CASE DESCRIPTION

A 29-year-old woman presented to her general practitioner (GP) with progressive dyspnea for 2 months. Her only relevant medical history was asthma, normally well-controlled on inhaled corticosteroids. Her physical examination was unremarkable, and her Wells score for pulmonary embolism, a clinical pretest probability score, was low. Her GP requested a D-dimer to exclude a pulmonary embolism, which was >4000 μg/L [cutoff <500 μg/L fibrinogen equivalent units (FEU)], using the Siemens INNOVANCE™ D-dimer immunoturbidimetric method. Because of her increased D-dimer, she was admitted to the hospital for investigation of suspected pulmonary embolism. Repeat D-dimer in hospital using the same assay was also >4000 μg/L. Other routine laboratory tests, including coagulation screen, full blood count, creatinine, liver function tests, C-reactive protein, glucose, and rheumatoid factor were normal. Her chest radiograph and subsequent computed tomography pulmonary angiogram were both normal.

She was discharged to the care of her GP with a course of prednisone for a presumed asthma exacerbation. The GP continued to monitor her D-dimer levels, which remained grossly increased (2670, 3020, and 2590 μg/L). Testing was carried out by a new community laboratory provider using the original assay and cutoff. Because the patient was concerned about her abnormal test results, she sought a second opinion from another doctor. Biochemistry staff were consulted, who suggested repeating her D-dimer with an alternative immunoturbidimetric method (STA®-Liatest® D-Di). This process returned a normal level of <220 μg/L (cutoff <500 μg/L FEU). To exclude the possibility that her D-dimer level had indeed been high and now returned to normal, repeat D-dimer measurements were performed simultaneously with both assays and a second alternative. The original assay was still increased (1950 μg/L); however, the 2 alternative assays were normal: 270 μg/L (STA-Liatest D-Di) and 341 μg/L (STA-Liatest D-Di Plus) (cutoff <500 μg/L FEU). Further, the sample was pretreated in a heterophilic blocking tube (HBT) for 1 hour and then tested for D-dimer using the original method alongside the untreated sample. The level of both samples was >6000 μg/L. Scantibodies Laboratories, the HBT manufacturer, advised us to incubate the HBT for 2 h. This was done 9 months after the first abnormal result, when the patient was in good health. Results were >6000 μg/L in the untreated sample and 1050 μg/L in the treated sample. At the same time, the untreated sample was manually diluted with saline buffer by 1 in 2 and 1 in 4 and automatically diluted by 1 in 8 with the Sysmex CA-7000. These dilutions returned results of >6000, 1960, and 1810 μg/L.
respectively. The previous results reported to the GP as 2670, 3020, and 2590 μg/L may have been automatically diluted and could have actually been >6000 μg/L.

**CASE DISCUSSION**

Our patient’s unexplained high D-dimer showed nonlinearity on dilution, was substantially reduced after HBT treatment and was normal with 2 alternative assays, suggesting the presence of an interfering endogenous antibody—either a specific human anti-animal antibody (HAAA) or a heterophile antibody (1, 2).

Heterophile antibodies are natural antibodies or autoantibodies, which react polyspecifically and weakly with heterogeneous antigens (1). Natural antibodies are low-affinity antibodies found in all people produced by the immune system before antigen exposure and can be “polyspecific” (combine with chemically different antigens), “idiotypic” (bind the variable region of other antibodies), or “natural rheumatoid factor” (bind the Fc portion of other immunoglobulins).

Autoantibodies are associated with autoimmune or chronic disease, are mostly autoimmune polyspecific or autoimmune rheumatoid factor, and tend to bind more strongly than natural antibodies (1). In contrast to heterophile antibodies, specific HAAAs are high-affinity antibodies produced against a specific animal protein, which bind specifically to the immunizing antigen (1). For example, specific HAAAs may be produced in response to mouse monoclonal antibodies injected for cancer treatment, injected antibody-targeted diagnostic imaging reagents, or exposure to animal antigens while working with animals or keeping them as pets (3). However, terminology in the literature remains ambiguous. “Heterophile” and “HAAA” are often used interchangeably, possibly because of the historical view that heterophile antibodies arise in response to animal allergens (1) and because the term “heterophile antibody” is often used to describe an antibody that interferes in immunoassays (2).

Both heterophile antibodies and specific HAAAs interfere in immunoassays by reacting with reagent antibodies in place of the analyte, causing false results (2, 3). Circumstantial evidence suggests that natural heterophile antibodies are the major cause of automated immunoassay interference, and for assays using blocking agents, the false-positive rate is about 0.05% (1). The clinical relevance of interfering antibodies is that they can affect a significant number of immunoassays, leading to incorrect diagnoses and unnecessary, potentially harmful treatments (2). As far as we can ascertain, there are only 5 previous case reports describing endogenous antibody interference with an immunoturbidimetric D-dimer assay (4–8). D-dimer is a circulating breakdown product of fibrin and is therefore increased in a range of diseases, such as venous thromboembolism (VTE) and aortic dissection (9). VTE comprises deep vein thrombosis and pulmonary embolism, both of which commonly present nonspecifically. However, since D-dimer has a high negative predictive value for VTE, it is a useful “rule-out” test in low-to-medium risk individuals like our patient with suspected pulmonary embolism (9). Heterophile antibodies or specific HAAAs are thought to interfere with immunoturbidimetric D-dimer assays by bridging the D-dimer specific mouse monoclonal antibodies that coat the latex microparticles, leading to agglutination of the microparticles, increased turbidity in the suspension, and a falsely positive D-dimer result (6).

Previously described cases also demonstrated a falsely increased D-dimer by obtaining a normal D-dimer with an alternative immunoturbidimetric or other immunoassay (4–6, 8), or after preincubation with HBT, 10% mouse serum, or dithiothreitol (5–7). Two cases were attributed to heterophile antibodies (5, 7); 1 to human antimouse antibodies (HAMA) (8); 1 to heterophile antibodies of the HAMA type (6); and 1 did not specify the source of antibody interference (4). Our patient had no his-
tory of treatment with injected animal immunoglobulins, but reported that as a child, she had kept a pet rabbit, guinea pig, and hamster and spent time with pet mice. Her interfering antibody could be a specific HAAA arising from vermin exposure, or a heterophile antibody, either capable of reacting with vermin (mouse monoclonal antibodies) in the assay (1, 3, 10). In cases of animal handling, if the interfering antibody shows a preference for the same species as the suspected immunizing species, it should be termed a specific HAAA (10). If this was the case, we would expect it to be a specific human antimouse antibody that reacts with mouse monoclonal assay antibodies (1, 10). Comparing the original assay, which included a mouse heterophilic blocking reagent, with the first alternative assay that did not include a blocker, her antibody reacted in the original assay in spite of the presence of a blocker, but was unreactive in the latter, which had no blocking capacity; thus, the antibody does not seem to be specific for mouse antibodies (1). Apart from the blocker, the other difference between these 2 assays was the specific monoclonal antibody (mAb) used. The interfering assay used mAb8D3, whereas both alternative assays used mAb8D2 and mAb2.1.16. The interfering antibody reacted distinctively with one mouse monoclonal antibody, but not with others, which often occurs in heterophilic interference due to distinctive, complementary interactions between idiotopes (1). This type of idiotypic interaction can also account for the interference that occurred in spite of the presence of a blocker. An idiotypic antibody can have distinctive binding sites that are reactive with an assay antibody but not the blocking agent, rendering the blocking agent ineffective (1).

Our case highlights the need to consider endogenous antibody interference when the clinical information is inconsistent with an increased D-dimer. This practice will prevent unnecessary patient distress, repeated investigations, and cost to the healthcare system. In cases where an

**TAKEAWAYS**

- Apart from venous thromboembolism, an increased D-dimer can be caused by aortic dissection, arterial thromboembolism (e.g., myocardial infarction and stroke), disseminated intravascular coagulation, infection, trauma/surgery, severe liver and renal disease, pregnancy, cancer, and interference from heterophile antibodies or specific human anti-animal antibodies.
- An interfering antibody should be suspected when the clinical information is inconsistent with a positive D-dimer.
- Immunoturbidimetric D-dimer assays using mouse monoclonal antibodies are subject to interference from endogenous antibodies, either heterophile antibodies (natural antibodies or autoantibodies) or specific human anti-animal antibodies, which can bridge assay antibodies, producing a falsely positive D-dimer.
- Exposure to animal antigens may cause the production of endogenous-specific human anti-animal antibodies, which can interfere in D-dimer assays, producing a falsely positive D-dimer.
- Where appropriate, D-dimer reports could include a caution that endogenous antibodies may interfere in immunoassays, producing false results. Their presence can be investigated by repeating the D-dimer with an alternative assay or after preincubation with a blocking agent, resulting in normal or reduced levels in most cases of interference. Suspected cases should be noted in the patient's clinical record.
interfering antibody is suspected, it must be documented in the patient's clinical record to avoid future confusion and unnecessary investigations, such as repeat computed tomography pulmonary angiogram. The importance of communication between clinicians and laboratory staff in the diagnostic process, particularly in cases of diagnostic uncertainty is also highlighted. Laboratory staff should understand the principles and pitfalls of their immunoassays, particularly in disciplines such as coagulation, where they have not traditionally been used. The use of middleware, to hold up above assay range and diluted results, for manual authorization, would alert operators to nonlinear dilutions and the possibility of interfering antibodies. This case and those reported previously also highlight the value of having a variety of D-dimer assays available in a particular locality. Lastly, we advocate that D-dimer reports note their method of testing, and where appropriate include a caution that endogenous antibodies may interfere in immunoassays, producing false results (1). We believe this will act as a necessary reminder, both to laboratory staff and clinicians, in cases of uncertainty.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Acknowledgments: We would like to thank Tao Meng of Labtests Auckland, the Coagulation Section of Hematology, LabPLUS, Auckland City Hospital, and the Coagulation Section of Hematology, North Shore Hospital, for undertaking further work on the sample.

REFERENCES