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CASE REPORT

A Case of False Elevation of D-Dimer and Elimination Methods

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SUMMARY

Background: **There are many methods for the detection of D-dimer in clinical laboratories. Immunoturbidimetric assays are widely used because of its high sensitivity and specificity [1-3]. However, this method may be affected by the interference of rheumatoid factor (RF), heterophilic antibodies, and other unknown proteins, and its falsity will increase, thus affecting clinical diagnosis.**
Methods: **This paper reports the cause analysis of a case of spurious D-dimer increase and four corresponding elimination methods: double dilution of the original specimen, detection of fibrin degradation product (FDP) level, addition of heterophilic blocking reagent, and comparison between different instruments.**
Results: **It was confirmed that there were special antibodies in the patient's body by four methods, which had non-specific reactions with D-dimer reagents, resulting in false increases of results.**
Conclusions: **When the coagulation function results of patients show isolated increases in D-dimer, or the results are inconsistent with clinical symptoms, laboratory personnel should consider the possibility of interference factors, and conduct effective treatment to obtain correct test results, and thus reduce the occurrence of medical adverse events caused by inaccurate test results.**
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KEYWORDS

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INTRODUCTION

D-dimer, a degradation product of crosslinked fibrin, is one of the markers that specifically reflects the hypercoagulable state and secondary hyperfibrinolysis *in vivo*, as well as an important marker for the diagnosis of deep vein thrombosis (DVT) formation, pulmonary embolism (PE), and disseminated intravascular coagulation (DIC) [4]. D-dimer plays an increasingly important role in clinical diagnosis. We report a case of false elevation of D-dimer caused by interference of immunoturbidimetric assays. The details are shown below.

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CASE PRESENTATION

The patient, a 38-year-old female, was admitted to the emergency room of our hospital on July 27, 2023, in a coma due to head injury and hemorrhage caused coma by a car accident. She was clinically diagnosed with an epidural hematoma and underwent intracranial hematoma removal. Plasma D-dimer was detected as 35.2 mg/L on the second day after surgery (the instrument used was Sysmex-CS5100, the reference range was less than 0.7 mg/L, after excluding the test data of instrument, quality control, reagents, and other factors that may affect the results). The attending doctor suspected that the patient had venous embolism. CT pulmonary artery angiography and special ultrasound examination of deep veins of blood vessels and limbs showed that no thrombosis was found. Therefore, the patient was not treated and was a review of the patient was advised on the next day. On July 29, 2023, the re-examination of the patient's D-dimer level was 30.0 mg/L, which was still much higher than the normal value, so the clinician contacted the laboratory to inform them that the patient's D-dimer result was inconsistent with the patient's clinical symptoms and imaging diagnosis. He requested a check on whether there was a detection error.

Laboratory staff immediately launched an investigation: First, we conducted a retest of the original sample on the original instrument, and the test result was 29.8 mg/L, which was consistent with the reported result. Secondly, we diluted the patient's plasma sample by double ratio, and the test result was not linearly reduced with the dilution ratio, and the dilution result had no linear change rule. In contrast, D-dimer in control serum (derived from plasma of patients with typical venous embolism) decreased linearly with dilution times (Table 1). D-dimer is one of the components of FDP, and we detected the FDP with a measurement result of 0.7 μg/mL (normal reference range is 0 - 5 μg/mL), which also suggests that the detection result of D-dimer may be falsely elevated. Finally, we added heterophilic blocking reagent (HBR: from Scantibodies Laboratory, Inc., CA, USA) for detection. The detection value was 2.5 mg/L, which was much lower than the result without heterophil blocking agent (Figure 1). In order to further clarify the test results, with the consent of the patient, peripheral blood was collected again on July 30, 2023, and some tests were carried out (Table 2). The D-dimer test result of the newly collected specimen still had a high value of 30.2 mg/L, and the specimen was sent to another hospital. A different detection system (Stago STA-RMAX, Paris, France) was used to detect the specimen, and the D-dimer result was 0.67 mg/L (the normal reference range of the instrument is < 0.5 mg/L), which was much lower than the detection result of our laboratory. We discussed this with the clinician and then informed the patient that the D-dimer test results were mildly elevated, and the previously reported results were falsely elevated due to heterophil antibody interference.

DISCUSSION

The accuracy of D-dimer detection results is increasingly important. Studies have shown that D-dimer has important application value in the early diagnosis of acute aortic dissection, tumor screening, and prognosis judgment [5,6]. Therefore, D-dimer is great significance for the clear diagnosis and therapeutic evaluation of clinical diseases. However, there are still many problems to be solved in the laboratory detection of D-dimer, such as poor comparability of results between different detection systems and poor accuracy of results due to many interference factors. Cases of false elevation of D-dimer have been reported, but these reports are not detailed enough in case analysis, eliminate methods, and interference cause analysis [7,8].

In this case, we confirmed the false increase in D-dimer detection results through four methods: specimen dilution method, detection of FDP in patients, use of heterophilic antibody blockers, and comparison between different instruments. Subsequently, we also carried out the interference cause analysis. We tested the patient's peripheral blood immunoglobulin, rheumatoid factor, prolactin, cardiolipin antibody, and other indicators. Combined with the analysis of the patient's case data, we found two possible causes of interference: First, the serum prolactin level of the patient reached 850 (mIU/L), much higher than the normal value, while the detection results of other items were within the normal range. It has been reported that hyperprolactinemia is associated with a variety of autoimmune diseases and can increase the production of special immunoglobulins in the body [9]. Therefore, we speculate that the false increase in D-dimer test results in this patient is most likely due to interference caused by the presence of special antibodies in the patient. Second, the patient had developed urticaria 60 days earlier, which resolved spontaneously after a week. Urticaria can cause the body to produce special immunoglobulin, lead to the activation of complement and a series of immune cell events in the body, resulting in the production of heterophilic antibodies [10]. Therefore, we suspect that the above two reasons may produce heterophilic antibodies that lead to interference in D-dimer detection.

Heterophile antibodies are a class of immunoglobulins, which are divided into natural antibodies and autoimmune antibodies. Heterophile antibodies have been reported to be produced after infection with viruses such as rubella, measles, and varicella zoster, and this phenomenon is due to the polyclonal activation of B lymphocytes following viral binding to complement receptor 2 (CR2) [11,12]. When D-dimer is detected by immunoturbidimetric assays, the heterophil antibody will react non-specifically with the detection reagent, resulting in an abnormal increase in the detection result. The occurrence of this non-specific reaction is related to the type of antibody in the detection reagent. The monoclonal antibodies targeted by different brands of reagents are different. STA-RMAX uses mouse anti-human D-

Table 1. D-dimer results with serial dilution of the sample, using Sysmex CS-5100.

	No dilution	1:2 dilution	1:4 dilution	1:8 dilution	1:16 dilution	1:32 dilution
Patient (mg/L)	30.00	3.79	1.260	2.29	0.38	0.14
Control (mg/L)	24.72	12.75	6.40	3.27	1.65	0.85

Table 2. Results of laboratory testing at patient admission.

	Patient results	Reference ranges
Immunoglobulin A (g/L)	2.47	0.70 - 5.00
Immunoglobulin G (g/L)	13.48	7.00 - 16.00
Immunoglobulin M (g/L)	2.42	0.40 - 2.80
Immunoglobulin E (KIU/L)	67.50	< 158.00
Total bilirubin (mmol/L)	14.20	5.00 - 21.00
Total cholesterol (Umol/L)	4.43	2.84 - 5.69
Triglyceride (mmol/L)	0.88	0.56 - 1.70
C-reaction protein (mg/L)	0.22	0 - 7.00
Anti-cardiolipin antibody G (Gplu/mL)	< 1.00	0 - 10.00
Anti-cardiolipin antibody A (Aplu/mL)	< 2.50	0 - 10.00
Anti-cardiolipin antibody M (Mplu/mL)	5.39	0 - 10.00
Anti-dsDNA antibody (IU/mL)	< 2.00	0 - 30.00
Fibrinogen (g/L)	2.34	2.00 - 4.00
Fibrin degradation product (μg/mL)	0.70	0 - 5.00
D-dimer (mg/L)	30.20 ↑	0 - 0.70
Rheumatoid factor (IU/mL))	6.20	0 - 14.00
Prolactin (mIU/L)	850.00 ↑	108.80 - 557.10

Note: The secretion of prolactin is pulsed, there are peaks and valleys in the day, and the detection value is not detected during the peak period.

dimer monoclonal antibody 8D2, and Sysmex CS5100 uses mouse anti-human D-dimer monoclonal antibody MA-8D3 [1,13]. Therefore, we can conclude that the heterophil antibody in this patient binds nonspecifically to the mouse monoclonal antibody MA-8D3 in the Sysmex CS5100 system, resulting in a false increase in D-dimer results. STA-RMAX uses mouse anti-human D-dimer monoclonal antibody 8D2 without interference from this non-specific antibody.

In summary, when a patient's coagulation function results show an isolated increase in D-dimer, or when the results are inconsistent with clinical symptoms and imaging results, laboratory personnel should take into account the possibility of interference factors and conduct effective investigation to obtain correct test results, and finally determine and analyze the possible causes of interference according to the patient's past and current history. Thus, the occurrence of medical adverse events caused by inaccurate test results can be reduced.

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Declaration of Interest:
All authors declare that they have no competing interests.

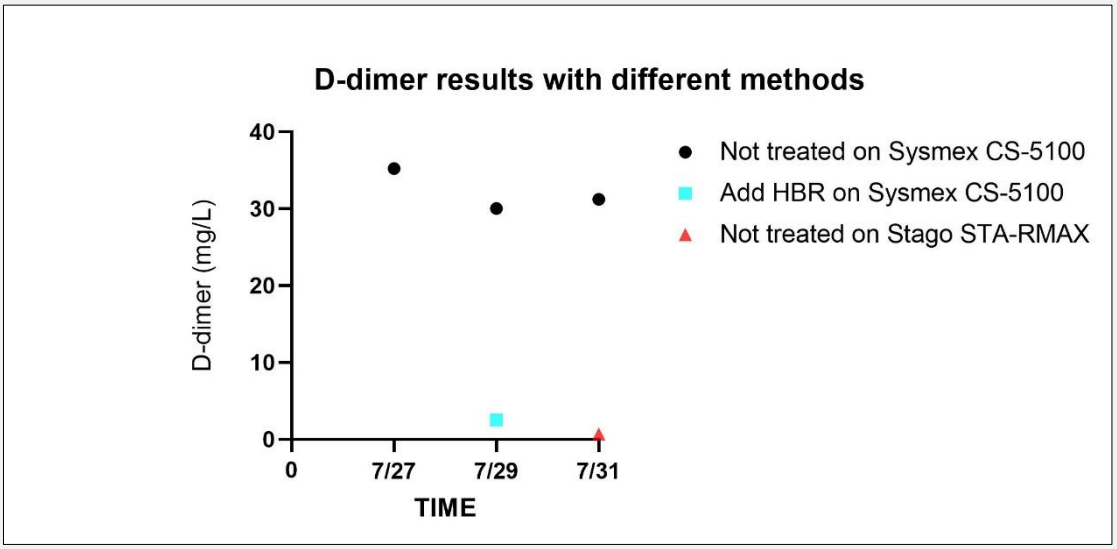

Figure 1. The results of D-dimer with different methods (treated or not treated with HBR) on the same specimen on the same day.

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