Evaluation of D-dimer levels measured by different analytical methods in COVID-19 patients

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Clinicians experience some challenges due to the lack of standardization of test, although D-dimer is a prognostic marker for COVID-19. We compared the clinical and analytical performances of D-dimer results obtained from different devices, kits and methods in patients with a diagnosis of COVID-19. Thirty-nine patients with a diagnosis of COVID-19 and 24 healthy individuals were included in the study. D-dimer levels were measured with Innovance D-DIMER kit (immunoturbidimetric method) on Sysmex CS-2500 and BCS XP and VIDAS D-Dimer Exclusion II kit (enzyme-linked fluorescence method) on mini VIDAS. The studies of precision, method comparison and clinic performance were performed. The variation coefficients in all systems were within the acceptable imprecision (7.8%). Bias%(12.5%) between BCS XP and Sysmex CS-2500 was lower than the acceptable Bias%(15.5%). Bias% values (19.2% and 33.3%, respectively) between Mini VIDAS with BCS XP and Sysmex CS-2500 were higher than the acceptable Bias%. The correlation coefficients among all systems were 0.89–0.98. For 500 ng/ml FEU, there was almost perfect agreement between BCS XP and Sysmex CS-2500, a moderate agreement between Mini VIDAS and BCS XP and Sysmex CS-2500. The cut-off values for distinguishing between individuals with and without COVID-19 were Mini VIDAS, Sysmex CS-2500 and BCS XP 529, 380 and 390 ng/ml FEU, respectively. The immunoturbidimetric method can be used as an alternative to the enzyme-linked fluorescent method because of satisfactory agreement at the different thresholds proposed for venous thromboembolism. However, it is recommended to follow up COVID-19 with the D-dimer results obtained by the same assay system. *Blood Coagul Fibrinolysis* 33:000–000

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Introduction

Although D-dimer is a fibrin degradation product and is used as a fibrinolytic marker for the risk of venous thromboembolism (VTE) and disseminated intravascular coagulation (DIC) [1], it has also been recommended as a marker for the severity and mortality of COVID-19 after pandemic [2–5]. However, methodological differences in studies on COVID-19 may create some challenges in evaluating clinical performance [1,6]. These differences can be classified as using different brands kits, calibrators and assay methods, differences in analytical performance, lack of standardization in units, absence of disease-specific threshold for D-dimer. The difference of used mAbs in D-dimer analysis and availability of a variety of fibrinogen and fibrin degradation products in plasma can adversely affect the specificity and sensitivity of the test [1,7]. Calibrator-based unit differences [D-dimer unit (DDU) and fibrinogen equivalent unit (FEU) or mg/l, μg/ml and ng/ml] may be overlooked during the evaluation of results.

D-dimer measurement has been used to decide on low-intensity or high-intensity anticoagulant therapy and to adjust the dose of anticoagulant therapy in patients with COVID-19 [8–10]. In this new use of D-dimer measurements, it is important to provide accurate, reliable and comparable results. False high levels may lead to unnecessary treatment, while false low levels may increase the risk of thromboembolism. It may be beneficial to compare the systems of D-dimer analysis and to investigate its effect on diagnosis or prognosis. For this reason, we aimed to evaluate the D-dimer results obtained by three different devices and two different kits in COVID-19 patients who had different stages, in terms of clinical and analytical performances.

Materials and methods

Participants

Thirty-nine patients diagnosed with COVID-19 and 24 healthy individuals not diagnosed with COVID-19 were included in the study. The average age of COVID-19 patients was 55.3 ± 16.9 years, of whom 16 (41.0%) were men and 23 (59.0%) were women. The average age of healthy individuals was 36.9 ± 7.9 years, of whom 10 (41.7%) were men and 14 (58.3%) were women.

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In healthy individuals without COVID-19 [COVID-19 (−)], those who received anticoagulant, fibrinolytic or thrombolytic therapy, and those with hematologic or liver disease were excluded. According to Clinical Spectrum of SARS-CoV-2 Infection of WHO, the patients [COVID-19 (+)] were classified as 'Asymptomatic infection', 'Mild, Moderate, Severe, and Critical' illnesses [11]. According to this classification, 'Mild and Moderate' and 'Severe and Critical' illnesses were combined to form two groups as 'Non-Severe' and 'Severe', respectively. The study was approved by the Tepecik Training and Research Hospital Ethics Committee (Decision No. 2021/05-25 dated 17.05.2021) and conducted in accordance with the Declaration of Helsinki.

Methods

Within the first 24 h of admission to the hospital, venous blood samples from individuals were taken into blood collection tubes containing 3.2% citrate (Becton Dickinson Vacutainer, Franklin Lakes, New Jersey, USA). After blood sampling, it was centrifuged at 1500 g for 10 min and the plasma was separated and aliquoted. Haemolyzed, icteric or lipemic samples were not included in the study. In the first step, plasma D-dimer measurement was performed using Innovance D-DIMER kits (Reference no. OPBP07; Siemens Healthcare Diagnostics, Marburg, Germany) on a Sysmex CS-2500 (Sysmex Corporation, Kobe, Japan) within 2 h. Remained plasma samples were stored at -20°C until they were analysed (at the latest 1 month according to the manufacturer's instructions).

In the second step, D-dimer levels in frozen and then thawed plasma were simultaneously measured using Innovance D-DIMER kits (Reference no. OPBP07; Siemens Healthcare Diagnostics) on a Sysmex CS-2500 (Sysmex Corporation, Kobe, Japan) and a BCS XP (Siemens Healthcare Diagnostics, Illinois, USA) instruments, and using VIDAS D-Dimer Exclusion II (Reference no. 30455-02; Biomerieux Diagnostic, Paris, France) kit on a mini VIDAS (Biomerieux Diagnostic, Marcy l'Etoile, France) instrument. The specifications for commercial D-dimer kits are given in Table 1.

The stability of the plasma samples was evaluated. Analytical precision and comparison studies were performed for D-dimer kits on different devices. Clinical performances of systems were compared for different clinical decision limits of D-dimer.

For D-dimer levels that were measured Sysmex CS-2500, Bias% values between results of 56 samples before and after freezing were calculated with the following formula. D-dimer levels in seven samples were measured without freezing.

$$100* \frac{(\text{Result after freezing}) - (\text{Result before freezing})}{(\text{Result before freezing})}$$

Plasma pools at two different levels (approximately <1000 and >1000 ng/ml FEU) were created. These plasma pools were run in three replicates for 5 days on all systems. Within-run, between-run and total coefficients of variation (CV%) were calculated.

The result of the VIDAS D-Dimer Exclusion II kit was accepted as a reference and compared to the results of the Innovance D-DIMER kit. Bias% values for D-dimer results ($n = 63$) were calculated with the following formula and averaged.

$$100* \frac{(\text{Innovance®\ D-DIMER result}) - (\text{VIDAS®\ } D-\text{Dimer Exclusion}^{TM}\ \text{II result})}{(\text{VIDAS®\ } D-\text{Dimer Exclusion}^{TM}\ \text{II result})}$$

Statistical analysis

Statistical analysis was performed with MedCalc Statistical Software version 19.1.3 (MedCalc Software Ltd, Ostend, Belgium). The normal distribution of data was evaluated with the Shapiro–Wilk test. According to the normal distribution, the data between groups were compared using Wilcoxon or the paired *t*-test. *P* value less than 0.05 was considered statistically significant. The relationship between groups was evaluated with Passing Bablok regression analysis and correlation coefficient. If the calculated Bias% between groups were lower than the acceptable Bias% ($Bias_a$%) (according to the European Society for External Quality Assessment, the bias was

Table 1 Specifications for commercial D-dimer kits

Reagent	Innovance D-DIMER	VIDAS D-dimer exclusion II
Assay Method	Particle-enhanced immunoturbidimetric assay Automated	Enzyme-linked fluorescent assay Semi-automated
Plasma tube	Sodium citrate	Sodium citrate
Antibody	Polystyrene particles covalently coated with 8D3 mAb	10B5E12C9 mAb coated on the solid phase and alkaline phosphatase-labelled monoclonal antibody 2C5A10
Antigen material for calibrator	Fibrin degradation products	Fibrin degradation products
Sample volume	50 µl for Sysmex CS-2500 15 µl for BCS XP	200 µl for mini VIDAS
Sample stability	4 weeks at ≤18°C	6 months at −25 ± 6°C
Analytical range	190–3520 ng/ml FEU for Sysmex CS-2500 170–4400 ng/ml FEU for BCS XP	45–10 000 ng/ml FEU
Clinical decision limit for venous thromboembolism	500 ng/ml FEU	500 ng/ml FEU

Table 2 The sample stability before and after freezing

$n=56$	Before freezing	After freezing
Mean ± SD, ng/ml FEU	1075 ± 867	1124 ± 884
Median (Min-Max), ng/ml FEU	820 (210–4020)	835 (220–3800)
Comparisons	Before vs. after freezing	
Bias%	6.1	
Differences of median, *P* value	0.001	
Intercept (95% CI)	5.7 (−25.8–40.0)	
Slope (95% CI)	1.0 (1.0–1.1)	
Linearity, *P* value	0.520	
Residual standard deviation (95% CI)	78 (−153–153)	
Spearman' s *r* (*P* value)	0.987 (<0.001)	

All D-dimer levels were measured using Innovance D-DIMER kits on a Sysmex CS-2500.

15.5%, which equals half of the total allowable error budget), and the total CV% were lower than the acceptable CV% (according to the European Society for External Quality Assessment, the CV% was 7.8%, which equals a quarter of the total allowable error budget), the values were supposed as acceptable.

According to the normal distribution of the data, Mann–Whitney test or unpaired *t*-test was used to compare D-dimer results between outpatient and inpatient or between COVID-19 (+) and (−). One-way analysis of variance (ANOVA) or Kruskal–Wallis test was performed to compare D-dimer results according to the severity of the disease. *P* value less than 0.05 was considered statistically significant. In addition, receiver operating characteristic (ROC) analysis was performed for those whose comparison results were significant. The cut-off value, the area under the ROC curve (AUC), sensitivity and specificity values were calculated. Considering the 500 and 1000 ng/ml FEU cut-off values used to exclude VTE [12], the inter-device agreement was evaluated by Kappa analysis. Kappa coefficient was interpreted as follows: 0–20 as none, 0.21–0.39 as minimal, 0.40–0.59 as weak, 0.60–0.79 as moderate, 0.80–0.90 as strong and 0.90–1.00 as almost perfect agreement [13].

Results

Out of 63 individuals, 39 (61.9%) were COVID-19 (+) and 24 (38.1%) were COVID-19 (−). Among COVID-19 (+) patients, 12 of them (19.0%) were 'Asymptomatic', 12 (19.0%) had 'Non-Severe' and 15 (23.9%) had 'Severe' symptoms.

The stability of samples after freezing and thawing is presented in Table 2. There was statistically significant difference between the results including before and after freezing. Yet, this difference was not clinically significant, and a high correlation was found between the results.

Within-run, between-run and total imprecision results of different kits and devices are summarized in Table 3. For less than 1000 ng/ml FEU, while the total CV% values of Innovance D-DIMER kits were close to each other, they were higher than those of the VIDAS D-Dimer Exclusion II kit. For more than 1000 ng/ml FEU, the total CV% values of Innovance D-DIMER kits were similar to VIDAS D-Dimer Exclusion II kit. For both levels, the total CV% of all devices were acceptable.

The comparison results of the kits and devices are presented in Table 4. Although there were statistical differences between Sysmex CS-2500 with mini VIDAS and BCS XP, there was no statistical difference between mini VIDAS and BCS XP. Bias% values between mini VIDAS with BCS XP and Sysmex CS-2500 were higher than $Bias_a$%.

Although the proportional error only between Sysmex CS-2500 and BCS XP was detected, they had a very high correlation ($r=0.98$, $P<0.001$) (Fig. 2). Between BCS XP vs. Sysmex CS-2500, mini VIDAS vs. BCS XP, and then mini VIDAS vs. Sysmex CS-2500, respectively, Residual Standard Deviation and Bias% increased, and the correlation coefficient decreased.

There was almost perfect and strong agreement between BCS XP vs. Sysmex CS-2500 at D-dimer decision limits

Table 3 Within-run, between-run and total imprecision results of kits and devices

Kits	VIDAS D-Dimer Exclusion II	Innovance D-DIMER	
Devices	mini VIDAS	BCS XP	Sysmex CS-2500
For <1000 ng/ml FEU			
Mean, ng/ml FEU	814	936	921
Within run SD, ng/ml FEU	15.9	19.8	42.6
Within run CV%	1.9	2.1	4.6
Between run SD, ng/ml FEU	16	58	39
Between run CV%	2.0	6.1	4.2
Total SD, ng/ml FEU	22.8	60.8	57.8
Total CV%	**2.8**	**6.5**	**6.3**
For >1000 ng/ml FEU			
Mean, ng/ml FEU	3093	3813	3959
Within run SD, ng/ml FEU	73	57	200
Within run CV%	2.4	1.5	5.1
Between run SD, ng/ml FEU	59	118	102
Between run CV%	1.9	3.1	2.6
Total SD, ng/ml FEU	93	131	172
Total CV%	3.0	3.4	4.3

D-dimer level was measured three times a day for 5 consecutive days. CV%, coefficients of variation. The acceptable imprecision (CV%) was considered as 7.8%.

Table 4 The comparison results of kits and devices

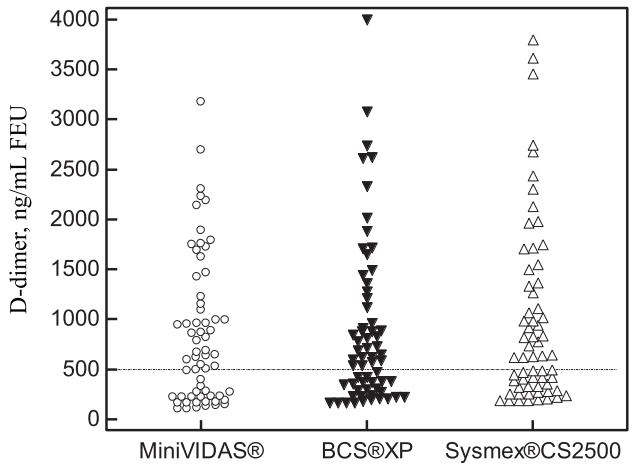
$n=63$	mini VIDAS	BCS XP	Sysmex CS-2500
Mean ± SD, ng/ml FEU	866 ± 737	892 ± 823	994 ± 899
Median (Min-Max), ng/ml FEU	651 (105–3181)	620 (170–4000)	640 (190–3800)
Comparisons	mini VIDAS vs. Sysmex CS-2500	mini VIDAS vs. BCS XP	BCS XP vs. Sysmex CS-2500
Bias%	33.3[a]	19.2[a]	12.5
Differences of median, *P* value	0.020[b]	0.787	<0.001[b]
Intercept (95% CI)	33.4 (−4.9 to 96.1)	13.1 (−26.7 to 77.3)	−4.3 (−37.8 to 24.3)
Slope (95% CI)	1.07 (0.95–1.20)	0.99 (0.85–1.11)	1.14 (1.10–1.19)[c]
Linearity, *P* value	0.130	0.390	0.800
Residual standard deviation (95% CI)	281 (−551 to 551)	249 (−488 to 488)	119 (−233 to 233)
Spearman' s r (*P* value)	0.89 (<0.001)	0.90 (<0.001)	0.98 (<0.001)
Kappa coefficient (95% CI) for 500 ng/ml FEU of CDL	0.67 (0.49–0.86)	0.70 (0.53–0.88)	0.96 (0.90–1.00)
Kappa coefficient (95% CI) for 1000 ng/ml FEU of CDL	0.74 (0.56–0.92)	0.72 (0.53–0.91)	0.85 (0.71–0.99)

$Bias_a$%, allowable Bias%; CDL, clinical decision limit; CI, confidence interval. [a] Higher than $Bias_{a\%}$ which is 15.5%. [b] *P* value <0.05 for Wilcoxon test. [c] Proportional error.

of 500 and 1000 ng/ml, respectively. The moderate agreement between mini VIDAS vs. BCS XP or mini VIDAS vs. Sysmex CS-2500 was found at two decision limits (Table 4). The distribution of D-dimer results is shown in Fig. 1.

The ROC analysis of systems to distinguish the presence or severity of disease is summarized in Table 5. With regard to the D-dimer results of all systems, there was no statistically significant difference between outpatient and inpatients with COVID-19 (+), or between different severity of COVID-19 (+). However, D-dimer results between COVID-19 (+) and (−) individuals were statistically significant different. When AUC values of systems were compared, the significant differences between mini VIDAS with Sysmex CS-2500 and BCS XP were found ($P=0.012$ and $P=0.047$, respectively). However, no significant difference was found between Sysmex CS-2500 and BCS XP ($P=0.073$).

Fig. 1



The distribution of D-dimer results of systems. The dashed line shows decision limits of 500 ng/ml FEU.

Discussion

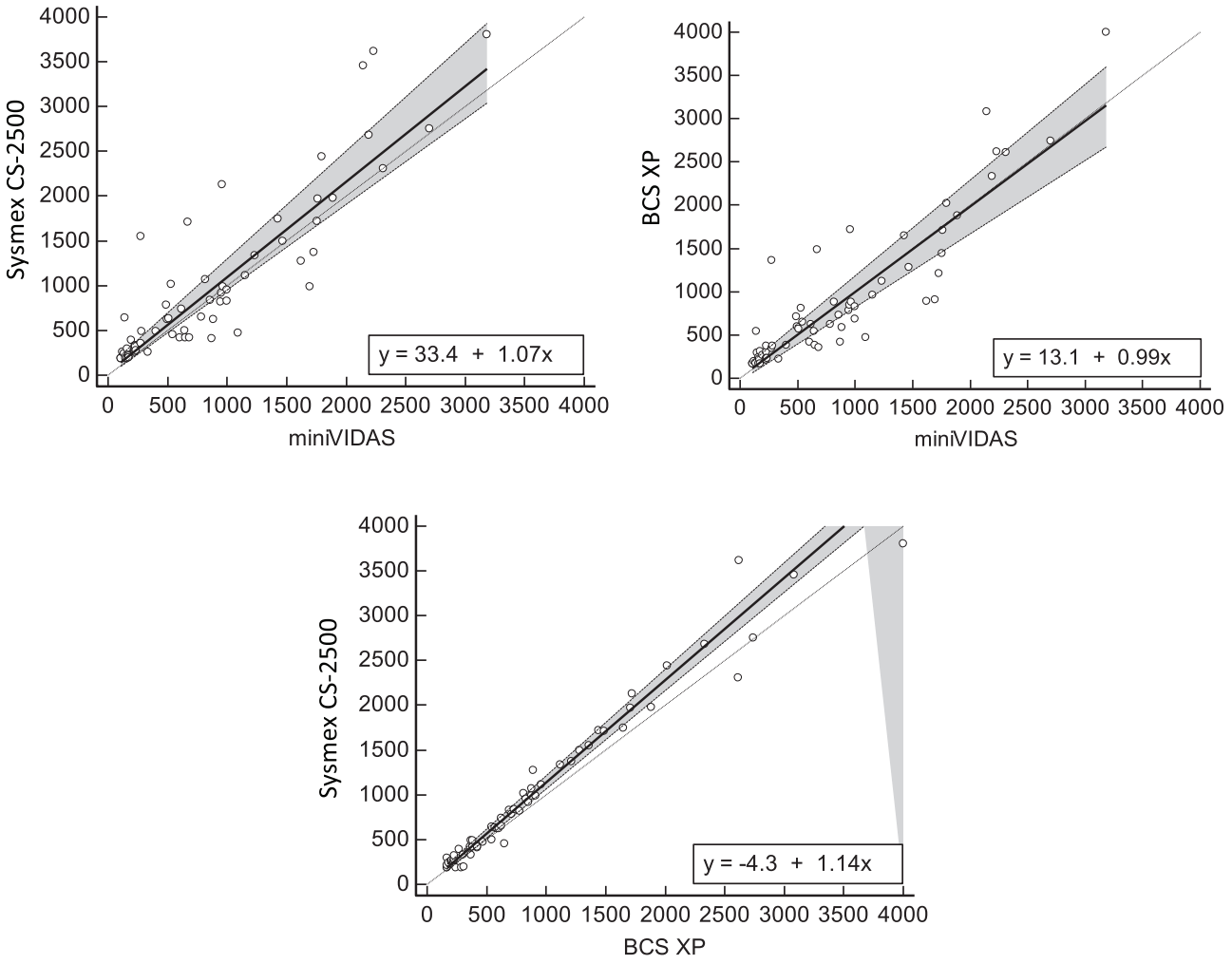
In the previous studies, D-dimer has been identified as a fibrinolytic marker for VTE and DIC. Although there is an opinion that D-dimer is a prognostic marker in COVID-19 patients, the use of follow-up treatment is still controversial because of the different methods and lack of standardization. Instead of standardization of the test, harmonization studies were conducted in patients with DIC due to VTE, acute arterial occlusive disease, severe liver failure, multiorgan failure or infections [14,15]. This study is the first study evaluated clinical and analytical performances of D-dimer results in COVID-19 patients measured by different assays.

The compatibility of the results including before and after freezing demonstrated sample stability, and allowed us to safely and simultaneously perform D-dimer

Table 5 The comparison of systems to distinguish the presence or severity of the disease

Kits Devices	VIDAS D-dimer Exclusion II mini VIDAS	Innovance D-DIMER BCS XP	 Sysmex CS-2500
Outpatient ($n=12$) vs. Inpatient ($n=27$) with COVID-19 (+) (*P* value for Mann–Whitney test)	0.199	0.753	0.578
Distinguishing of asymptomatic ($n=12$), nonsevere ($n=12$) and severe ($n=15$) ill with COVID-19 (+) (*P* value for Kruskal–Wallis test)	0.360	0.335	0.383
COVID (−) ($n=24$) vs. COVID (+) ($n=39$)			
P value for Mann–Whitney test	<0.001	<0.001	<0.001
Cutoff value (ng/ml FEU)	>529	>380	>390
AUC (*P* value)	0.956 (<0.001)	0.898 (<0.001)	0.875 (<0.001)
Sensitivity% / Specificity%	87.2 / 91.7	89.7 / 79.2	94.8 / 70.8

Fig. 2



Passing Bablok regression graph shows the relationship between two systems.

measurement on all devices. In our study, although CV% values of Sysmex CS-2500 and BCS XP at D-dimer less than 1000 ng/ml FEU were higher than those of mini VIDAS, they were within acceptable imprecision limits.

In the study of Oude Elferink *et al.*, the correlation coefficients between mini VIDAS with Sysmex CS-1500 and BCS XP were 0.72 and 0.75, respectively, and there was no systematic or proportional error. A high correlation ($r = 0.94$) was found between Sysmex CS-1500 and BCS XP [16]. Similarly, in our study, the correlation coefficients between mini VIDAS vs. Sysmex CS-2500, mini VIDAS vs. BCS XP and Sysmex CS-2500 vs. BCS XP were 0.89, 0.90 and 0.98, respectively.

Lapic *et al.* [17] found a high correlation ($r = 0.93$) between mini VIDAS vs. Sysmex CS-5100. For the 500 ng/ml FEU of D-dimer, the Kappa coefficient (0.85) between VIDAS and Sysmex CS-5100 was higher than the Kappa coefficient (0.67) between VIDAS and Sysmex CS-2500 in our study. This may have been due to different populations or devices.

At 500 and 1000 ng/ml FEU of D-dimer, we found a strong and almost perfect agreement between Sysmex CS-2500 and BCS XP by using the same kits, respectively. This can be explained by the use of the same mAbs and calibrators. Also, the agreement between enzyme-linked fluorescent assay (ELFA) and immunoturbidimetric methods was moderate, namely satisfactory.

The proportional error and statistically significant difference between Sysmex CS-2500 vs. BCS XP showed that the different devices using same kits may affect D-dimer results. However, it was not impaired clinical compliance.

Although the combination of infection-induced inflammatory changes, thrombocytopenia, prolonged prothrombin

time and increased D-dimer in COVID-19 suggests DIC, it is different from DIC seen in sepsis [18]. In sepsis, thrombocytopenia is usually more pronounced, and the D-dimer level is lower than those in COVID-19 [2,18]. In fact, most COVID-19 patients are not classified as DIC according to the DIC scoring system of the International Society for Thrombosis and Hemostasis [2,18]. In addition, increased D-Dimer in COVID-19 indicates secondary fibrinolysis. It is stated that fibrin formation helps in the defense against the influenza virus [19]. Therefore, fibrinolysis can be potentially induced following severe COVID-19 infection. Current evidence suggests that COVID-19-associated coagulopathy is a combination of low-grade DIC and localized pulmonary thrombotic microangiopathy, and has a significant impact on organ dysfunction in severe disease [18].

Studies are emphasizing that a higher threshold is required to exclude VTE in patients with COVID-19 [20]. Although there is a low probability of VTE for the 2000 ng/ml FEU of cut-off value, 2.6% of patients with VTE may be overlooked. Some algorithms used to determine the type of heparin therapy in COVID-19 have suggested 1000 and 3000 ng/ml FEU as a threshold [21]. Thus, it may be important to the clinical validation at the different cut-off values to exclude VTE in COVID-19 patients. As a limitation of our study, we did not evaluate the performance characteristics of D-dimer analysis systems according to the VTE status of the patients.

In a multicentre study [22], D-dimer levels upon admission for the prediction of in-hospital mortality in COVID-19 patients were harmonized. Harmonized D-dimer yielded an AUC of 0.66, with an optimal cut-off value of 945 ng/ml FEU. Patients with harmonized D-dimer at least 945 ng/ml FEU had a higher mortality rate, but had limited performance as prognostic test. However, D-dimer results have not been harmonized among systems for the diagnosis of VTE in COVID-19 patients.

In our study, there was no difference in D-dimer results of outpatient and inpatient COVID-19 (+) patients or 'Asymptomatic infection', 'Nonsevere' and 'Severe' COVID-19 (+) patients. However, D-dimer results in COVID-19 (+) patients were higher than in COVID-19 (−) individuals. VIDAS D-Dimer Exclusion II had the highest AUC to distinguish COVID-19 (+) patient from COVID-19 (−) individual. The cut-off value of the VIDAS D-Dimer Exclusion II kit (529 ng/ml FEU) was higher than those of Innovance D-dimer kits (380 and 390 ng/ml FEU). To distinguish COVID-19 (+) patients from COVID-19 (−) individuals, it may be preferable to perform D-dimer measurements with the same analytical systems as in other immunological tests.

However, the immunoturbidimetric method may be used as an alternative to the ELFA method due to satisfactory agreement in the different cut-off values for VTE. In addition, the immunoturbidimetric method may be preferred in daily laboratory applications due to full automation, the simultaneously D-dimer analysis with other coagulation tests, low sample volume, short measurement time and high test capacity. Kit and/or device-specific cut-off value can be determined with multicentre clinical studies in large populations or D-dimer results can be harmonized among systems for the diagnosis of VTE in COVID-19 patients.

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Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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