False-positive high sensitivity troponin I on Alinity i

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ABSTRACT

Background: High sensitivity troponin I (Hs troponin I) is a precise and sensitive marker of myocardial injury. However, interferences by antibodies are not uncommon in immunoassays. Macrotroponin is one such phenomenon, in which immune complexes are formed between troponin and circulating antibodies, giving rise to false-positive results.

Case Presentation: This is a case of a 30-year-old male with elevated high sensitivity troponin I results of 419 ng/l (99th percentile upper reference limit for males is 34.2 ng/l). The test was performed on Abbott Alinity i immunoassay analyzer (Abbott Gmbh & Co. KG, Wiesbaden, Germany). Electrocardiogram, exercise tolerance test, and echocardiogram were all unremarkable. The results of creatinine kinase-myocardial component, troponin T, and conventional troponin I on VITROS ECi Immunodiagnostics System (Ortho-Clinical Diagnostics, Rochester, NY) were within normal limits. Laboratory experiments were carried out to evaluate the case, and the investigations conducted indicated the presence of macrotroponins in the specimen, which demonstrated cross immunoreactivity with the Alinity (Abbott) assay but not with the VITROS (Ortho) assay.

Conclusion: Clinicians and laboratorians should be aware of the possible interference by macrotroponin in their assays and should be alerted when there is a discordance between the laboratory and clinical findings.

Keywords: Macrotroponin, cardiac biomarkers, autoantibody, heterophile antibody, immunoassay interferences, case report.

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Background

High sensitivity troponin I (Hs troponin I) is a precise and sensitive marker of myocardial injury and is being increasingly used for early rule in and rule out of acute coronary syndrome. However, interferences by antibodies are not uncommon in immunoassays. Macrotroponin is one such phenomenon. Macrotroponin is a complex of immune-reactive troponin with a circulating antibody, which gives a falsely high troponin result on various analytical platforms. High sensitivity troponin assays are even more vulnerable to the phenomenon than the conventional assays. As more and more laboratories continue to adopt the high sensitivity assays, the problem is expected to increase. Both clinicians and laboratorians should be wary of the condition as it can lead to potentially unnecessary investigations and interventions. This is the first reported case of macrotroponin I in Pakistan and on Abbott Alinity i immunoassay analyzer.

Case Presentation

A 30-year-old male, medical laboratory technologist by profession, while performing a study on the verification of 99th percentile upper reference limits for high sensitivity troponin I assay on Abbott Alinity i immunoassay analyzer, demonstrated a value of 419 ng/l (claimed 99th percentile URL 34.2 ng/l) on his plasma specimen. The technologist was asymptomatic but gave a past medical history of palpitations and chest pain and was referred to the Emergency Department by the laboratory personnel. Cardiologist was called in. Electrocardiogram, echocardiography, and exercise tolerance test (ETT) were done and were all unremarkable. Creatinine kinase-myocardial component (CK-MB) was performed, which was 24 IU/l (reference interval < 25 IU/l). A qualitative troponin T assay on Roche immunochromatographic device was negative. Repeat high sensitivity troponin I after 2 and 4 days was still high at 423 and 366 ng/l, whereas the technologist remained asymptomatic. The specimen was tested on VITROS ECi (Ortho-Clinical Diagnostics) for conventional troponin I, and the result was < 0.012 ng/ml (negative).

Considering possible interference by heterophile antibodies or macrotroponin, a linearity study was carried out. The results were found to be linear across a dilution range of 1:1 until 1:4. The test was then repeated after precipitation with 25% polyethylene glycol. The specimen was incubated for 10 minutes with an equal quantity of 25%

solution of polyethylene glycol. It was then centrifuged at 5,000 rpm for 10 minutes, and the supernatant was analyzed for troponin I. The result after accounting for the dilution factor was 1.26 ng/l, which is below the limit of quantification of Alinity high sensitivity troponin I assay. The recovery as calculated by dividing the percentage of troponin I in the supernatant with the patient's original result was less than 1%, compared to 70.4% recovery in the internal control solution. These investigations indicate the presence of macrotroponins in the specimen causing falsely elevated high sensitivity troponin I results on Alinity i.

The case reinforces the fact that potential interferences exist with high sensitivity troponin assays. Clinicians should be aware of these interferences and interpret the results in correlation with the clinical findings.

Discussion

Cardiac troponins are now considered to be the best biomarkers of cardiac injury. High sensitivity cardiac troponin assays have been recently advocated because of their superior detection limit and high precision. These biomarkers are the cornerstone in diagnosing acute myocardial infarction as per its definition, whereby demonstrating a rise or fall of the biomarker along with evidence of ischemia is required for the diagnosis [1].

The cardiac troponins, troponin I, troponin T, and troponin C make up the myofilaments which are responsible for cardiac muscle contraction. Troponin I occurs as three isomers. There is an isomer in the cardiac tissue with 209 amino acid residues and molecular weight 23-24 kDa, whereas an isomer of troponin I is produced each in slow- and fast-twitch skeletal muscle fibers, having an approximately 40% homology with the cardiac troponin I [2]. Therefore, the assays which are developed for cardiac troponin I should have a high degree of specificity. In addition, the high sensitivity troponin assays have an increased requirement for lower detection limits and greater precision [3].

As with other laboratory tests, variations can occur in the troponin assays due to pre-analytical, analytical, biological, and pathological factors [4]. Among the analytical considerations, interference by heterophile antibodies is one of the most important. Heterophile antibodies are antibodies which occur in the serum against unidentified antigens and are cross-reactive to a variety of antigens. They can cause interference in immunoassays by binding to the Fc component of the assay antibody, thus giving a false-positive result. Heterophile antibodies can develop in patients after blood transfusions, treatment with monoclonal antibodies, exposure to animals, vaccination, or the presence of autoimmune disease [5,6]. The incidence of heterophile antibodies has been quoted to be around 3.1% in the literature [4].

Antibodies to troponins can also exist causing a false-negative result by inhibiting the binding of assay antibodies to cardiac troponin and thereby generally going unnoticed [4]. The incidence of troponin I autoantibodies has been quoted as between 2% and 20% in the normal population [7,8]. On the other hand, autoantibodies can also cause an increase in troponin I by causing cardiotoxicity [9]. Around 5% of discrepancy between troponin I on various assays could be due to the presence of autoantibodies accounting for assay interferences as shown by Warner [8].

Macrotroponin is a special form of interference in immunoassays, in which an autoantibody binds to the analyte of interest, thus forming a large molecule which is difficult to excrete from the bloodstream and gives a persistent false-positive result. This was commonly seen for some other protein analytes such as amylase and prolactin, but now troponin assays are shown to be susceptible to this interference as well, with high sensitivity assays being more vulnerable. A recent study [10] has stated the prevalence of macrotroponin as 55% which is an alarming figure if analytical concerns are not met. Among the various assays available, Abbott's high sensitivity assay on Architect has shown a preponderance of this interference [8,11–13], suggesting that the Abbott assay somehow, due to reasons not yet clear, develops more cross binding to the macrotroponin complex than other assays. Alinity series is a newer analytical platform launched by Abbott Diagnostics. The report is the first such reported case on Alinity i in the region. Macrotroponins can also give falsely low results if the targeted epitope in the assay is not captured [10].

Various methods are employed in the laboratory to work up for the potential interferences in immunoassays [4,6,12]. For the suspected cases with heterophile antibodies, antibodies blocking agents can be added to the specimen before analysis. Another way to identify cross-reacting heterophile antibodies is to serially dilute the specimen [14]. If the dilutions fail to give a linear recovery, it is likely to be due to the presence of heterophile antibodies. Incubation with 25% polyethylene glycol and then re-centrifuging the specimen leads to precipitation of the high molecular weight molecules, including macro troponins if any, and low recovery of the analyte in the supernatant. Performing troponin I by a different platform using varied antibody target epitopes and performing tests for alternate biomarkers, for example, troponin T or CK-MB can give additional clues. In this case, repeated troponin I assays by the same method on Alinity i remained high, whereas results were linear on serial dilution and gave very low recovery after precipitation with polyethylene glycol. This pointed toward the presence of macrotroponin in the specimen, causing falsely elevated results. Further confirmation was done by performing cardiac troponin T and

CK-MB, which were both within reference limits, and performing troponin I on an alternate method, VITROS ECi, which too was negative.

Although the phenomenon of heterophile antibodies and macrotroponins is known in the literature, it is highly likely that many such cases go unnoticed and unreported. This can lead to a potential harm to the patient with unnecessary investigations, interventions, and treatment. This is only one case, which has been brought to the light of the laboratory, but it is likely that there are more which have not been picked so far. It is proposed that if there is a lack of clinical correlation with the results, including the characteristic temporal rise and fall of the troponins, as seen in this case, the physicians should suspect the presence of macrotroponin or other antibody interferences and should communicate with their laboratory colleagues for the specimen to be worked up.

There were certain limitations in the study. We did not have the resources to study the effect of addition of heterophile antibody blocking reagents or immunoglobulin binding proteins such as protein A or G to the specimen or to do analysis by gel filtration. This is a single case only. A well-defined protocol needs to be established for laboratories to follow whenever discordant results of high sensitivity troponin I are observed.

Conclusion

Clinicians and laboratorians should be aware of the possible interference by macrotroponin in their assays and should be alerted when there is a discordance between the laboratory and clinical findings.

What is new?

Selected cases of interferences in troponin assays have been reported in the literature. High sensitivity troponin assays are becoming increasingly common in the region and are more prone to these interferences. The manuscript describes a case of macrotroponin I on alinity I, a newly launched testing platform by Abbott Diagnostics.

Consent for publication

Written informed consent was taken from the patient.

Ethical approval

Ethical approval is not required at the institution for publishing an anonymous case report.

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Summary of the case

1	**Age**	30 years
2	**Gender**	Male
3	**Symptoms**	None
4	**Diagnosis**	Macrotroponin I
5	**Investigations**	High sensitivity troponin I, troponin T, CK-MB, conventional troponin I, electrocardiogram, echocardiography, ETT, linearity studies, precipitation studies
6	**Treatment**	None
7	**Specialty**	Cardiology, Clinical Chemistry