

Assay what? The troponin masqueraders— a case report

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Abstract

Guidelines defining the universal diagnosis of myocardial infarction recommend the rise and/or fall of a cardiac biomarker, preferably troponin, above the 99th percentile of a healthy population in patients presenting to hospital in conjunction with chest pain and/or electrocardiographic changes. This report considers the case of a 44-year-old woman with ongoing chest pain and chronic troponin elevation. Highlighting the importance of a delta change of troponin, with a rise and fall in blood level, in conjunction with clinical symptoms; without which interpretation of laboratory tests in the assessment of patients presenting with possible cardiac ischemic injury can be fraught with uncertainty. We describe sources of potential assay interference, as demonstrated in this case, and how such cases can be approached.

Keywords: acute myocardial infarction; cardiac troponin; assay interference; heterophile antibodies; delta change.

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Case Report

Case report

A 44-year-old female with a history of ongoing chest pain and cardiac troponin T (cTnT) elevation presented to the outpatient department of St Thomas' hospital, London, United Kingdom. She had a past medical history of antiphospholipid syndrome, but no other risk factors for coronary disease. Her medication regime was Warfarin, Plaquenil and Mycophenolate Mofetil. Her presenting complaint was that of ongoing atypical chest pains, occurring intermittently, not always associated with exertion. Her index admission was to another local hospital three years earlier, after complaining of left sided chest and arm pain. A laboratory measured cTnT was elevated at 0.5ng/ml (cut-off for diagnosis of myocardial infarction (MI) >0.03ng/ml, cTnT Elecsys E170, Roche). Interestingly, an ECG showed normal sinus rhythm at 85 beats per minute with T wave inversion in the chest leads V4-V6, with no ST-segment abnormalities; with these changes appearing static. A chest x-ray showed a normal cardiothoracic ratio with clear lung fields. Other blood tests performed included a full blood count, renal and liver function, glucose, CK, CK-MB and myoglobin, which were all within normal limits.

In view of the presenting symptoms and elevated cTnT treatment for a possible Acute Coronary Syndrome (ACS) was commenced, and a diagnostic coronary angiogram was subsequently performed. A delta change of cTnT was not considered as the initial result was high above the threshold for diagnosis of MI. Coronary angiography revealed a possible ostial

circumflex artery lesion of 50% diameter stenosis, but otherwise angiographically normal epicardial coronary arteries. However, the cardiologist performing the catheterization procedure noted severe chest pain upon each injection of intra-coronary contrast. The patient was transferred to the local interventional center for consideration of percutaneous coronary intervention (PCI) to the circumflex artery stenosis. Repeat angiography at the tertiary center, including intravascular ultrasound (IVUS) assessment of the ostial circumflex lesion, revealed normal mean luminal areas with no suggestion of a significant flow limiting stenosis, in this, or any of the other, coronary vessels. Therefore revascularization with PCI was not performed. Despite the reassuring nature of the angiogram, the patient continued to be troubled with chest pain, and repeated cTnT measurements continued to be elevated. Thus an echocardiogram and CT-pulmonary angiogram (CTPA) were performed to further investigate potential alternative causes of her symptoms. The echocardiogram, however, revealed normal left and right ventricular size and function and normal valve function and the CTPA excluded the possibility of a pulmonary embolus. The patient was discharged on aspirin, ramipril long-acting diltiazem and a GTN spray in addition to her prior medications. The cTnT concentrations were 0.5ng/ml at presentation and remained elevated, peaking at 0.9ng/ml (Fig. 1).

Unfortunately the patient continued to experience chest pains and returned again to the Emergency Department (ED) with left sided arm and chest pain, with her accompanying ECG being identical to her previous admission. A cTnT 12-hours after symptom onset was elevated at 0.7ng/ml and therefore a further coronary angiogram was performed. This did not

demonstrate any new lesions and the suspicion of assay interference was raised. However, no confirmatory tests on the serum were performed and thus a diagnosis was not established for the patient.

Following further specialist reviews she presented to the outpatient department here with a cTnT measured on the high-sensitivity Roche assay of 52ng/ml (Roche Elecsys, high-sensitivity cTnT, 99th percentile 14ng/ml). Examination was normal and her ECG remained unchanged. A cardiac MRI showed no evidence of wall motion abnormality, myocardial scarring or fibrosis. Other bloods remained normal, but an NT-pro BNP was mildly elevated at 422ng/L (cut-off for interval outpatient echocardiogram is 400). As a first line of testing, serial dilutions of the samples were made which demonstrated a non-linear response. Furthermore, samples were incubated with blocking reagents (HBR) containing a mixture of animal immunoglobulins (Igs) and the concentrations of cTnT were normal after this treatment. This further emphasizes the likely presence of interfering heterophile antibodies (HAs). Also, samples were tested for cTnI and CK-MB, which did not show elevated concentrations. The patient had a chronic autoimmune condition and rheumatoid factors (RhF) were present in her blood. This finding further supported the presence of interfering antibodies in the serum. However, RhF antisera was not used in this case.

Comment

The cardiac troponins (cTn), I (cTnI) and T (cTnT), by virtue of their biologically mediated high cardiac specificity have become the preferred biomarkers for myocardial cell death. They are incorporated into the 2007 "Universal definition of Myocardial Infarction" where a rise and/or fall above a threshold is part of the diagnosis of MI [1]. However, the clinical context is paramount to interpretation, a fact stressed in the Universal definition of MI, but often forgotten in the vim of the ED environment. Clinical evidence of myocardial ischemia is necessary because serum cTn elevations are not necessarily due to an ACS – cTn does not convey etiology [2]. Among patients with a high pretest probability of athero-thrombotic coronary heart disease (CHD), the diagnostic and prognostic value of cTn is clear [3, 4]. On the contrary, in patients with a low pretest probability of CHD, cTn elevations can be nonspecific and may divert attention from the true underlying clinical problem

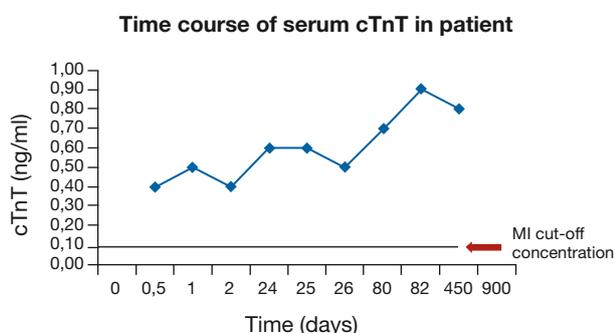


Fig. 1 Demonstration of the chronicity of elevation of cTnT in the patient over several admissions and without any obvious rise and fall.

[4]. This leads to unnecessary cardiac evaluation, invasive testing and inappropriate medication. cTn elevation, but absence of significant coronary disease on coronary angiography may be termed a false positive [5]. Several differential diagnoses must be entertained in such instances [6]. Among this list of differentials are analytical issues pertaining to the specific assay being used to measure cTn concentration: interference can produce a false positive result [Table 1]. Such cases have been reported previously [7] and there are certain recommendations for laboratory staff to follow in order to confirm such false positive results [8]. Awareness of this possibility may assist the physician in the management of the patient and may spare the patient additional, potentially invasive, diagnostic procedures. It is also noted that these concentrations often are at the limits of analytic sensitivity where their presence can blur the separation between clinically important and interference.

Interference can result from a variety of sources but HAs are one common source [9, 10]. The incidence of interference from HAs alters depending on assay and study group varying from 0.2% to 40% [10, 11] and the magnitude of the interference varies from sample to sample, as well as within a patient over time. HAs are endogenous antibodies that cross-react with immunoglobulins of different species causing cross-linking of capture and detection antibodies in immunoassays (Fig. 2a, 2b). Their main interfering effects are seen in two-site immunometric assays. This cross-linking by the HAs can thus lead to positive or negative interference with cTn measurement [12]. Mouse monoclonal antibodies are often used in these assays (to aid specificity) and development of human anti-mouse antibodies (HAMAs) can follow, for instance, the use of murine monoclonal antibodies for therapeutic or imaging purposes [13]. A variety of

methods from transfusions, vaccinations or autoimmune diseases can induce broadly reactive HAs that bind Igs from other species [7]. These antibodies are multispecific antibodies, with weak affinity usually of IgG class directed toward the Fc portion of the antigen.

The pertinent element of this case is the lack of rise and fall in the cTn concentrations that would be characteristic (and required) for MI [14] but given the initial high level treatment was commenced on the first positive sample, as it should be according to guidelines. This case also describes a patient with co-existent antiphospholipid syndrome (APS): which typically represents a syndrome of recurrent venous or arterial thrombosis and/or fetal losses in which persistently elevated concentrations of antibodies directed against membrane phospholipids are noted [15]. APS may

Human anti-mouse antibodies (HAMAs) Heterophile antibodies Autoantibodies Rheumatoid factor Haemolysed samples Clots Macrocomplexes of troponin with IgG
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Table 1 Causes of assay interference (potential false positive results).

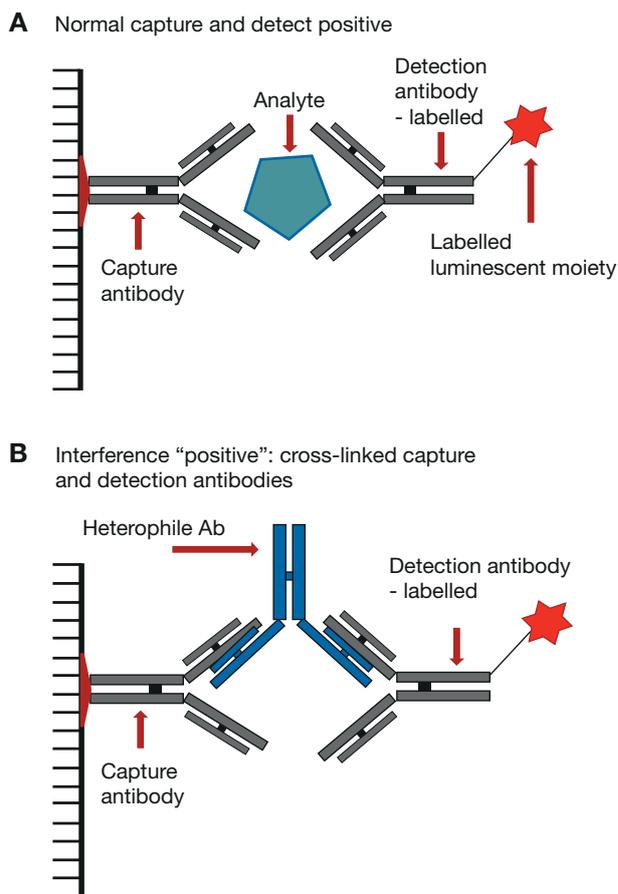


Fig. 2 A: Usual method of binding with analyte across capture and detection antibodies to generate a fluorescent or chemiluminescent signal for detection and **B:** Interference from a heterophile antibody or HAMA to cross-link the sandwich antibodies and cause signal generation even in the absence of analyte (adapted with permission from Scantibodies, USA).

contribute to an increased incidence of MI. APS is also associated with circulating Rheumatoid Factor (RhF) [16] and in a series Dasgupta et al. [17], report false positive cTnI results in 12 patients with positive RhF (5% of healthy patients are RhF positive). 7 of these patients had measurable cTnI concentrations, 4 were above the diagnostic cutoff for MI but none had indication of MI on invasive tests. By using polyclonal antisera against RhF, cTnI concentrations were normalized, interestingly, none of these specimens had detectable cTnT.

Conclusion

This case highlights the importance of communicating with our clinical chemistry colleagues. If suspicion of interference is raised it may be necessary to repeat the test, dilute the sample to check linearity of results or re-assay on another manufacturer's platform. The use of blocking antibodies to pre-treat the sample (Heterophile blocking reagents, HBRs, Scantibodies, California, USA) can also be tried. When HBR binds to the human heterophilic antibody, the blocking may be accomplished by steric hindrance. Parallel analysis of CK-MB to observe a rise and fall may also help establish a cause. The laboratory may need heterophile blocking tubes, protein A columns or be able to send to another lab to measure cTnT or I as appropriate [18]. Many of the modern assays will now incorporate steps or reagents to minimize the cross-reactivity and interference but they are not always infallible and it remains the clinicians' responsibility to alert the laboratory to any potential discrepancy between the assay measurement and the clinical picture. However, when clinical decisions are sometimes forced to be made at the technical limits of assays misinterpretation is inevitable and may well increase in the era of high-sensitivity assays. Therefore, as in this case, a false-positive cTn result should serve as a reminder that, although the assays are an integral part in the diagnosis of ACS in contemporary clinical practice, other diagnostic modalities, including a careful clinical history and electrocardiographic markers of ischemia, continue to be important in accurately aligning the clinical

scenario with elevated cTn results in confirming the diagnosis of an ACS. •

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