Case report

Interference by heterophilic antibodies in immunoassays: wrong increase of myoglobin values

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Abstract. Aim of this work is to illustrate how analytical interference in immunoassay may produce serious errors in clinical laboratory results. The sophisticated quality assurance schemes used in many laboratories do not identify erroneous results arising from aberrant samples. Recently attention has been focused on the incidence and implication of false-positive results arising from the presence of certain substances in a patient's serum that interfere with one or more steps in immunoassays. In this paper, we present the case of a 92 year-old woman whose plasma myoglobin concentrations falsely increased when measured using the Beckman Access assay. We demonstrated that heterophilic antibodies accounted for the falsely increased myoglobin values, and we suggest how to resolve such situations. (www.actabiomedica.it)

Key words: Heterophilic antibodies, immunoassays, myoglobin values, blocking tube

Introduction

Analytical interference in immunoassay may produce serious errors in clinical laboratory results. Clinicians need to be aware of these limitations, since immunoassay results are used for both disease diagnosis and for monitoring response to treatment (1-3).

Laboratorians have introduced and use numerous sophisticated quality assurance schemes in their routine testing schedules to identify erroneous results, but these schemes do not identify erroneous results arising from aberrant samples.

Attention has been recently focused on the incidence and implication of false-positive results arising mainly, but not exclusively, from the presence of certain substances in a patient's serum that interfere with one or more steps in immunoassays (4, 5).

Heterophilic interference problem

The presence in a patient's serum of human anti-animal antibodies (HAAA), especially those directed against mouse monoclonal antibodies, is of particular importance.

A "heterophilic sample" is a serum or plasma sample which contains antibodies that are able to bind to the animal antibodies used in immunochemistry assays.

The clinical use of monoclonal mouse antibodies (e.g., for radioimaging, in the treatment of some cancers) often produces human anti-mouse antibodies (HAMA). Anti-animal antibodies may also arise as a result of incidental or occupational exposure to foreign proteins (e.g. veterinarians, farm workers, food preparers) or due to the presence of domestic animals in the home environment.

The presence of high titres of these antibodies may lead to analytical errors in commonly used " sandwich" immunoassays by crosslinking the capture and label antibodies in the absence of a specific analyte (6, 7).

This paper reports the case of a 92 year-old woman whose plasma myoglobin concentrations falsely increased when measured using the Beckman Access assay.

We demonstrated that HAAA accounted for the falsely increased myoglobin, and we suggest how to resolve such situations.

Case report

G.G., 92 years old, affected by known chronic coronaropathy, atrial fibrillation, type 2 diabetes and primary hypothyroidism, under chronic dietetic and pharmacological treatment with insulin, digoxin, torasemide, isosorbide-5-mononitrate, enalapril, thyroxine and acenocoumarol, was admitted to our hospital due to pneumonia and chest pain.

Her medical history included two coronary-aortic bypass, and short, sporadic episodes of angina. The patient had been affected by cough and dyspnea for two days and was admitted to our hospital due to the onset of stenocardia. On arrival at the Emergency Dept, her chest X-ray showed a left basal consolidated area with moderate homolateral pleural effusion; the ECG tracing confirmed the already known chronic atrial fibrillation with moderate ventricular response, and complete left bundle branch block. The acute disease had caused severe metabolic (glycaemia = 4.65 g ‰; natremia = 131 mEq/l; uric acid = 10.1 mg %) and hemodynamic failure, with renal insufficiency (oliguria; creatininemia = 2.3 mg %; azotemia = 1.01 g ‰), but, above all, hypoxemia (pO_2 = 51.0; pCO_2 = 42.3), which, as a result of the discrepancy mechanism, had destabilised the angina. The remaining hematochemical tests, performed urgently, showed a significant increase in LDH (1311 UI/l) and of myoglobin (1981 mg %), while the other cardiac profile parameters (AST, ALT, CPK, CK-MB mass, troponin I) were found to be normal. The typicalness of the stenocardia symptoms, the unreliability of the ECG tracing, due to the aforementioned left bundle branch block, and the alteration of some parameters of the cardiac profile, induced the medical staff to start intensive therapy with parenteral feeding, continuous intravenous infusion of insulin and nitro derivatives, decoagulant maintenance therapy, wide-spectrum antibiosis and monitoring of the cardiac profile every eight hours (Tab. 1). The routine tests, performed the day after, showed a marked increase in inflammation indices (ESR = 80 at the first hour; CRP = 114 mg/l; fibrinogen = 699 mg %; alpha-2 globulin = 13.5%; leukocytes = 12.640, with 79% of neutrophils).

During the first day, with the improvement of the metabolic and hemodynamic balance and of the renal function, a change in the chest pain occurred which became atypical: from accessional it became virtually continuous, worsening with deep inspiration, and shifting to the retrosternal region of the left hemithorax. These symptoms seemed more correlated with pleurodynia (left basal metapneumonic effusion) than with the coronary-related origin. The ECG tracing, although not discriminating, remained unvaried throughout the repeated daily check-ups and LDH values also re-entered normal limits within 36 hours. The constantly elevated myoglobin values remained isolated. After 48 hours, in view of the favourable clinical evolution, the frequency of the hematochemical checks was reduced and intensive treatment was suspended. In the following days, the metabolic and cardiocirculatory balance was completely restored, re-absorption of the pleural effusion progressively took place, renal parameters (creatinine = 1.1 mg %; azotemia = 0.49 g ‰), and inflammation parameters returned to normal, and air bronchogram testified to healing of pneumonia. Nonetheless, the high values of the myoglobin persisted (Tab. 1), although there were no signs of myoglobinuria and/or tubular distress. The direct and indirect Coombs test produced negative results, as did the search for autoantibodies (ANA, AMA, ASMA, LKM, APCA, anti-native DNA, anti-thyroglobulin and anti-thyroperoxidase) and circulating immune complexes. At this stage, the Laboratory was contacted in the certainty that what the investigation had revealed could not be myoglobin.

In order to evaluate whether the high myoglobin values were caused by heterophilic antibody interference, the patient serum was examined both after pretreatment with heterophilic blocking tube (HBT) (Scantibodies Laboratory) and after scalar dilution. At the same time, the control serum was pre-treated with HBT.

The HBT allows for the rapid and simple elimination of false positive heterophilic interference in plasma or serum for sandwich immunoassays (8).

Table 1. Parameters of cardiac profile

		AST (IU/l)	ALT (IU/l)	LDH (IU/l)	CPK (IU/l)	CKMB (mass) (ng/ml)	Myoglobin (ng/ml)	Troponin I (ng/ml)
Admission		48	20	1311	18	0,7	1981	0,07
Day	Hour							
I	6	21	23	273	22	0,6	1657	0,06
	14	19	21	361	21	0,3	1522	0,03
	22	26	21	577	47	0,7	1522	0,04
II	6	20	18	404	24	0,3	2018	0,07
	14	20	19	287	22	0,5	2301	0,07
	22	17	16	270	22	0,8	2090	0,08
III	6	17	15	307	37	0,5	2056	0,07
	22	22	12	344	43	0,1	2216	0,08
IV		19	13	275	28	0,1	2181	0,07
V		18	11	269	29	0,6	1895	0,08
VI		19	11	322	45	0,0	1656	0,04
VIII		23	13	350	48	0,0	1563	0,05
X		26	13	281	37	0,0	1562	0,05
XIII		21	12	274	39	1,5	1588	0,04
XVII		22	14	352	37	1,9	1767	0,04

HBT represents a pre-treatment assay intended to confirm or contradict the original non pre-treatment assay results, and it contains a unique blocking reagent composed of specific binders which inactivate heterophilic antibodies.

Once the specific binder binds to the heterophilic antibodies, the antibodies are no longer able to cause immunoassay interference.

In this case report, the pre-treatment sample assay result was lower than the untreated sample result, while the value of the control serum was still elevated, confirming the heterophilic antibody interference.

Moreover, the diluted samples from both the patient and from the positive calibrator revealed a non-parallel course.

These results strongly suggest that heterophilic antibody interference is the cause of the falsely increased myoglobin values.

Discussion

With reference to the immunoassay systems, attention was originally directed towards the specificity of the primary antibody for a definite analyte.

Currently, the role of interfering substances that are present in the patient's sample but not in calibrator or quality control samples continues to haunt clinical laboratories.

Recent biomedical literature includes case reports of misdiagnosis or improper treatment resulting from analytical errors (1-3).

Analytical errors arising from the presence of antibodies to mouse immunoglobulins in the patient's plasma or serum have received great attention but are just one of the many causes of interference in immunoassays (9).

The consequences of analytical errors, especially when seemingly confirmed by successive results from the same laboratory or from external laboratories using the same (which as usually occurs) or a different methodology, may be disastrous for patients.

This case report confirms the limitations of immunoassays in the routine clinical setting.

Although the risk of diagnostic errors cannot be abolished, it could be reduced through a closer and better informed communication between the physicians and the clinical laboratory on unexpected immunoassay test results.

The laboratory can help in several ways. It can provide up-to-date information on assay performance and discuss test limitations with the physician.

Further testing should be arranged to confirm the test result (i.e. repeating the analysis on the same spe-

cimen, retesting a second specimen, or arranging for analysis by another method).

In addition, dilution or blocking studies may be performed to confirm the suspected presence of analytical interference.

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