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CASE REPORT

Heterophilic antibodies leading to falsely positive D-dimer concentration in an adolescent

Danielle Verboogen PhD[1] | Bernd Granzen MD[2] | Ciska Hudig PhD[3] | Daan van de Kerkhof PhD[4] | Paul Verhezen BSc[5] | Douwe de Boer PhD[5] | Yvonne Henskens PhD[5]

[1]Department of Clinical Chemistry and Hematology, Elisabeth-TweeSteden Hospital, Tilburg, The Netherlands

[2]Department of Pediatrics, Maastricht University Medical Center, Maastricht, The Netherlands

[3]LabWest, Department of Clinical Chemistry, Haga Teaching Hospital, The Hague, The Netherlands

[4]Departement of Clinical Chemistry, Catharina Hospital Eindhoven, Eindhoven, The Netherlands

[5]Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, The Netherlands

Correspondence
Y.M.C Henskens, Central Diagnostic Laboratory, Maastricht University Medical Center, Postbus 5800, 6202 AZ Maastricht, The Netherlands.
Email: yvonne.henskens@mumc.nl

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Abstract

Background: We present the case of a 15-year-old adolescent with suspected pulmonary embolism and repeatedly elevated D-dimer levels.

Key Clinical Question: We aim to determine the cause for elevated D-dimer levels in a patient without venous thromboembolism.

Clinical Approach: When the D-dimer measurement was repeated with different assays, D-dimer levels were within the normal reference interval. Dilution series with assay diluent or low-affinity antibody blocking reagents either did not or only partially decreased the D-dimer value using the original reagent kit.

Conclusion: Analyses suggested the presence of interfering heterophilic antibodies in patient plasma, a known phenomenon with immunoturbidimetric D-dimer assays, which is rarely described. Prior to drawing this conclusion, the patient underwent extensive diagnostic testing, which led to uncertainty and discomfort for the health care providers, the patient, and their family.

KEYWORDS
adolescent, case report, D-dimer assay, heterophilic antibody, pulmonary embolism

Essentials

- D-dimer is a laboratory test conducted for evaluation of possible venous thromboembolism.
- We present an adolescent with suspected venous thromboembolism with high D-dimer levels but normal imaging studies.
- Persistent high D-dimer level with negative clinical work-up could suggest laboratory test interference.
- In this case, D-dimer level was elevated because of heterophilic antibody interference.

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1 | INTRODUCTION

The result of activation of coagulation is the formation of a hemostatic plug. Degradation of the hemostatic plug occurs by fibrinolysis, the cleaving into fragments called fibrin degradation products. The most widely analyzed degradation product is D-dimer, which is used in diagnostic evaluation of venous thromboembolism (VTE), such as deep vein thrombosis (DVT) and pulmonary embolism (PE). However, elevated D-dimer levels are not disease-specific (Table 1) and may be caused by any process that activates coagulation [1]. Therefore, for VTE diagnosis, pretest clinical probability of VTE should be considered before ordering D-dimer analysis. A common approach, which evaluates pretest probability, is the Wells score for DVT or PE [2,3]. When less than 2 or 4 points are scored, a D-dimer measurement is performed, which increases the negative predictive value of the algorithm. If D-dimer concentration is low, DVT or PE can be excluded in adults. If D-dimer concentration is high, imaging to rule out DVT or PE is required. In the following case report, we aim to determine the cause for an elevated D-dimer level in a patient without VTE.

KEY CLINICAL QUESTION

What is the cause for an elevated D-dimer level in a patient without venous thromboembolism?

2 | CASE HISTORY

A 15-year-old girl was referred by their general practitioner to the emergency department for suspected PE. At the emergency department, the patient had pleuritic left flank pain. Clinically, there were no signs of infection. She had no exercise intolerance, dyspnea, cough, or hemoptysis and had no history of malignancy, immobilization, or recent surgery. She had started an oral contraceptive (ethinylestradiol, 0.03 mg/levonorgestrel, 0.15 mg) 3 months ago. Between the age of 4 and 15, she was seen repeatedly in the hospital for recurrent urinary tract infections and glomerulonephritis. Her family history was negative for bleeding and thrombotic disorders. The YEARS clinical decision rule for PE was negative. This decision rule contains 3 items (clinical signs of DVT, hemoptysis, and whether PE is the most likely diagnosis). The initial D-dimer concentration was 1851 μg/L (upper limit of normal = 500 μg/L). Therefore, a computed tomography angiogram of the thorax was performed and found negative for PE. The patient and her parents were informed and sent home.

Two months later, the adolescent presented to the emergency department with acute pain around the lower ribcage during breathing. In addition, she had a painful leg, which was not swollen or red. Again, D-dimer level was elevated (1829 μg/L), and a computed tomography angiogram of the thorax was repeated. No explanation was found for elevated D-dimer level and pain. The medical team performed a duplex ultrasound, to rule out DVT, which came out negative. Abdominal ultrasound was also negative.

Over the following 14 months, the patient was seen repeatedly by her primary care provider or in the hospital because of recurring pain in her torso, and D-dimer concentration was repeatedly abnormally elevated (Figure 1). The clinical laboratory was contacted, and the possibility of interference in the analytical assay was raised.

3 | LABORATORY APPROACH

The initial D-dimer concentration was measured using Innovance D-dimer assay (Siemens). As interference was suspected, D-dimer analysis was repeated on 1 sample using the STA-Liatest D-Di plus (Stago) and Tina-quant D-dimer Gen.2 (Roche Diagnostics) assay. All are latex-enhanced immunoturbidimetric immunoassays.

Dilution experiments were performed with Innovance assay diluent or LowCross blocking buffer (CANDOR Bioscience). Rheumatoid factor Immunoglobulin (Ig) A and IgM were measured on serum (Thermo Fisher Scientific). Data were analyzed with Excel 2016 (Microsoft) and Prism 6.0 (GraphPad).

TABLE 1 Causes of higher-than-normal D-dimer concentration.

Physiological	
Deep vein thrombosis	Pregnancy
Pulmonary embolism	Heart disease
Disseminated intravascular coagulation	Recent surgery
Infection	Trauma
Stroke	Advanced age
Malignancy	Thrombophilias
Preanalytical	
Hemolysis of sample	Interference of heterophilic antibodies

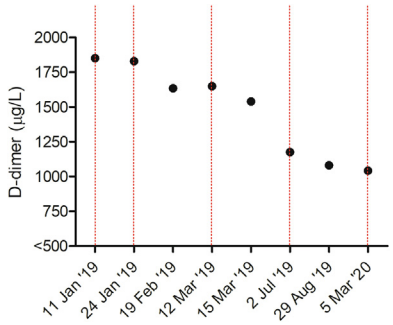

FIGURE 1 Overview of repeatedly elevated D-dimer concentrations (reference value <500 μg/L). Red lines indicate hospital visit

4 | RESULTS

Dilution of control plasma using assay diluent did not change the D-dimer concentration corrected for the dilution factor. On the contrary, dilution of the patient's plasma led to a ±50% decrease in D-dimer concentration (Figure 2A). The dilution series suggested assay interference due to heterophilic antibodies. Therefore, the dilution series was repeated using a LowCross blocking buffer, a buffer capable of neutralizing low-affinity interactions between the target and possible heterophilic antibodies. With LowCross blocking buffer, the concentration difference in patient plasma was ±56% (Figure 2B), implying that the interference could not be fully blocked using LowCross blocking buffer. Rheumatoid factor is a well-known group of interfering antibodies; however, both rheumatoid factors IgA and IgM were negative in the patient's serum.

Patient plasma was then sent to 2 laboratories that used a different assay for D-dimer analysis. In both laboratories, a value <500 µg/L was reported (Table 2), suggesting that the D-dimer level was not elevated *in vivo*.

5 | CONCLUSION AND DISCUSSION

In this adolescent patient with symptoms suggestive of possible VTE, who had recently started an oral contraceptive, repeatedly elevated D-dimer values led to extensive unnecessary diagnostic investigations. This led to uncertainty and discomfort for the patient, her parents, and health care providers. After repeated evaluations with no explanation for elevated level of D-dimer, the clinical laboratory investigated analytical interference in the D-dimer assay.

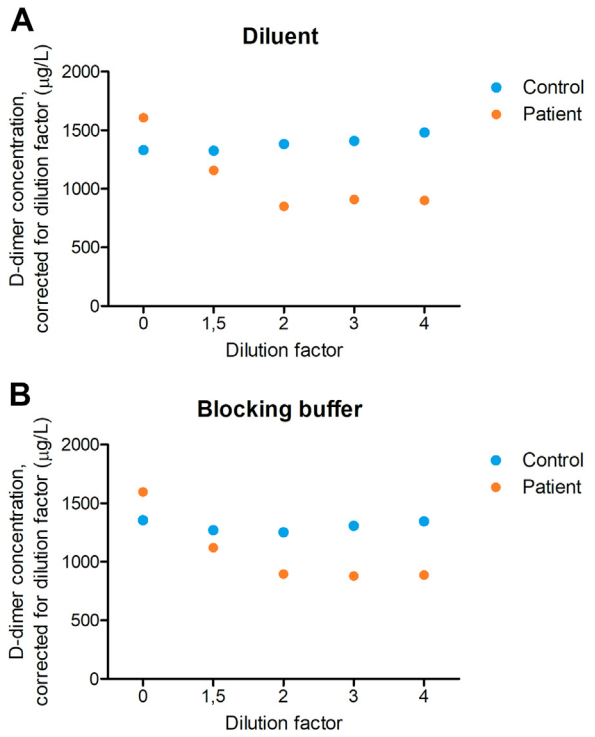

FIGURE 2 (A) Dilution series of patient and control plasma with assay diluent. (B) Dilution series of patient and control with LowCross blocking buffer

TABLE 2 D-dimer values as measured with assays from different suppliers.

Method	Result	Reference value
Innovance D-dimer	1176 µg/L	< 500 µg/L
STA-Liatest D-Di plus	320 µg/L	< 500 µg/L
Tina-quant D-dimer Gen.2	130 µg/L	< 500 µg/L

When the possibility of a falsely elevated D-dimer value, due to interference in the assay, was raised, dilution with a buffer blocking low-affinity antibodies was used. This only slightly lowered the reported D-dimer values. Subsequent analysis with different assays showed normal D-dimer values, indicating interference caused by likely higher affinity antibodies, although the exact cause was not found. Analytical interference in antibody-based tests is a known concept in laboratory medicine. Immunoassays, including assays for human chorionic gonadotropin, troponin, or thyroid hormones, are known to be sensitive to interference from heterophilic antibodies. These are naturally occurring polyreactive antibodies, autoantibodies, human anti–animal antibodies, or rheumatoid factor [4]. Published reports on antibody interference in D-dimer assays are rare. Cases described in the literature showed that interference in D-dimer assays occurs in both men and women of different ages (3-86 years) and with or without underlying diseases that could impact autoantibody status [5–8]. Interestingly, all described cases showed interference on latex-enhanced immunoturbidimetric D-dimer assays and occurred with monoclonal antibodies of different manufacturers, different antibody epitopes, and in reagents with different blocking agents [7]. All manufacturer package inserts of assays used in this report state that the assays are designed to minimize interference from heterophilic antibodies, for example, by addition of blocking agents or addition of F(ab)′2 fragments against a D-dimer epitope [9]. The small differences between assay reagents could explain why, in this patient, D-dimer values were abnormally elevated only in the Innovance assay. For the other assays (Table 3), no literature was found describing interference due to heterophilic antibodies. However, these assay types are less often used in high-throughput laboratories, and therefore, interference may not be detected or described.

Determination of the origin of heterophilic antibodies is challenging. The occurrence of antibody interference and the lack of a clearly definable cause for this interference makes considering the pretest probability of VTE in the context of D-dimer analysis even more important. D-dimer testing is sensitive but not specific to VTE (Table 1). Therefore, D-dimer analysis is mainly useful in excluding PE or DVT when it is suspected and the pretest probability is low. For this reason, several pretest probability algorithms can be used, like Wells, GENEVA, or YEARS criteria [11]. However, these algorithms do not perform well in children or adolescents and can lead to unnecessary further clinical evaluation, with a high psychological impact on vulnerable patient group [12,13].

TABLE 3 Types of D-dimer assays [10] and their susceptibility to interference of heterophilic antibodies.

Method	Susceptibility
Latex-enhanced immunoturbidimetric immunoassay	12 cases described in literature
Enzyme immunoassay, chemiluminescence	No cases described in literature
Polystyrene microparticle agglutination assay	No cases described in literature
Time-resolved fluorometry	No cases described in literature

In this case report, it is important to stress the negative psychological impact on an adolescent patient and her family due to the repeatedly elevated D-dimer value, continued suspicion of PE or DVT, and absence of final diagnosis. However, when repeated imaging does not indicate VTE, and there is no clinical cause of elevated D-dimer level, heterophilic antibodies should be considered.

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AUTHOR CONTRIBUTIONS

D.V. interpreted data and wrote the manuscript and F.H. and D.K. analyzed the data. B.G., P.V., D.B., and Y.H. contributed to the concept, design, analysis, and interpretation. All authors approved the final version of the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

INFORMED PATIENT CONSENT

The patient and her parents gave informed consent.

ORCID

Danielle Verboogen https://orcid.org/0000-0003-2368-9755
Bernd Granzen https://orcid.org/0000-0002-9567-0239
Ciska Hudig https://orcid.org/0000-0001-5298-7212
Daan van de Kerkhof https://orcid.org/0000-0002-0126-4695
Paul Verhezen https://orcid.org/0000-0002-5926-9384
Douwe de Boer https://orcid.org/0000-0003-2008-3328
Yvonne Henskens https://orcid.org/0000-0003-0217-6045

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