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Case Report

A false positive case of high-sensitivity cardiac troponin in a patient with acute chest pain: Analytical study of the interference

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ABSTRACT

We report a case of a heterophile antibodies interference in a new high-sensitivity troponin commercial immunoassay (cTNIH Siemens), observed in a patient with possible acute coronary syndrome (ACS). The analytical interference was investigated with standard laboratories procedures. The false positive result was found with different troponin methods and kits. We also investigated the protein sequence of cTnI and no sequence variants were detected. The discordance between clinical pictures and high concentration of cTnI, together with the collaboration between clinicians and laboratory staff avoided possible erroneous diagnosis and further invasive investigations to the patient.

1. Introduction

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) are currently considered the biomarkers choice for the diagnosis of ACS, and the fourth Universal Definition of Myocardial infarction (MI) has recently updated the definition of MI in order to accommodate the increased use of high-sensitivity cardiac troponin (hs-cTn) [1]. The high-sensitivity assays are able to measure cardiac troponins with a high degree of analytical sensitivity and a low level of imprecision at the low measuring range, allowing more rapid approaches for ruling in or ruling out ACS diagnosis. However, this increase comes at the cost of a reduced specificity of the biomarker, as more and more patients with other causes of acute or chronic myocardial injury or with non AMI-related pathologies are also been detected [2]. When an increased cTn value is found, in the absence of ACS, a careful investigation for other possible etiologies should be undertaken. However, one of the overlooked reasons of elevated troponin, although rare, is a false-positive test, which is due to analytical interference from other substances in the patient's blood [3,4].

2. Case presentation

A 52-year-old male arrived at the Policlinico A. Gemelli, Emergency Department with chest pain, mostly in the epigastric area, radiating to left arm; started during a rest period 3–4 h before. He didn't have cardiovascular risk factors or a history of coronary or cerebral artery disease. The family history was negative for coronary heart disease and stroke, no drugs or vitamins were taken beforehand (biotin included) and there was no presence of significant diseases in anamnesis [5]. The physical examination was normal, with no presence of dyspnea, palpitations or nausea. The electrocardiogram (ECG) showed normal sinus rhythm and no significant ST or T wave alterations. The blood pressure was elevated to 170/90 mmHg. The patient laboratory parameters, including creatinine and creatine kinase were within the reference ranges.

The cardiac troponin measured by the conventional assay cTnI-ultra Siemens (cutoff 0.040 μg/L), routinely used in our Laboratory (Policlinico Agostino Gemelli) on serum sample, were negative both at the admission and 3 h afterwards (0.012 and 0.008 μg/L respectively). As part of our Laboratory continuous evaluation program of analytical performances of new methods, we were examining the high-sensitivity TNIH Centaur XPT Siemens kit and therefore assaying troponin, simultaneously with cTnI-ultra and cTNIH kits. In our case, interestingly, we found high troponin levels (129 and 140 ng/L) using TNIH kit (cut-off 47 ng/L) in the same serum samples of the patient, at basal time and 3 h later. We had already compared about 100 patients before this finding and since we were concerned about the discrepancy between cTnI-ultra and cTNIH concentrations we contacted the clinicians. The troponin values are decisive when we record uncertain and suspicious cases; moreover the high-sensitivity of the cTNIH kit could have provided a better description of the patient conditions for the myocardial injuries compared to cTnI-ultra. Even if the patient was apparently in

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Table 1
Patient cTnI results using different kits on a same serum sample; methods characteristics and analytical performances of assays (manufacturer's data).

Kits	*Results*	*LOD*	*Cut-off*	*Method*	*Antibodies source and characteristics*
cTnI Ultra Siemens μg/L	0.012	0.006	0.040	*CLIA*	Polyclonal goat and two biotinylated monoclonal (mouse)
TNIH Centaur XPT Siemens ng/L	129	2.2	47	*CLIA*	Streptavidin-coated magnetic latex biotinylated (mouse, sheep) monoclonal R recombinant monoclonal Fab (sheep)/acridinium ester
Pathfast cTnI ng/L	9	1	27	*CLEIA*	Magnetic particles coated monoclonal (mouse) Alkaline phosphatase (calf intestine) monoclonal (mouse)
Dimension Vista Siemens ng/L	114	2.0	57	*LOCI*	Beads with biotinylated (mouse and sheep) monoclonal Streptavidin Sensibead
Vidas bioMérieux ng/L	36	1.5	19	*ELFA*	Alkaline phosphatase monoclonal (mouse) fluorescent substrate
Singulex ng/L	1.8	0.14	8.67	*SMC*	Biotinylated monoclonal conjugated to paramagnetic microparticles fluorescent label
Roche hs TnT ng/L	7	5	14	*ECLIA*	Biotinylated monoclonal mouse Mouse-human chimeric

good condition and the pain was no longer reported, the clinicians decided to admit him to the Observation Unit to monitor and undergo a complete investigation. During this observation no features of cardiac pathology was identified; the echocardiogram showed normal ventricular contractility and no evidence of myocardial ischemia and the ECG stress test was negative (no ECG alteration or angina). The troponin levels, respectively after 6 and 12 h from the first examination and 3 h after the ECG stress test, were always stably negative using cTnl-ultra kit. On the contrary, markedly increased levels (132 and 128 and 131 ng/L at 6 and 12 h and after stress test, respectively) with cTNIH kit were confirmed. As the clinical picture and the instrumental investigations (ECG, echocardiogram and stress ECG test) were incongruous with high troponins concentrations, we suspected that an analytical interference could be the cause of this discrepancy, in agreement with the cardiologist's opinion. The patient was discharged after 24 h, but additional samples of plasma and serum were obtained after informed consent and stored at −80 °C, for further investigation to better recognize the presumable interference.

3. Discussion

Interference is defined as the effect of a substance present in a sample that alters the correct value of the result [3]. Analytical interference from various nonspecific causes is a common problem that affects, to some extent, almost all immunoassays, producing false-positive or false-negative results [6]. A possible discrepancy between serum and heparinized plasma samples can occur if the antibodies are affected by heparin. Since the cTnl molecule has a high positive charge, it will attract negatively charged molecules such as heparin, which in turn can interfere with the antibody antigen interaction [7].

However in our case, the troponin concentration was always less than the cut-off level with cTnl-ultra kit, on the contrary, it was always high with the cTNIH kit, both in plasma heparin or EDTA and in serum. Moreover, the patient laboratory findings, including creatinine, AST, ALT, protein electrophoresis, alkaline phosphatase, immunoglobulins lgA, lgG, lgM, leukocytes were within the reference range. The most frequently described interfering mechanisms are those caused by autoantibodies (anti-troponin), rheumatoid factors, complement and human anti-animal antibodies, which include human anti-mouse antibodies (HAMA). A person could also develop anti-reagent antibodies after a continuous exposure to animal antigens, or after multiple transfusions or with the administration of monoclonal antibodies for therapeutic reasons. Additionally, sometimes, the mechanism is poorly defined (heterophilic antibodies) [3,4]. In our patient there was no clinical or laboratory evidences of infection, autoimmune diseases or skeletal muscle damage; the C-reactive protein, ANA, ENA, myoglobin, and CK-MB mass being within normal values. The rheumatoid factor was negative (< 10 IU/mL) and therefore this wasn't part of the cause. However, autoantibodies to cTnl mainly cause a negative interference (no false positive values) by blocking the binding of the assay antibodies to the mid-fragment of cTnl.

A test that is usually applied to detect the presence of interference is the lack of dilution linearity. Therefore, the patient serum was serially diluted with a serum sample with undetectable concentration of troponin by cTnl-ultra kit and 1.5 ng/L by TNIH kit (Limit of Detection 2.2 ng/L) [9], in order to check for linearity of results. The serial dilutions demonstrated that the values obtained by TNIH kit were non-linear (1:2 55 ng/L; 1:4 33 ng/L; 1:8 21 ng/L; 1:16 12 ng/L; 1:32 9 ng/L), thus suggesting the presence of an interfering substance within the patient sample.

The polyethylene glycol (PEG 6000) precipitation performed on the patient serum suggested the possible presence of a high-molecular-mass interference; in fact, after PEG with cTNIH kit we obtained a recovery of troponin value of 30%. This result is above recovery range of 20%, often used as the cut-off [8], but it was much lower than recovery (88%) observed in a serum from a patient with STEMI, assayed in parallel as control. The falsely elevated troponin was also confirmed by treatment of the patient's serum with a heterophile-blocking reagent (Scantibodies Laboratory, Santee, CA, USA), a formulation of immunoglobulins targeted specifically to neutralize their interference. Upon treatment cTnl decreased to a level below the LOD of the method [9], while the block tube did not alter TNIH value in the control serum of the patient with STEMI, thus indicating that the initial result was falsely elevated, due to interfering heterophile antibodies.

Interferences can have varying degrees of impact depending on the assay protocol used. Therefore, we decided to investigate the behavior of the patient's serum on different troponin kits, with the aim to fully comprehend where the interfering molecule influenced the analytical signal. Then, the serum sample with cTnl ultra 0.012 μg/L and TNIH Centaur 129 ng/L was re-assayed with the following methods: hsTnT (Elecsys Roche), hs Pathfast cTnl (Mitsubishi), Vidas hsTnl (BioMérieux), Singulex Clarity cTnl (Singulex) and TNIH Dimension Vista (Siemens). Troponin values were less than cut-off using hsTnT, Pathfast and Singulex kits; on the contrary, high concentrations were observed using Vidas and TNIH Dimension kits (Table 1).

These assays use different epitopes across the cTnl molecule (Fig. 1) and different analytical methods (Chemiluminescence CLIA, Electro-ChemiLuminescence ECLIA, Chemiluminescence enzyme CLEIA, Luminescent Oxygen Channeling LOCI, Enzymelinked fluorescence ELFA, Single Molecule Counting SMC TM).

The molecular heterogeneity of the cTnl molecule causes challenges in the selection of antibodies for cTnl assays. The troponin undergoes different post-translational modifications, with proteolytic degradation and various cTnl complexes are found in the circulation. The sequence located between amino acid residues 30 and 110 of cardiac troponin l (cTnl) is the most stable region of the cTnl: this is the area most often targeted for antibody detection for immunoassays, as recommended by IFCC [2]. Therefore, different manufacturers' assays can be variably or similarly influenced, depending on the epitopes targeted, even if in the last years the introduction of the international cTnl standard (SRM 2921) and the gradual standardization of the epitopes detected in commercial assays has been applied. Most kits utilize antibodies that

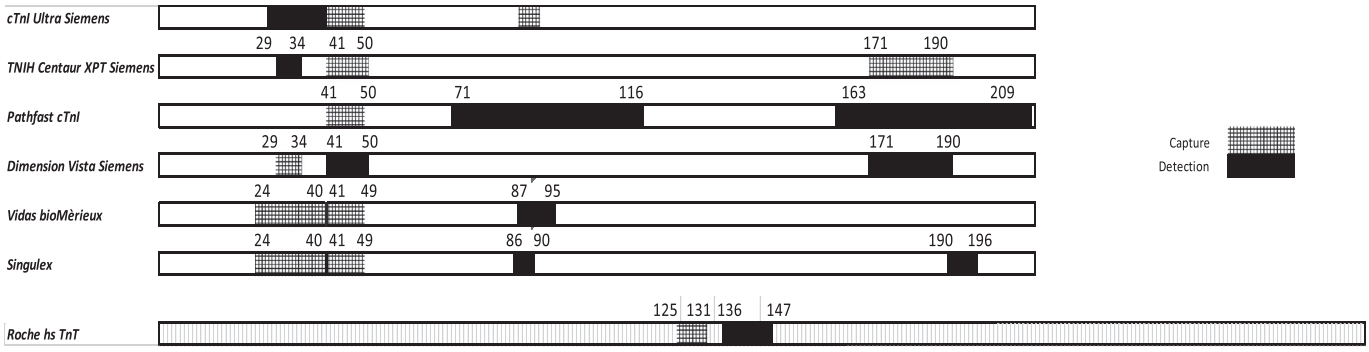

Fig. 1. Epitopes of the antibodies utilized in cTnI immunoassays (9).

are specific to three regions of the cTnl molecule: 23–43, 41–56 and 83–93 [10].

In our patient, the interference was observed with both TNIH Siemens (Centaur XPT and Dimension Vista) using capture and detection antibodies specific to same regions, but also with Vidas hsTnl kit using antibodies targeted to same epitopes of Tnl ultra kit, with whom there was not interference; however they have different assay architecture. The origin of the heterophilic antibodies is not so clear and in our patient the antigens that gave rise to the anti-animal antibodies remains unknown. In fact, there is no clear correlation between the antibodies source and the reported interference. These antibodies can bind to immunoglobulins of other species, including the species used to generate the antibodies used as reagents for immunoassays. The heterophilic antibodies can interfere by bridging the capture and signal antibodies of sandwich immunoassays in the absence of the antigen to be detected, giving a false-positive test result, as presumably happened in our patient with TNIH Centaur, TNIH Dimension and Vidas kits.

Moreover, we investigated the protein sequence of the cardiac troponin l of our patient to search if a sequence variant was present in the binding site of the molecule, involving any epitopes recognized by kits antibodies. It could have explained the different results and interferences among different assays [11]. Genomic DNA was isolated from patient peripheral blood sample, the extracted DNA was amplified by PCR and then sequenced using the following primers: 6F-ACAACACACACCACGTICCT/6RGAGACCAAGTCCCAGCCATC and 7F-GTGTAGGATGGAGGAGTIGGG/7RAGGCCTAGGGTIGTIGGCA for exon 6 and exon 7 respectively. However, no single nucleotide polymorphism or other variants were observed. At six months follow-up, the patient clinical course remained without additional events and laboratory findings were unchanged: ECG and echocardiogram within normal limits whereas cTnl-ultra and TNIH values confirmed our previous results.

In conclusion, even if cTnl assays have been optimized to reduce erroneous results by heterophilic antibodies, the analytical interference is always possible; managing these cases requires a close and strong collaboration between clinicians and laboratory. In this work we observed an antibody interference in three high-sensitivity troponin kits (Vidas hs Tnl, TNIH Centaur and TNIH Dimension Vista), in a patient with no underlying genetic variant and no cardiac injury.

In our case, thanks to the effective collaboration with the Emergency Department we managed to highlight the discordance between the clinical setting and the laboratory findings and consequently to identify the underlying cause of elevated cTnl since the very first results, thus avoiding unnecessary invasive investigations and continued anxiety to the patient.

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