We report the case of a 46 yo woman, working in a post-office, who has consulted her GP for unexplained asthenia and diffuse muscular pain. She felt stressed and tired but did not report any "real" feeling of sickness. In front of these non specific symptoms, the physician ordered a "general" blood analysis, including hormonal and serological tests. The results obtained by the Verviers Hospital laboratory, a general primary and secondary healthcare facility, were strictly normal, but an unexpected high level of fasting insulin at 147 µUI/mL (expected values: 6-27 µUI/mL) was found with the Immulite 2500 immunoanalyzer (Siemens Healthcare Diagnostics, Deerfield, IL). The glucose and C-peptide levels were normal, at 4.50 mmol/L and 1.1 ng/mL, respectively. This high level of insulin was further confirmed on two different samples (139 and 143 µUI/mL), that had been drawn respectively two and four weeks before and kept at -20°C in a blood bank.

According to these results, the patient was admitted to the CHU de Liège, a tertiary healthcare hospital, for a 72 hours observation period. During the stay, the patient underwent different blood samplings at different times for insulin, C-peptide and glucose determinations. No hypoglycaemia was observed, and the C-peptide and insulin levels, determined with the Roche Modular (Roche Diagnostics, Mannheim, Germany) never reached a pathological level, with the highest insulin level observed at 5 µUI/mL (expected value: <15 µUI/mL). The patient was then allowed to leave the hospital.

Retrospectively, we thus considered the Immulite results as spurious and we treated the three incriminated samples with a heterophilic antibody blocking tube (HBT, Scantibodies, Shantee, CA) that captures potential heterophilic antibodies in the patients' samples. We also incubated the samples with different sera from mouse, rat, guinea-pig and sheep origin (40 µl of animal serum in 400 µl of sample) to determine the possible origin of the interfering antibody. We observed that the results dramatically fell at 2 and 30 µUI/mL after HBT and mouse serum treatments, respectively. Treatments with serum of other animal species did not

change the initial results. We thus concluded that the Immulite 2500 Insulin assay had presented an interference due to a human anti-animal antibody (HAAA). This interfering antibody is not species specific as, in one part, it interferes with the Immulite insulin, a two-sites model constituted with monoclonal murine and polyclonal sheep antibodies. On the other part, it does not affect the Roche Modular assay, even if the monoclonal antibodies used in this test are also from murine origin.

It is well known that two-site immunoassays can be influenced by HAAA [1] that can spuriously increase laboratory results. Different publications have already underlined this phenomenon in several laboratory tests [2-8]. To the best of our knowledge, we report here the first case of heterophilic antibody interference with the Immulite 2500 Insulin immunoassay.

Even if the manufacturers write in the inserts of the kits that this kind of interference remains possible, one should be careful when interpreting the results of any immunoassay. We must keep in mind the tragic outcomes that can be observed with these interferences [9]. In the presently described case, these spurious results have led to unnecessary expensive and stressful extra-investigations. To overcome these problems, we think that a strong collaboration between each healthcare partner is important. Manufacturers should continuously improve their reagents to overcome these interferences, by adding always more efficient blocking substances in the conjugates of their assays. Clinical Chemists have to validate an increasing number of results. In routine practice, it is not always obvious for them to detect the discrepant result from the pathological one. When possible, different validation strategies that take HAAA interferences into account might be applied [10]. Finally, we think that the dialogue between Physicians and Clinical Chemists is of greatest importance, particularly in front on an unexpected result, or a result that does not fit with the patient's clinic or anamnesis.

In summary, we reported spuriously increased insulin levels due to human anti-animal antibodies with the Immulite which has led to a three-day expensive and stressful hospitalization.

Conflict of interests: None

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