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When lab tests lie … heterophile antibodies

Background

Health professionals rely on the accuracy and validity of laboratory investigations in managing patients. Occasionally the results of laboratory investigations do not correlate with the clinical scenario. Incorrect pathology results may lead to unnecessary further investigation, inappropriate therapeutic interventions, and considerable anxiety for the patient and doctor. Heterophile antibodies are endogenous antibodies in human serum/plasma that may interfere with immunoassays resulting in false elevation, or rarely false depression of measured values.

Objective

To alert health professionals to clinical situations in which heterophile antibodies may result in misleading results and potentially compromise patient care.

Discussion

Heterophile antibodies may interfere with a number of immunoassays commonly used in clinical practice. Awareness of the possibility of interference by heterophile antibodies is important to prevent inappropriate management on the basis of erroneous laboratory results.

Keywords

antibodies, heterophile; sensitivity and specificity; immunoassay

Case 1

A boy aged 10 years was referred to a paediatric endocrinology clinic following abnormal thyroid function tests performed because of fatigue and short stature. Free thyroxine (FT4) was 12 pmol/L (reference interval 10–26) and thyroid stimulating hormone (TSH) was elevated at 17.2 mIU/L (reference interval 0.1–4). Tests for anti-thyroid peroxidase and anti-thyroglobulin antibodies were negative. The boy was commenced on thyroxine replacement. Despite a progressive increase in his dose of thyroxine to 100 µg per day and a rise in FT4 to 25 pmol/L, TSH remained elevated. His parents were asked about medication adherence at each visit. After 2 years of thyroxine treatment, testing with heterophilic antibody-blocking studies confirmed interference by a heterophile antibody, TSH being normal with treated serum. The patient's thyroxine treatment was ceased and his thyroid function remained normal.

Case 2

A man aged 42 years with no risk factors for ischaemic heart disease presented to his general practitioner with atypical chest pain. An electrocardiogram was normal; however, his serum cardiac troponin I (cTnI) was elevated at 0.22 µg/L (reference interval <0.04). He was admitted to hospital with a diagnosis of non-ST elevation myocardial infarction. Ongoing chest pain led to urgent coronary angiography, which showed normal vessels. Echocardiography revealed normal left ventricular size and function and normal right ventricular systolic pressure. Over the ensuing days the patient's cTnI remained elevated. Testing confirmed interference by a heterophile antibody and normal serum troponin levels were seen when blocking antibodies were used.

Case 3

A man of 55 years presented to his general practitioner for an annual review. His serum prostate-specific antigen (PSA) level was measured for the first time and was markedly elevated at 42.4 µg/L (reference interval 0–4). Digital rectal examination revealed a normal prostate on palpation, and transrectal ultrasound did not reveal any abnormality. Repeated measures of serum PSA on the same assay remained elevated. Computerised axial tomography of the abdomen and pelvis and a bone scan were unremarkable. The patient was commenced on androgen deprivation therapy. Three months later his prostate was atrophic on rectal examination and his serum testosterone was undetectable; however, his serum PSA remained markedly elevated.

A repeat PSA using an alternative assay revealed a normal result, and evaluation with heterophilic antibody blocking studies confirmed interference in the original assay. Androgen deprivation therapy was ceased and regular surveillance serum PSA levels over the ensuing 2 years remained normal.

Discussion

The prevalence of heterophile antibodies is 0.17–40% in the general population.[1] In eight automated tumour marker immunoassays, the prevalence of heterophile antibodies was 0.2–3.7%.[2] Sources proposed for the induction of heterophile antibodies include exposure to mice and mouse products, immunisation, blood transfusion, autoimmune diseases, dialysis and maternal transfer. Two-site immunometric or sandwich assays are particularly susceptible to analytical error in the setting of heterophile antibodies. These assays use at least two antibodies directed against different epitopes of an antigen: one bound to a solid-phase, the other in solution and tagged with a signal moiety. The antigen in the sample bridges the two antibodies so that the amount of labelled antibody that binds to the solid-phase is proportional to the antigen concentration in the sample (*Figure 1*). Heterophile antibodies can also bridge the two antibodies independently of antigen, resulting in an increase in labelled antibody concentration (*Figure 2*).

Heterophile antibodies may affect a wide array of laboratory tests, resulting in false elevation of tumour markers, endocrine tests, cardiac injury markers and drug levels (*Table 1*). Heterophile antibodies may also cause false depression of serum cortisol levels, resulting in incorrect diagnosis of hypothalamic-pituitary-adrenal axis insufficiency and inappropriate and potentially harmful replacement with glucocorticoids. False lowering of thyroglobulin levels may also occur. Where suspected, the presence of heterophile antibodies interfering with the accuracy of investigations can be addressed through analysis by an alternative analytical platform, non-linearity of results on serial dilution or the use of heterophile-blocking reagents or immunoglobulin-blocking reagents.

A study of patients investigated for cTnI, because myocardial infarction was suspected, found heterophile antibodies caused false positives in 5.5% of those with raised cTnI and 14% of those with raised cTnI and normal creatine kinase.[3] The universal international definition of myocardial infarction requires the detection of a rise or fall of cardiac biomarker values together with symptoms of ischaemia, new changes on electrocardiography, evidence on imaging of new loss of viable myocardium or identification of an intracoronary thrombus.[4] Acute elevation of cTnI may also occur with sepsis, pulmonary embolism, myopericarditis,

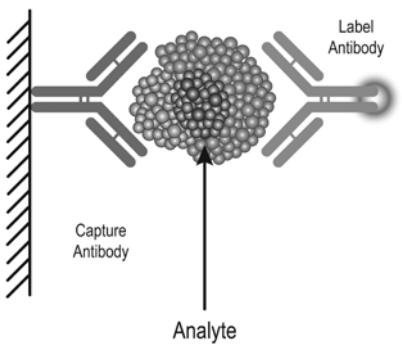

Figure 1. 2-site immunometric (sandwich) assays

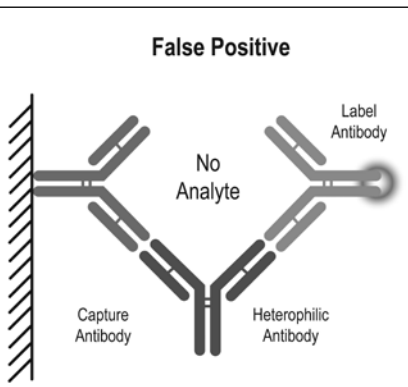

Figure 2. Interference by heterophile antibodies

Table 1. Immunoassays potentially affected by heterophile antibodies

Cardiac markers
- Troponin, creatine kinase-MB isoenzyme, B-type natriuretic peptide (BNP)

Tumour markers
- Prostate-specific antigen (PSA)
- Carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA 19-9), cancer antigen 125 (CA 125)
- Alpha-fetoprotein (AFP), beta human chorionic gonadotropin (ßhCG)
- Calcitonin
- Thyroglobulin
- Chromogranin A

Endocrine tests
- Follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, oestradiol, progesterone, testosterone
- Thyroid-stimulating hormone (TSH), free thyroxine (FT4), Free triiodothyronine (FT3)
- Adrenocorticotropic hormone (ACTH), cortisol
- Parathyroid hormone (PTH)
- Inhibin A

Drug levels
- Digoxin
- Cyclosporine, tacrolimus

Other
- Tryptase
- Myoglobin
- Antiphospholipid antibodies
- Erythropoietin
- Human immunodeficiency virus (HIV)

stroke, chemotherapy, strenuous exercise and acute heart failure.[5] In addition to heterophile antibodies, chronic persistent elevation of cTnI may occur with renal dysfunction, chronic obstructive pulmonary disease, pulmonary hypertension, left ventricular hypertrophy and obstructive sleep apnoea.

Heterophile antibody interference with tumour markers is particularly important as it may lead to a false diagnosis of malignancy (either primary or residual post-treatment) and unnecessary surgery and/or chemotherapy. Rotsmensch and Cole described 12 women incorrectly diagnosed with postgestational choriocarcinoma on the basis of persistently positive human chorionic gonadotropin (hCG) levels. Most of the women underwent extirpative surgery or chemotherapy without diminution in hCG titre and were found to have false-positive hCG values due to heterophile antibodies.[6] The authors concluded that protocols for the diagnosis and treatment for choriocarcinoma should include a compulsory test for hCG in urine as the immunoglobulin-hCG complex is not cleared by the kidney. It has been suggested in clinical scenarios where there is a low rate of true positives and a higher likelihood of interference, adding a heterophilic antibody blocker to all positive tests may be a more cost-effective than retesting positive samples.[7] An example of this is measuring serum calcitonin levels in patients with thyroid nodules. A study of 378 subjects found 5 patients (1.3%) with falsely elevated calcitonin levels as a result of heterophile antibodies, whereas none of the patients had medullary thyroid cancer.[8] The use of tumour markers in screening should be discouraged not only because of interference by heterophile antibodies, but because of the significant rate of false positive and false negative results with these tests due to tumour biology.

Conclusion

Heterophile antibodies are a cause of falsely elevated or depressed laboratory values and should be considered and sought whenever there is incongruity between a clinical scenario and the results of pathology investigations.

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