Case Report

Tülay Çevlik*, Rana Turkal, Goncagül Haklar and Önder Şirikçi

A case of falsely elevated D-dimer result

https://doi.org/10.1515/tjb-2021-0262
Received December 7, 2021; accepted June 9, 2022;
published online August 24, 2022

Abstract

Objectives: Heterophile antibodies can cause interferences in immunometric assays. While many tests are shown to be affected by interference from heterophilic antibodies, the D-dimer test has rarely been reported to be affected. With this case, we report an elevated D-dimer measurement which was not compatible with the clinical presentation.
Case presentation: A 41-year-old patient who was admitted to hospital with heart palpitations had a D-dimer elevation irrelevant to his clinical condition. D-dimer measurements were repeated in new samples directly and after being treated in heterophilic blocking tube with two different reagent lots of a latex-based automated immunoturbidimetric assay and an immunoturbidometric assay. D-dimer values were normalized (0–0.5 mg/L) when we used a new lot of reagent on the same instrument or measured by an immunoturbidometric method on the chemistry analyzer. After treatment with HBT, all samples revealed D-dimer results within the reference ranges.
Conclusions: The presence of heterophile antibodies in a sample should be considered when an elevated D-dimer value that is not compatible with the clinical presentation is encountered. Apart from the patient's HA's causing false results, sporadic susceptibility of the reagents should also be kept in mind as a possibility.

Keywords: coagulation testing; D-dimer; heterophilic antibody; immunoassay; interference.

***Corresponding author: Tülay Çevlik**, Biohemistry Laboratory, Marmara University Pendik Education and Research Hospital, Istanbul, Turkey, E-mail: tulaycevlik@gmail.com. https://orcid.org/0000-0002-3871-8072
Rana Turkal, Biochemistry Laboratory, Marmara University Pendik Education and Research Hospital, Istanbul, Turkey. https://orcid.org/0000-0002-0042-7261
Goncagül Haklar and Önder Şirikçi, Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey. https://orcid.org/0000-0003-4037-283X (g. Haklar). https://orcid.org/0000-0002-7286-4989 (Ö. Şirikçi)

Introduction

D-dimer is a product of fibrin degradation which forms during fibrinolysis. It is most used for the diagnosis of deep vein thrombosis (DVT), pulmonary embolism (PE), dissection of the aorta, sepsis, infection, and disseminated intravascular coagulation (DIC). D-Dimer can also be measured in low amounts in healthy individuals [1]. Whole blood, plasma, and serum samples can be used for analysis. At least 30 commercial D-dimer assays based on three other methods are available to determine D-dimer qualitatively and quantitatively. These methods are enzyme-linked immunosorbent assays (ELISA), immunofluorescent assays and latex-based automated assays with immunoturbidometric readings. The common point of these methods is the use of monoclonal antibodies against epitopes on D-dimer fragments. Each of these methods has its specific considerations and limitations [2].

Paraproteinemia, icterus, lipemia, and hemolysis are common factors interfering with coagulation tests at the preanalytical phase. The interference will also depend on the analytical method used [3].

Heterophile antibodies (HA) are a source of interference in immunoassays. HAs interact weakly or strongly with the antibody present in the reagent or antigen-antibody complex formed during the immunoassay analysis, which may cause false-positive or false-negative results. HAs interact weakly or strongly with the antibody or antigen-antibody complex at the end of the reaction and cause false-positive or false-negative results. Many reports are available regarding the effect of HA on different immunometric assays but reports of interference with D-dimer assays are rare [4].

In this case report, we investigated the reason behind an elevated D-dimer result which was not compatible with the clinical presentation of the patient who was admitted to our hospital with heart palpitations.

Case presentation

A 41-year-old male patient was admitted to Marmara University Pendik E&R Hospital with a complaint of heart palpitations. The patient's history revealed sleep disturbances, and he had stated to be passing through a stressful

period. The patient had no medical history of illness, except for high triglycerides and cholesterol levels, and the patient was on a lipid-lowering diet. There was no history of any drug use. His physical examination and routine laboratory tests were normal except for an elevated triglyceride level and an unexpected D-dimer elevation (1.21 mg/L; reference range 0–0.5 mg/L) which had persisted on a repeated sample on the same day (1.13 mg/L). His ECG, Holter monitoring records and echocardiography were normal. COVID-19 PCR (Polymerase Chain Reaction) and antibodies against SARS-CoV-2 were all negative. CT (Computed Tomography) angiography was normal. Heart palpitations were attributed to the patient's stressful period. His physician consulted the laboratory for the elevated D-dimer levels. An elevated D-dimer result in a 41 years old patient without compatible clinical presentation on admission and status on follow-up has led us to search for other causes than thrombosis.

New samples were drawn to determine D-dimer levels directly and after being treated in a heterophilic blocking tube (HBT, Scantibodies Laboratories, Santee, CA) with two different reagent lots of a latex-based automated immunoturbidimetric assay (STA-R, Diagnostica Stago, USA) and an immunoturbidometric assay (Olympus AU400 Chemistry Analyzer, Beckman Coulter, USA). Additionally, triglyceride and anti-rheumatoid factor (RF) tests were ordered reflectively.

For HA interference screening, 500 µL of patient's plasma was added to the HBT that contains a pellet of blocking reagent. The tube was then incubated for 1 h at room temperature. The untreated and treated plasma samples were assayed with two different lots of reagents on the hemostasis analyzer STA-R and Olympus AU400 Chemistry Analyzer, simultaneously.

Table 1: Laboratory test results.

	Patient results		Reference range
	At admission (30.12.2020)	Follow-up (30.12.2020)	
Leukocytes, $\times 10^3$/µL	8.3	ND	4.0–10
Red blood cell, $\times 10^6$/µL	5.6	ND	3.5–5.7
Hemoglobin, g/dL	16.8	ND	12–17
Hematocrit, %	48.9	ND	36–50
Platelets, $\times 10^3$/µL	231	ND	150–440
D-dimer, mg/L	1.21	1.13	0–0.5
Troponin T-hs, ng/L	4	ND	0–14
Fibrinojen, mg/dL	306	ND	200–400
Glucose, mg/dL	95	ND	65–110
Triglycerides, mg/dL		334	30–200
Bilirubin, mg/dL	0.31	ND	0–1.13
Creatinine, mg/dL	0.73	ND	0–1.12
ALT, U/L	26	ND	10–40
AST, U/L	21	ND	10–37
COVID-19 PCR	Negative		
Rheumatoid factor, IU/mL	ND	13	0–14

ND, not determined.

Table 2: D-dimer[a] results obtained from the new sample before and after HBT treatment.

Analyzer	Untreated sample	After HBT treatment
Hemostasis analyzer STA-R (new sample, current lot)	0.94 mg/L	0.34 mg/L
Hemostasis analyzer STA-R (new sample, new lot)	0.30 mg/L	0.13 mg/L
Chemistry analyzer olympus AU400	0.22 mg/L	0.31 mg/L

[a]Reference range, 0–0.5 mg/L.

Results

The preanalytical and analytical conditions of previous samples were requestioned for any factor that could account for the elevated D-dimer results, and all operations were found to be in accordance with the procedures. The internal and external quality control results ~~to~~ pertaining to days of elevated results were reviewed and were found to be within acceptable limits. Except for triglycerides, all test results were within the normal reference range (Table 1). His RF was negative, and his triglyceride level was 334 mg/dL (reference range <200 mg/dL). The samples' hemolysis, icterus, and lipemia indices were within normal limits.

The new sample still revealed an elevated D-dimer result with the current reagent lot used in the STA-R hemostasis analyzer. However, D-dimer results were within reference ranges when measured with a new lot of reagents on the same instrument and with the immunoturbidometric method on the chemistry analyzer. After treatment with HBT, all samples' D-dimer results were within reference ranges (Table 2).

Discussion

D-Dimer is a fibrin degradation product, and elevated levels could be an alarming sign indicating thrombus formation, a potentially fatal situation, which can also be encountered in COVID-19 patients. Elevated D-dimer concentrations may also be observed in many conditions unrelated to thrombosis, including preanalytical and analytical interactions, and differentiation of these conditions is a challenge [3]. In our case, the lack of compatible

clinical presentation with a high D-dimer result upon admission, lack of any indicator of thrombosis in cardiovascular tests and radiological investigations, a negative COVID-19 PCR, lack of clinical signs of thrombosis during follow-up led us to non-thrombotic reasons, mainly to preanalytical and analytical reasons interfering with the test result.

D-Dimer measurements might be affected by hemolysis, icterus, and lipemia, but the repeating samples' hemolysis, icterus, and lipemia indices were all within acceptable limits. The patient's triglyceride concentration was elevated (334 mg/dL), but it was still below the threshold of lipemia interference of 670 mg/dL on Stago hemostasis analyzers as stated by the manufacturer [5]. Elevated RF is another molecule that can interfere with D-dimer assays, but the patients' RF was found to be negative.

Another increasingly recognized cause of interference in immunoassays is the presence of HA, which are multispecific immunoglobulins. The formation of HA is triggered by known or unknown antigenic stimulants encountered as a result of exposure to animals and/or products containing antigenic molecules of animal source, after immunization by vaccines, following blood transfusions, autoimmune diseases, and maternal metastases [4]. Depending on the design of the assay, interference due to HA may lead to falsely low or high analyte levels in several immunoassays. When suspected, the presence of HA can be verified by following the methods, checking for linearity of results in serially diluted samples, re-analyzing the sample by a different method or by using heterophilic antibody blocking reagents/tubes [6]. Wu et al. [4] and Sun et al. [7] have also reported heterophilic antibody interference with very high D-dimer results. In both cases, the patients were over 70 years of age with underlying diseases (including hypertension, diabetes, bronchial asthma and chronic heart failure). In both cases, the heterophilic antibody interference was shown by treating the samples with heterophilic antibody blockers; the D-dimer levels had decreased after adding heterophilic antibody blockers but were still above the reference ranges. Whereas, our patient was a young and healthy individual, who had a slightly elevated result above the reference range but well within the measuring range. After treating with heterophilic antibody blockers, the D-dimer level had returned to within the reference range interval. Getting normalized D-dimer results with HBT treated samples led us to conclude that there was an HA interference in our patients' samples, causing a false elevation.

The prevalence of HA in the general population can be as high as 40% [8]. False results due to HA interference has the potential to have medical and legal consequences. Therefore, IVD companies try to decrease the frequency of interferences by adding HA blocking agents to reagents [9]. These agents are usually nonimmune (irrelevant) globulins or normal animal sera in nature. This practice has dramatically decreased the frequency of interference observed in current assays to 0.05% (10 per 20,000) [10]. In addition to the demonstrated HA in patient's plasma, the fact that normal results had been obtained both with another lot of reagent on the same hemostasis analyzer and with an immunoturbidometric assay on the chemistry analyzer has shown us that the current reagent lot used on the hemostasis analyzer was more susceptible to interference with HA, leading to the elevated D-dimer results.

Conclusions

Although D-dimer is a marker for fibrinolysis and the test is run on hemostasis analyzers in many laboratories, it should be remembered that the assays are immunoassays in principle. The presence of HA in a sample should be considered when an elevated D-dimer value that is not compatible with the clinical presentation is encountered. Apart from the patient's HA's causing false results, sporadic susceptibility of the reagents should also be kept in mind as a possibility.

Points to remember:

- It should be noted that D-dimer measurements are also antibody-based methods.
- Keep in mind the possibility of interference by heterophile antibodies.
- Maintain open communication with clinicians so they can freely reflect on inappropriate test results.

Research funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions: ÖŞ and GH conceived the study. TÇ and RT abstracted the data and participated in the data interpretation. RT and TÇ performed data interpretation, statistical analysis and reported the results. TÇ wrote the original draft of the paper. ÖŞ and GH proof-read and corrected the initial manuscript. All authors have read and approved the final version.

Conflict of interest: The authors declare no conflicts of interest regarding this article.

Informed consent: Not applicable.

Ethics declarations: Ethics approval and consent to participate. All methods were carried out in accordance with relevant guidelines and regulations.

Data availability: The dataset analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Weitz JI, Fredenburgh JC, Eikelboom JW. A test in context: D-dimer. J Am Coll Cardiol 2017;70:2411–20.
2. Johnson ED, Schell JC, Rodgers GM. The D-dimer assay. Am J Hematol 2019;94:833–9.
3. Favressea J, Lippib G, Roy PM, Chatelaina B, Jacqmina H, Cate H, et al. Preanalytical, analytical, post-analytical variables, and clinical applications. Crit Rev Clin Lab Sci 2018;55: 548–77.
4. Wu Y, Xiao YX, Huang TY, Zhang XY, Zhou HB, Zhang XX, et al. What makes D-dimer assays suspicious–heterophilic antibodies? J Clin Lab Anal 2019;33:22687.
5. Ramzi M, Esteve F, Grimaux M. D-dimer measurements and Lipemia Interference of STA-Liatest D-Di on diagnostica stago analyzers. A new approach for interference testing and lipid removal by high-speed centrifugation. Diagnostica Stago; 2018: 7 p. Report No. 301478.
6. Çokluk E, Tuncer FB, Şekeroğlu MR, Gözükara C, Ağaç S, Gözükara Sİ. Effect of heterophyll antibody interference on CA 19-9 measurement: a case report. Sakarya Tip Dergisi 2019;9:558–64.
7. Sun HX, Ge H, Xu ZQ, Sheng HM. Clinical laboratory investigation of a patient with an extremely high D-dimer level: a case report. World J Clin Cases 2020;8:3560–6.
8. Bolstad N, Warren DJ, Nusta K. Heterophilic antibody interference in immunometric assays. Best Pract Res Clin Endocrinol Metab 2013;27:647–61.
9. STA®-Liatest® D-Di Plus, less interferences for even more confidence. Available from: https://www.stago.com/products-services/new-products/detail/article.
10. Levinson SS, Miller JJJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta 2002;325:1–15.