, Hennen G. Transient neonatal hyperthyrotropinemia: A factitious syndrome due to the presence of heterophilic antibodies in the plasma of infants and their mothers. J Clin Endocrinol Metab. 1981;53:387–93.
11. Berglund L, Holmberg NG. Heterophilic antibodies against rabbit serum causing falsely elevated gonadotropin levels. Acta Obstet Gynecol Scand. 1989;68:377–8.
12. Falchuck KR, Iselbacher KJ. Circulating antibodies to bovine albumin in ulcerative colitis and Crohn's disease: Characterization of the antibody response. Gastroenterology. 1976;70:5–8.
13. Azrin MA. The use of antibodies in clinical cardiology. Am Heart J 1992; 124:753–68.
14. Hoffman T. Anticipating, recognizing, and preventing hazards associated with *In vivo* use of monoclonal antibodies: Special considerations related to human anti-mouse antibodies. Cancer Res. 1990;50:1049–50.
15. Wilder RB, DeNardo GL, DeNardo SJ. Radioimmunotherapy: Recent results and future directions. J Clin Oncol. 1996;14:1383–400.
16. Boscato LM, Stuart MC. Heterophilic antibodies; A problem for all immunoassays. Clin Chem. 1988;34(1):27-33.
17. Reinsberg J. Interference by human antibodies with tumor marker assays. Hybridoma. 1995;14:205–208.
18. Klee GG. Human anti-mouse antibodies. Arch. Pathol. Lab. Med. 2000;124(6):921–3.
19. Kricka LJ. Human anti-animal antibody interferences in immunological assays. Clin. Chem. 1999;45:942-956.
20. Fitzmaurice TF, Brown C, Rifai N, Wu AHB, Yeo KJ. False increase of cardiac Troponin I with heterophilic antibodies. Clin Chem. 1998;44:2212-4.
21. Cassin M. Two cases of false troponin I increase in patients with heterophile antibodies. Ital Heart J Suppl. 2002;3(2):235-8.
22. Ringdahl EN, Stevermer JJ. False-positive troponin I in a young healthy woman with chest pain. J Am Board Fam Pract. 2002;15(3):242-5.
23. Bionda C. Unnecessary coronary angiography due to false positive troponin I results in a 51-year-old man. Clin Chim Acta. 2007;378(1-2):225-6. Epub 2006 Oct 14.
24. Makaryus AN, Makaryus MN, Hassid B. Falsely elevated cardiac troponin I levels. Clin Cardiol. 2007;30(2):92-4.
25. Lippi, Giuseppe, et al. Interference from heterophilic antibodies in troponin testing. Case report and systematic review of the literature. Clinica Chimica Acta. 2013;426:79-84.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=864&id=32&aid=8178