

LETTER TO THE EDITOR

Interference of D-dimer levels from heterophilic antibody in COVID-19: A serious concern in treatment and follow-up of patients

Dear Editors,

COVID-19 has become a pandemic since 11 March 2020 and caused devastating effects with high death rates worldwide. COVID-19 has led to severe coagulopathy, and thus, routine coagulation assays have been recommended to evaluate coagulation deficiency.¹ D-dimer has been used as a biomarker of coagulopathy from the beginning of the pandemic, which has become an important tool for follow-up in COVID-19 as the elevation of D-dimer has been directly correlated with poor prognosis.² D-dimer levels guide a physician to determine which patients should receive LMWH therapy.^{2,3}

From the beginning of the pandemic, we dealt with very high levels of D-dimer (>20.000 ng/mL) in some of the COVID-19 patients during their hospitalizations. Those D-dimer tests were re-assayed, and serial dilutions were made, but the results were still elevated and were higher than 20.000 ng/mL D-dimer Units (D-DU). In some of them, D-dimer levels were persistently elevated despite no abnormalities were found related to this elevation. It has been reported that, along with some laboratory tests such as ACTH, troponin and prostate-specific antigen, false D-dimer elevations could be seen in patients with viral infections because of heterophilic antibodies but data on COVID-19 were not available up to now.^{4,5} Heterophilic antibodies are polyclonal, heterogenous antibodies that bound to animal antigens. They can either be IgG or IgM type and may be induced by viral, bacterial infections, malignancies, autoimmune disorders, after vaccinations or produced without any apparent cause.⁶ They are more common in debilitated and hospitalized patients (0.2%-15%) but in general population, its prevalence is about 0.5%-3%.⁶ In this paper, we want to represent two PCR-positive COVID-19 cases with isolated and persistently elevated D-dimer without obvious cause after discharge from hospital.

D-dimer was analysed with ACL TOP (Instrumentation Laboratory) using HemosIL D-dimer test which is an automated latex enhanced turbidimetric immunoassay for determination of D-dimer in human citrated plasma. The latex reagent of the assay is a suspension of polystyrene latex particles of uniform size coated with the F(ab')₂ fragment of an mAb (MA-8D3) that is highly specific for the cross-linked D-dimer domain in FDPs or derivatives. The use of the F(ab')₂ fragment produces greater specificity in the detection of the D-dimer antigen while avoiding interference from endogenous factors such as RF and the reagent also contains a blocking agent to HAMA.⁷

In both cases, serial dilutions (1/2, 1/10 and 1/20) were performed to rule out assay interference, and similar recovery percentages (90%-120%) were shown which reflected us that there was no assay interference (Table 1). Tests for lupus anticoagulant, anti-nuclear, anti-cardiolipin antibodies and rheumatoid factor which might be responsible for positive interference were also analysed and were negative in both patients. Finally, a suspicion of heterophilic antibody interference was discussed with clinical laboratory. The same plasma analysed for D-dimer in autoanalyzer was used for heterophilic antibody existence, which was incubated in heterophilic antibody-blocking tube (HBT) (Scantibodies Laboratory, Inc.) for an hour. Afterwards, the supernatant of plasma was transferred to a tube and analysed for D-dimer once again. In both cases, significant decrease in D-dimer levels was shown.

In the first case, a 54-year-old male patient, who had no history of chronic illness, was hospitalized with complaints of fever and shortness of breath and his chest computed tomography (CT) revealed bilaterally interstitial infiltration compatible to COVID-19 pneumonia. Complete blood count (CBC) was normal at the beginning, but D-dimer was elevated (Hb: 14.6 g/dL, Leu: $4.9 \times 10^9/L$, Plt: $135 \times 10^9/L$, D-dimer: 325 ng/mL (D-DU) [69-243]). Despite treatment with favipiravir, levofloxacin, methylprednisolone and LMWH therapy during hospitalization, his clinical condition deteriorated and Ferritin and C-reactive protein levels along with IL-6 levels were found to be elevated (181 pg/mL [0-7]), but procalcitonin levels were in normal range. The progression of lung infiltrations and laboratory condition (high CRP, ALT, Ferritin and IL-6 levels, but normal procalcitonin) excluded secondary bacterial infection and led to the diagnosis of macrophage activation syndrome and tocilizumab for a total of 600 mg were applied to patient. Dramatic improvement was seen on the general clinical condition of the patient after tocilizumab administration. However, D-dimer level did not decrease simultaneously; instead, it was elevated to 19 537 ng/mL (D-DU) despite better clinical condition and no radiologic evidence of thrombosis. He was discharged on the 9th day of tocilizumab therapy in good clinical condition with a D-dimer value of 21 390 ng/mL (D-DU) and followed up on LMWH therapy. The decline in D-dimer continued in a slower manner, but no signs of COVID-19-associated pulmonary complications or thrombus were observed (Figure 1). On the 46th day of his discharge, D-dimer level was still 817 ng/mL (D-DU). After the suspicion of

	First measurement	Dilutions			After HBT treatment
		½	1/10	1/20	
Patient 1	817 ng/mL	505	99	37	382 ng/mL
Recovery %		123.62%	121.17%	90.57%	46%
Patient 2	1979 ng/mL	1005	252	89	92 ng/mL
Recovery %		101.56%	127.3%	89.9%	4.6%

TABLE 1 Biochemical procedures in cases with elevated D-Dimer level

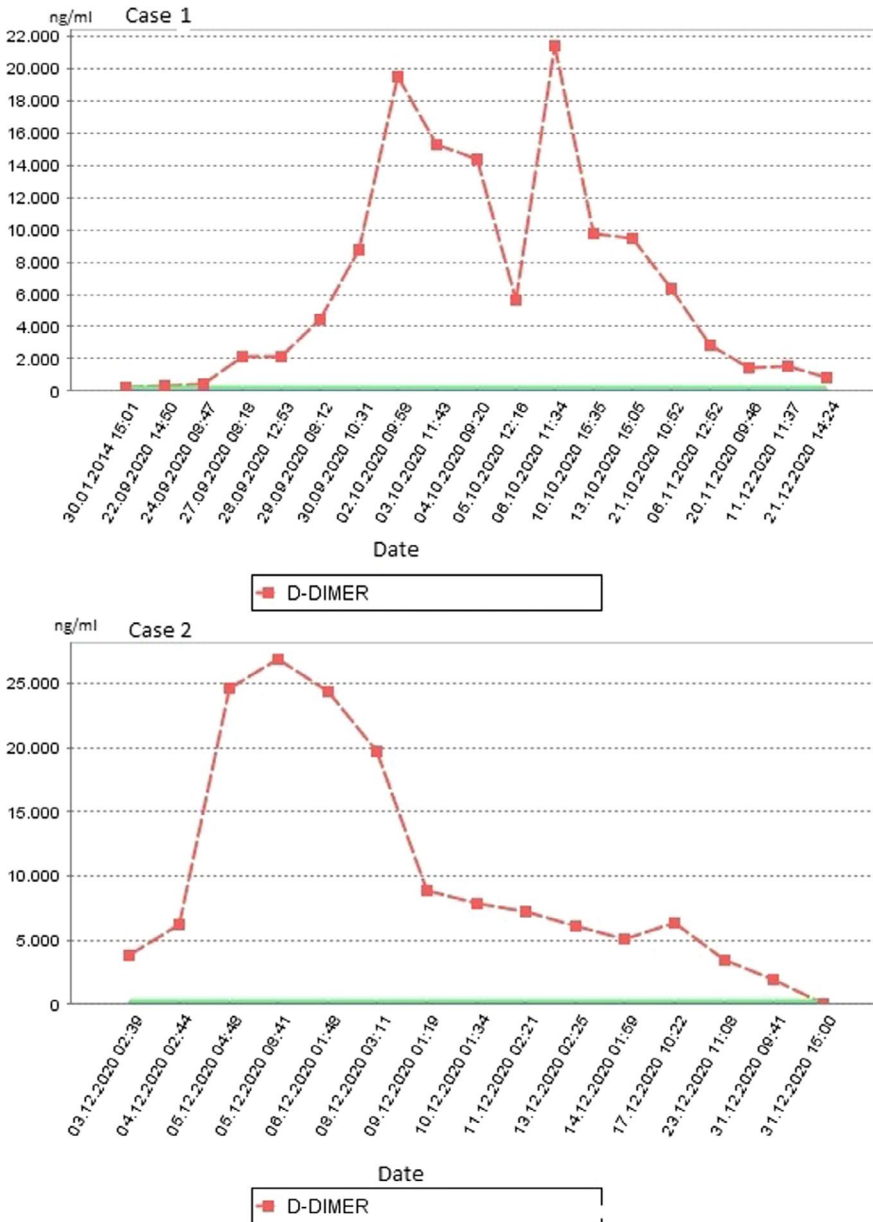


FIGURE 1 Course of pseudo-elevated D-Dimer levels during the follow-up of the patients

heterophilic antibody interference and HBT incubation, plasma D-dimer level fell to 382 ng/mL (D-DU), by which 46.7% recovery was shown. This decline is highly indicative for interference with heterophilic antibodies during and/or after COVID-19. In the light of these findings, we decided to stop LMWH therapy and began to follow up the patient with aspirin (100 mg/d).

The second case was a 42-year-old woman with a history of mitral valve disease who presented to the emergency department complaining of dry cough. She was diagnosed as SARS-CoV-2 pneumonia on chest CT and given favipiravir, moxifloxacin, therapeutic dose of LMWH and N-acetyl cysteine therapy since then. CBC was normal (Hb: 12.8 g/dL, Leu: $3.9 \times 10^9/L$, Plt: $169 \times 10^9/L$), but liver

enzymes, CRP and D-dimer were slightly elevated (AST: 38.6 IU/L, ALT: 41.9 IU/L, GGT: 71.94 IU/L, CRP: 15.09 mg/L and D-dimer: 380 ng/mL (D-DU)). On the 5th day of hospitalization, CRP decreased and had good clinical condition. But ALT, GGT and D-dimer levels steadily increased (199 IU/L, 167 IU/L and 24 566 ng/mL (D-DU), respectively) during her hospitalization (Figure 1). To rule out acute hepatitis, serological investigation, imaging tests revealed no abnormalities. She was discharged in good state of health with decreasing trend for all blood tests except D-dimer and liver function tests. Seventeen days after discharge, she was devoid of any symptoms of thrombosis despite a D-dimer level of 1979 ng/mL (D-DU). A re-evaluation of D-dimer testing with HBT revealed a normal D-dimer level of 92 ng/mL (D-DU) with 4.64% recovery. LMWH therapy was stopped, and aspirin (100 mg/d) was started as a result.

Various tests analysed with monoclonal antibodies including D-dimer has been interfered by heterophilic antibodies.⁴ Elevation of troponin I, prostate-specific antigen or human chorionic gonadotropin elevation has been well defined before, and there are a few reports describing heterophilic antibody-mediated D-dimer elevation^{3,8} as well. Viral infections have been suggested to be responsible in most of cases, and also, blood transfusions, exposure to immune materials, dealing with animals or its products and dialysis have also been closely related to induce heterophilic antibodies as well.⁵ In those cases, D-dimer levels have dramatically returned to normal after re-testing with antibody-blocking methods.^{3,8} The same mechanism may also be the fact in COVID-19.⁹ In both cases, patients had high levels of D-dimer while testing for heterophilic antibody, and we suspected antibody interference while the usual normalization process of D-dimer prolonged. In case one, the D-dimer levels decreased but did not decline to normal level; however, in case two, D-dimer levels became normal after testing with heterophilic antibody blocking as in other cases related with viral infections.⁸ We suggested that the difference in decrease rate in our cases may be related to heterogeneous nature of heterophilic antibodies and the differences in concentrations among individuals. In both cases, a steady but slow decline in D-dimer levels urged us to evaluate other possibilities as the patients had no proven thrombosis, sepsis, malignancy or other causes of elevated D-dimer levels. D-dimer levels steadily increased above 20 000 ng/mL (D-DU) and slowly declined but did not reach to normal levels. It has been reported that false D-dimer elevations could be seen in patients with viral infections as a result of heterophilic antibodies, and there is also a hypothesis is suggested that in viral infections, heterophilic antibodies are produced because of polyclonal activation of B lymphocytes following the binding of virus to complement receptor CR2.^{3,8-10}

Although a small but not insignificant complication of bleeding in two retrospective studies was reported, LMWH therapy significantly reduced mortality in COVID-19 and had major therapeutic and immunomodulatory effects in COVID-19 beyond anticoagulation.^{11,12} Unawareness of the pseudo-elevation of D-dimer by heterophilic antibodies could lead to unnecessary treatment with LMWH and devastating results because of major haemorrhage.

In conclusion, elevated D-dimer levels in COVID-19 without any signs of thrombotic disease should prompt the physician to

evaluate the case for heterophilic antibody. In these cases, somehow a kind of immunologic reaction may be triggered and caused de novo synthesis of antibodies against virus which may cause positive interference in D-dimer analysis. We cannot rule out that D-dimer plasma levels were actually elevated to some extent in those patients; heterophilic antibodies would have led to an overestimation; urgent studies are needed to explore the incidence and etiology of this phenomenon.

KEYWORDS

coagulation, COVID-19, D-dimer, heterophilic antibodies

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CONFLICT OF INTEREST



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AUTHOR CONTRIBUTIONS

Demircan Ozbalci has been responsible for writing and editing of the article. Duygu Kumbul Doguc, Fevziye Burcu Sirin, Fusun Zeynep Akcam, Onder Ozturk and Gulruhsar Yilmaz have been responsible for editing of the article. Duygu Kumbul Doguc and Fevziye Burcu Sirin have also added information about D-dimer analysis.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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