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Serum sample containing endogenous antibodies interfering with multiple hormone immunoassays. Laboratory strategies to detect interference

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ABSTRACT

Objectives: Endogenous antibodies (EA) may interfere with immunoassays, causing erroneous results for hormone analyses. As (in most cases) this interference arises from the assay format and most immunoassays, even from different manufacturers, are constructed in a similar way, it is possible for a single type of EA to interfere with different immunoassays. Here we describe the case of a patient whose serum sample contains EA that interfere several hormones tests. We also discuss the strategies deployed to detect interference.

Subjects and methods: Over a period of four years, a 30-year-old man was subjected to a plethora of laboratory and imaging diagnostic procedures as a consequence of elevated hormone results, mainly of pituitary origin, which did not correlate with the overall clinical picture.

Results: Once analytical interference was suspected, the best laboratory approaches to investigate it were sample reanalysis on an alternative platform and sample incubation with antibody blocking tubes. Construction of an in-house 'nonsense' sandwich assay was also a valuable strategy to confirm interference. In contrast, serial sample dilutions were of no value in our case, while polyethylene glycol (PEG) precipitation gave inconclusive results, probably due to the use of inappropriate PEG concentrations for several of the tests assayed.

Conclusions: Clinicians and laboratorians must be aware of the drawbacks of immunometric assays, and alert to the possibility of EA interference when results do not fit the clinical pattern.

Abbreviations: ACTH, adrenocorticotropic hormone; EQAS, external quality assurance schemes; FSH, follicular stimulating hormone; EA, endogenous antibodies; fT4, free thyroxine; HCU, Hospital Clínico Universitario "Lozano Blesa"; MRI, magnetic resonance imaging; LH, luteinising hormone; PEG, polyethylene glycol; QC, quality control; TSH, thyrotropin

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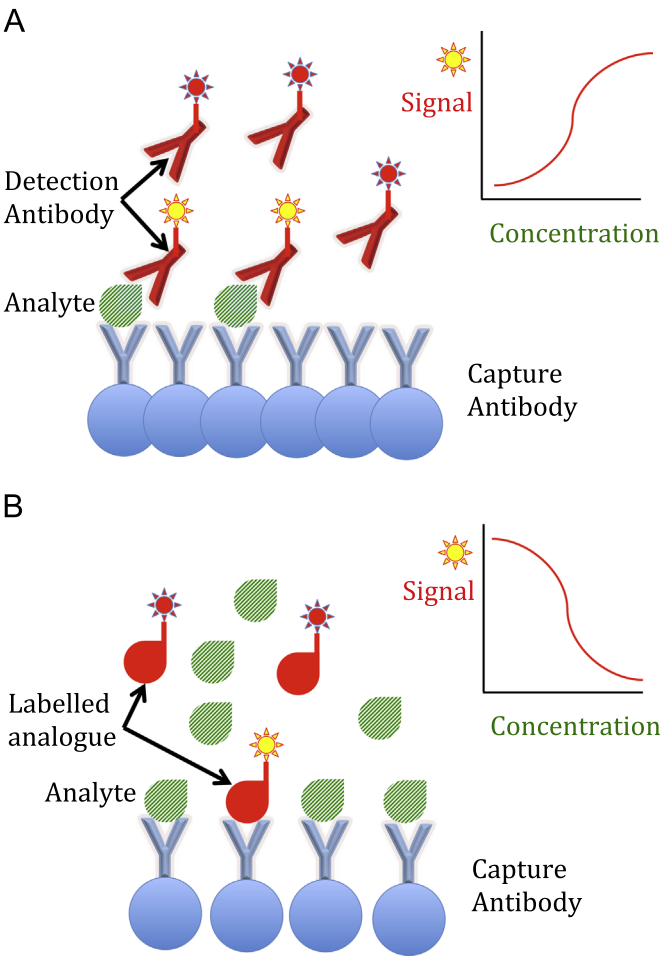

Fig. 1. Examples of immunoassay formats. (A) Sandwich immunoassay: the reaction kit includes both capture and labelled detection antibodies that bind different epitopes of the analyte. The higher the amount of analyte, the greater the signal developed. (B) Competitive immunoassay: the reaction kit includes a capture antibody and a labelled analogue of the analyte that competes for the capture antibody. The higher the amount of analyte, the lesser the signal developed.

1. Introduction

Two basic immunoassay formats are currently available for hormone (analyte) measurement. Total hormone quantification for large molecules is generally based on immunometric sandwich assays [1], while competitive assays are often used for analysis of free hormones and/or small molecules [2]. Examples of both types of assay format are depicted in Fig. 1, although there are many variations from the general scheme shown.

Endogenous antibodies (EA) (either heterophile antibodies, human antianimal antibodies or autoantibodies [3]) may cause interferences in these immunoassays, which often translate into erroneous tests results. Heterophile antibodies are considered to be naturally occurring because they are produced without exposure to specific immunogens. Human anti-animal antibodies are species-specific and produced following acute or chronic chronic exposure to the animal protein (immunoglobulin). Finally, autoantibodies bind specific analytes, such as anti-thyroglobulin and anti-insulin antibodies.

The mechanism by which EA cause interference is different depending on the type of antibody and the immunoassay format. Heterophile and anti-animal antibodies, for instance, usually work by cross-linking capture antibodies with detection antibodies in the absence of antigen (that is, the analyte to be measured), thus resulting in a false positive result (although also false negative results are possible, depending on the assay construction and the site of interference). Therefore, this kind of interference is more common with sandwich assay protocols (see Fig. 2A). However, although more rarely reported, even competitive assays may suffer from the presence of EA, as depicted in Fig. 2B. Interestingly, it appears that current competitive immunoassays may be more susceptible to EA interference than older competitive radioimmunoassays, due to the combination of components used in today's reagents [2].

It has been reported that up to 40% of people may have antibodies with affinity to animal antibodies, although it is thought that most of them will not create problems in immunoassays [1]. However, this kind of interference, along with others derived from preanalytical factors (e.g., physiological factors, medications, adherence to sample collection and sample