Clin. Lab. 2024;70:XXX-XXX

CASE REPORT

A Case of False Decrease of Plasma D-Dimer

Minggang Lu [1, *], Liangfeng Hu [1, *], Lihong Zhang [1], Ye Yang [2]

** Minggang Lu and Liangfeng Hu contributed equally to this work*
[1] Clinical Laboratory, Shaoxing People's Hospital, Shaoxing, P.R. China
[2] Pathology Department, Shaoxing People's Hospital, Shaoxing, P.R. China

SUMMARY

Background: **D-dimer, a specific product of cross-linked fibrin degradation, is of great clinical value in the early diagnosis of thrombotic diseases and in monitoring the efficacy of thrombolysis; therefore, the accuracy of D-dimer test results is crucial.**
Methods: **This article reports a case of a patient with disseminated intravascular coagulation (DIC) who experienced a false decrease in D-dimer due to the hook effect.**
Results: **The three D-dimer test results for DIC patients were 1.09 mg/L, 0.93 mg/L, and 1.43 mg/L. After sample dilution, the results were: first time (1:128) 842.24 mg/L, second time (1:128) 1,505.28 mg/L, third time (1:32) 415.68 mg/L. There was a significant difference in the three test results before and after dilution, because the D-dimer concentration was too high, exceeding the detection range and causing the hook effect, which falsely lowered the D-dimer value.**
Conclusions: **When the D-dimer value of DIC patients does not match the clinical situation, the possibility of the hook effect should be considered, and the false decrease can be ruled out by the sample dilution method. In this way, accurate clinical results can be obtained to avoid delaying the diagnosis and treatment of DIC patients.**
(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2024.240223)

Correspondence:
Ye Yang
Pathology Department
Shaoxing People's Hospital
No. 568 North Zhongxing Road
Shaoxing City 312000
Zhejiang Province
China
Phone: + 86 13819581386
Email: nmhot_2004@163.com

Case Report accepted February 25, 2024

KEYWORDS

D-dimer, DIC, hook effect, false decrease

INTRODUCTION

The main characteristics of DIC are manifested as an increase in systemic coagulation activity, leading to the formation of thrombi in the microvasculature, thereby affecting organ blood perfusion. In addition, due to the large consumption of coagulation factors and platelets during the formation of thrombi, secondary fibrinolysis function is enhanced, and the body will have hemostatic and coagulation dysfunction [1]. D-dimer is a specific degradation product of cross-linked fibrin hydrolyzed by fibrinolytic enzymes, and it is an important reference index in the diagnosis and treatment of DIC [2]. The hook effect is a phenomenon of false negatives caused by inappropriate antigen-antibody ratios during the process of immune analysis. This article introduces the phenomenon of falsely reduced D-dimer values in DIC

Table 1. D-dimer reported values, retest values, and dilution values at different time points.

Test mode	D-dimer (mg/L)			Reference range (mg/L)
	First result January 5	Second result January 6	Third result January 7	
Test values	1.09	0.93	1.43	0 - 0.7
Retest values	1.06	0.91	1.42	0 - 0.7
Dilution 1:8	exceed detection limit	exceed detection limit	exceed detection limit	-
Dilution 1:16	exceed detection limit	exceed detection limit	exceed detection limit	-
Dilution 1:32	exceed detection limit	exceed detection limit	12.99	-
Dilution 1:64	exceed detection limit	exceed detection limit	7.76	-
Dilution 1:128	6.58	11.76	4.61	-
Conversion values	842.24	1505.28	415.68	0 - 0.7

Table 2. Results of coagulation indices, platelets, 3P test, and blood culture at different time points.

Test items	First result January 5	Second result January 6	Third result January 7	Reference range
Prothrombin time (PT)	17.5	> 180	52.6	10 - 13.5 seconds
Activated partial Thromboplastin time (APTT)	57.0	> 180	51.3	23.9 - 33.5 seconds
Thromboplastin time (TT)	26.1	> 160	54.7	14.5 - 21.5 seconds
Fibrinogen (Fbg)	1.17	< 0.1	0.48	2.0 - 4.0 g/L
Platelets (PLT)	83	23	41	125 - 350 x 10^9/L
3P test	positive	-	-	negative
Blood culture	gram-negative bacillus positive	negative	-	negative

The blood culture result is the sample submission time.

patients due to the hook effect. The details are as follows:

CASE PRESENTATION

The patient is a 33-year-old male, on the night of January 5, 2024, due to a fever for 3 days and, a coma for more than 1h, he was admitted to our hospital emergency department for treatment. On the night of January 7, 2024, due to the critical condition, the family voluntarily give up the treatment and then discharged from the hospital. Three D-dimer tests were performed in the course of the patient's treatment. The instrument used was a Sysmex fully automatic coagulometer (model: CN-6000), and the method used was latex-enhanced immunoturbidimetry. The first time after admission (January 5, 2024, 22:56) D-dimer: 1.09 mg/L, the second time (January 6, 2024, 12:03) D-dimer: 0.93 mg/L, and the third time (January 7, 2024, 12:19) D-dimer: 1.43 mg/L. On January 7, 2024, at about 13:00, the clinician contacted our department (Department of Clinical Laboratory), stating that, in combination with other test data and the patient's clinical presentation, the patient's diagnosis was DIC, and expressed doubt about our D-dimer results. We retested the three D-dimer results and found that all the D-dimer reaction curves (before and after the retest) were normal without any abnormalities, and there was no significant difference between the results after the retest and the originally reported results, as shown in Table 1. At the same time, we reviewed the patient's other test data, as shown in Table 2, and found that the patient's blood cultures were positive (Gram-negative bacillus positive), positive plasma protamine

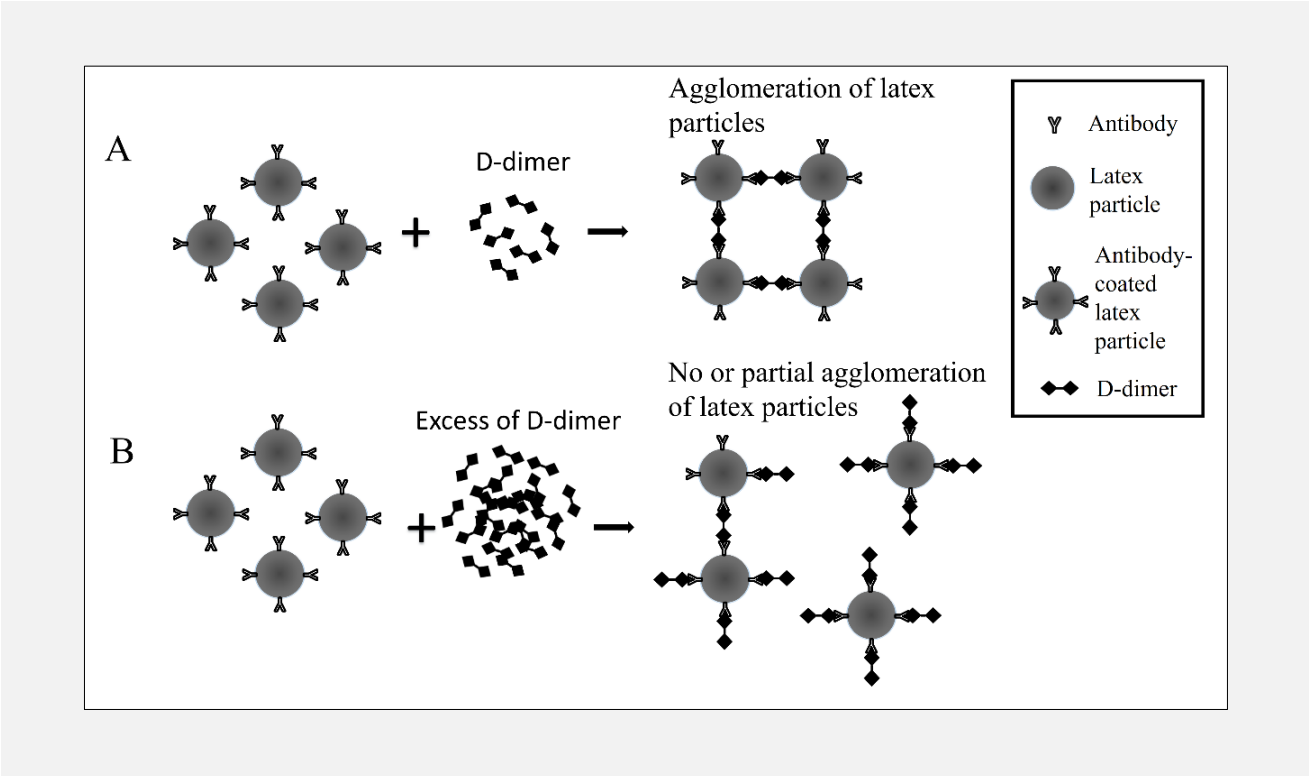

Figure 1. Schematic diagram of D-dimer detection.

A - The normal process of latex-enhanced immunoturbidimetric detection of D-dimer, where D-dimer binds to specific antibodies on latex particles, causing the latex particles to agglomerate.
B - The process of the hook effect occurring in the latex-enhanced immunoturbidimetric detection of D-dimer, where an excess of D-dimer saturates the specific antibodies on the latex particles, causing the latex particles to not agglomerate or partially agglomerate.

paracoagulation test (3P test), platelets, and coagulation factors were heavily depleted. All the test results were consistent with the diagnosis that the patient was DIC. However, the D-dimer result did not support this diagnosis, could it be that there was a hook effect in the testing process? With the question, we tested the specimen after double dilution (1:8, 1:16, 1:32, 1:64, 1:128) with D-dimer diluent. Finally, the first-time D-dimer: 842.24 mg/L (after 1:128 conversion), the second-time D-dimer: 1,505.28 mg/L (after 1:128 conversion), and the third-time D-dimer: 415.68 mg/L (after 1:32 conversion), and the detailed data are shown in Table 1. The results of the D-dimer after dilution were hundreds or thousands of times higher than those before dilution, and we confirmed that the hook effect caused the false decrease of D-dimer. Finally, we communicated with the clinician, who approved of our post-dilution D-dimer results.

DISCUSSION

DIC is a serious syndrome that can pose a threat to the patient's life if not detected and treated in time. Therefore, it is crucial to detect DIC early and start appropriate treatment [3]. D-dimer is a specific degradation product of cross-linked fibrin clots. Fibrinogen or non-crosslinked fibrin does not produce D-dimer after degradation. Therefore, D-dimer is considered a reliable marker of pathological coagulation and can be used for the diagnosis and therapeutic monitoring of DIC [4]. The half-life of D-dimer in the blood is about 6 to 8 hours [5,6]. Currently, the commonly used method for detecting D-dimer in the laboratory is latex-enhanced immunoturbidimetry. The principle of this method is (as shown in Figure 1A) that when D-dimer is added, the latex particles coated with D-dimer specific antibodies will agglomerate, causing the absorbance of the solution to increase, and the absorbance is directly proportional to the concentration of D-dimer. However, latex-enhanced immunoturbidimetry is also affected by various interfering factors. Numerous reports have pointed out that this method may show a false increase in D-dimer, while the false decrease in D-dimer caused by the hook

effect is less common [7-9]. This paper reports a case of false decrease in D-dimer detection by latex-enhanced immunoturbidimetry caused by the hook effect. The D-dimer reagent used by the Sysmex CN-6000 fully automatic coagulation analyzer is produced by SIEMENS. This reagent contains a sufficient number of latex particles coated with monoclonal-specific antibodies. When an appropriate excess of D-dimer is added, the hook effect will not occur, and the detection system will prompt "> 35.2 mg/L" or "antigen excess". However, when detecting abnormally high D-dimer or when the amount of D-dimer far exceeds the detection limit of the instrument, the hook effect will still occur, the instrument has no prompts, and the reaction curve is also normal. This phenomenon is not common and is difficult to detect, which can easily lead to misdiagnosis and mistreatment in clinical practice. The reason for this phenomenon is that the D-dimer structure has two D-region fragments [10]. When an abnormally large amount of D-dimer is added, each D-dimer-specific antibody can only bind to one binding site of D-dimer, and the other binding site has no chance to bind with the specific antibody, causing the latex particles to only partially aggregate or not aggregate, the measured absorbance will correspondingly decrease, and the calculated D-dimer value will also decrease accordingly (as shown in Figure 1B). Our laboratory personnel can eliminate interference by diluting the sample when encountering this rare and important hook effect. At the same time, we need to pay attention when the clinical diagnosis is inconsistent with the D-dimer value, or when the patient has obvious consumption of coagulation factors, platelets, fibrinogen, etc., and the D-dimer value is still relatively low. The D-dimer value at this time may show a false decrease caused by the hook effect, which needs to be dealt with in time to provide accurate results to clinicians.

In summary, this case reminds laboratory personnel to be vigilant regarding the possibility of the hook effect causing a false decrease in D-dimer values when encountering DIC patients whose D-dimer values are inconsistent with clinical diagnoses. Specific measures can be taken to eliminate interference by diluting the sample, thereby providing accurate results to the clinic as soon as possible and avoiding delays in the diagnosis and treatment of DIC patients.

Source of Support:
The study was supported by the Project of Health Science and Technology Program of Shaoxing Municipal Health Commission (2022KY021).

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

References:

1. Papageorgiou C, Jourdi G, Adjambri E, et al. Disseminated Intravascular Coagulation: An Update on Pathogenesis, Diagnosis, and Therapeutic Strategies. Clin Appl Thromb Hemost Dec 2018; 24(9_suppl):8s-28s. (PMID: 30296833)

2. Riley RS, Gilbert AR, Dalton JB, Pai S, McPherson RA. Widely Used Types and Clinical Applications of D-Dimer Assay. Lab Med May 2016;47(2):90-102. (PMID: 27016528)

3. Mammen EF. Disseminated intravascular coagulation (DIC). Clin Lab Sci 2000 Fall;13(4):239-45. (PMID: 11586511)

4. Weitz JI, Fredenburgh JC, Eikelboom JW. A Test in Context: D-Dimer. J Am Coll Cardiol 2017 Nov 7;70(19):2411-20. (PMID: 29096812)

5. Toulon P, Metge S, Hangard M, Zwahlen S, Piaulenne S, Besson V. Impact of different storage times at room temperature of unspun citrated blood samples on routine coagulation tests results. Results of a bicenter study and review of the literature. Int J Lab Hematol 2017 Oct;39(5):458-68. (PMID: 28481034)

6. Böhm-Weigert M, Wissel T, Muth H, Kemkes-Matthes B, Peetz D. Long- and short-term in vitro D-dimer stability measured with INNOVANCE D-Dimer. Thromb Haemost Feb 2010;103(2):461-5. (PMID: 20126827)

7. Ma H, Chen CH, Li YM, Wang JJ, Hu ZD. Falsely elevated D-dimer partially caused by heterophilic antibodies: A case report. Clin Chim Acta 2022 Oct 1;535:140-2. (PMID: 35988779)

8. Zhang L, Bao G, Zheng Z. Two Cases of Pseudoelevation of Plasma D-Dimer Levels. Clin Lab 2021 Sep 1;67(9). (PMID: 34542980)

9. Verboogen D, Granzen B, Hudig C, et al. Heterophilic antibodies leading to falsely positive D-dimer concentration in an adolescent. Res Pract Thromb Haemost 2023 Jan;7(1):100017. (PMID: 36785755)

10. Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and future prospects. Blood 2009 Mar 26;113(13):2878-87. (PMID: 19008457)