



Harmonized D-dimer levels upon admission for prognosis of COVID-19 severity: Results from a Spanish multicenter registry (BIOCOVID-Spain study)

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Abstract

Coagulopathy is a key feature of COVID-19 and D-dimer has been reported as a predictor of severity. However, because D-dimer test results vary considerably among assays, resolving harmonization issues is fundamental to translate findings into clinical practice. In this retrospective multicenter study (BIOCOVID study), we aimed to analyze the value of harmonized D-dimer levels upon admission for the prediction of in-hospital mortality in COVID-19 patients. All-cause in-hospital mortality was defined as endpoint. For harmonization of D-dimer levels, we designed a model based on the transformation of method-specific regression lines to a reference regression line. The ability of D-dimer for prediction of death was explored by receiver operating characteristic curves analysis and the association with the endpoint by Cox regression analysis. Study population included 2663 patients. In-hospital mortality rate was 14.3%. Harmonized D-dimer upon admission yielded an area under the curve of 0.66, with an optimal cut-off value of 0.945 mg/L FEU. Patients with harmonized D-dimer \geq 0.945 mg/L FEU had a higher mortality rate (22.4% vs. 9.2%; $p < 0.001$). D-dimer was an independent predictor of in-hospital mortality, with an adjusted hazard ratio of 1.709. This is the first study in which a harmonization approach was performed to assure comparability of D-dimer levels measured by different assays. Elevated D-dimer levels upon admission were associated with a greater risk of in-hospital mortality among COVID-19 patients, but had limited performance as prognostic test.

Keywords COVID-19 · D-dimer · Harmonization · Prognosis · Mortality

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Introduction

Coronavirus disease (COVID-19), which results from infection with the RNA virus SARS-CoV-2, was identified in late 2019 in China and characterized as a pandemic from March 2020. The role of clinical laboratories in this viral outbreak

includes staging, prognosis and therapeutic monitoring of individuals with COVID-19 [1]. Several biomarkers have been reported to identify infected patients at higher risk of progression to severe disease [2]. From the onset of the pandemic, D-dimer was identified as one of the predictors of severity in COVID-19 patients [3].

Coagulopathy is a key finding of SARS-CoV-2 infection and is correlated with a poor prognosis [4]. Coagulopathy most commonly manifests as a pro-thrombotic state with increased incidence of both venous and arterial thrombosis [5]. Elevated D-dimer levels have consistently been shown as an important feature of severe COVID-19 patients [6–11]. Therefore, the International Society on Thrombosis and Haemostasis (ISTH) has recommended measuring D-dimer upon admission in all patients who present with COVID-19 [12]. However, several authors and scientific societies have highlighted significant problems across the medical literature with D-dimer reporting, creating confusion and potentially misleading data interpretation [13–15], as much of the published research does not include information on the analytical methods used for D-dimer testing [16]. In addition to the limited information on whether D-dimer units (DDU) or fibrinogen equivalent units (FEU) were used, and the inconsistencies in the magnitude of units reported, the main limitation of studies on the prognostic role of D-dimer in COVID-19 is that most failed to identify the manufacturer or type of D-dimer assay [14]. High inter-method variation is accepted as a major drawback of D-dimer assays, mainly caused by the heterogeneity of fibrin degradation products in patient samples, as well as the variable specificity of the different antibodies used in these assays [17]. This represents a potential source of bias, whereby standardization of D-dimer measurement is a key aspect in test result interpretation [14]. Besides, according to Lippi et al. [18], the adoption of the cut-off values reported in some studies for D-dimer in COVID-19 patients is unfeasible and unadvisable, due to the multiple analytical techniques that are currently available for the measurement of this biomarker. The complexity of target analyte and the variability among D-dimer assays specificity hamper assay standardization. Therefore, harmonization has been proposed to improve the comparability of results obtained with different assays [14], which could be achieved by conversion of D-dimer values from different assays to a common scale, by applying a validated conversion factor [16, 17].

BIOCOVID-Spain study is an initiative by Laboratory Medicine professionals in Spain to generate a multicenter cohort database focusing on laboratory tests, including D-dimer. In this line, we aimed to evaluate the prognostic value of D-dimer levels in hospitalized COVID-19 patients, measured upon admission to the Emergency Department (ED), in hospitalized COVID-19 patients. Given the multicenter design of this study, and the use of four different

FDA-approved immunoturbidimetric assays for D-dimer, results were converted into a harmonized value to ensure comparability.

Methods

Study setting

BIOCOVID-Spain study is a multicenter, retrospective observational study including hospitalized patients with a diagnosis of COVID-19, recruited in 32 hospitals of the National Health System in 9 autonomous communities of Spain. The recruitment period for the current study was from March 1st, 2020, to April 30th, 2020. The follow-up censoring date was May 20th, 2020. The study was approved by the Ethics Committee of all participating hospitals. Because of the retrospective design, we received the approval for data collection with waiver of informed consent.

This study was endorsed by Spanish Association of Medical Biopathology and Laboratory Medicine (AEBM-ML), Spanish Association of Clinical Laboratory (AEFA) and Spanish Society of Laboratory Medicine (SEQC-ML).

Patient eligibility

All consecutive adult patients (≥ 14 years) discharged or dead after hospital admission, with SARS-CoV-2 infection, were eligible for inclusion in the study, as previously described [19]. COVID-19 was diagnosed by a positive result of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) testing of a nasopharyngeal specimen or by a positive result of serological testing and a clinically compatible presentation.

Exclusion criteria were: (a) patients < 14 years; (b) pregnant women; (c) patients transferred from or to another hospital; (d) patients transferred from nursing homes; (e) patients discharged from the ED for at home treatment; (f) patients with Intensive Care Unit (ICU) admission criteria who were not admitted due to lack of availability; and (g) patients in whom D-dimer levels were not measured on admission to ED.

Data collection

Data collection was performed retrospectively from electronic medical records and laboratory information systems by two researchers for each hospital. For eligible patients, we extracted the demographics (age and gender), preexisting comorbidities (hypertension, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD) and diabetes mellitus) and laboratory tests. For measurement of D-dimer levels, four

Table 1 Assays for measurement of D-dimer in BIOCOVID study

Assay	Units ^a	Cut-off value	n (%)
Siemens Innovance® D-dimer	mg/L FEU	0.5 mg/L	513 (19.3)
Stago STA Liatest D-Di	µg/mL FEU	0.5 µg/mL	211 (7.9)
HemosIL D-Dimer HS	ng/mL DDU	230 ng/mL	324 (12.2)
HemosIL D-Dimer HS-500	ng/mL FEU	500 ng/mL	1615 (60.6)

^aIn our study, D-dimer levels, expressed in conventional units (ng/mL and µg/mL), were converted to SI units (mg/L) and expressed as FEU units

Adapted from Favaloro et al. [13]

immunoturbidimetric assays were used (Table 1). The primary outcome of interest was all-cause in-hospital mortality.

Harmonization

For the harmonization of results, four citrate plasma pools were prepared with a range of D-dimer levels. Normal pooled plasma was prepared from blood samples collected from 10 individuals without known thrombotic events or prior antiplatelet or anticoagulant treatment. Blood from the cubital vein was collected into tubes containing sodium citrate as anticoagulant, thoroughly mixed and immediately centrifuged at 2000×g for 15 min. As pathological pooled plasma, a set of three plasma pools with increasing concentration of D-dimer were prepared, collecting for each pool blood samples from 10 patients with recent known thromboembolic events. Pools were analyzed within two hours of collection using the ACL TOP 700 analyzer (Instrumentation Laboratory, US) and HemosIL D-Dimer HS assay. Results were converted to FEU units.

Plasma was immediately separated, aliquoted and frozen at -20 °C to be transported to other participating laboratories using the other D-dimer assays included in BIOCOVID study (Table 1). These laboratories were blinded to D-dimer concentrations. The storage time of the pools was less than 1 week until analysis. After thawing, samples were analyzed in duplicate within two hours.

In this study, the strategy for harmonization was based on a mathematical transformation of regression lines through the assay-specific values of a set of plasma samples with different D-dimer levels to a reference regression line [17, 20, 21]. Because HemosIL D-Dimer HS-500 was the assay mostly used in the BIOCOVID study, this was taken as reference and four lines were generated for the values previously reported (Table 2). The harmonization model was based on a linear transformation of the majority method's harmonized reference line ($y_h = 2.197x_h - 2.843$) and calculating x_h by means of the line obtained in each method different from the reference one as $x_h = [(y_m - b_m)/a_m]$, according to the method described by Meijer et al. [21].

Table 2 Assay specific mean D-dimer levels (mg/L FEU) in normal and pathological plasma pools and lines obtained

Assay ^a	Pool 1	Pool 2	Pool 3	Pool 4
Siemens Innovance® D-dimer ($y_m = 2.966 x_m - 3.980$)	0.525	1.160	1.885	10.170
Stago STA Liatest D-Di ($y_m = 2.655 x_m - 3.495$)	0.460	1.120	1.960	9.030
HemosIL D-Dimer HS ($y_m = 1.964 x_m - 2.525$)	0.374	0.950	1.472	6.748
HemosIL D-Dimer HS-500 ($y_h = 2.197 x_h - 2.843$)	0.393	1.075	1.582	7.542
Overall mean value	0.438	1.076	1.724	8.372

^aSlope and intercept expressed as mg/L

For the external validation of the harmonization model, results were collected from two levels of quality control for D-dimer included in the External Quality Assurance (EQA) scheme, provided by the Spanish Society of Hematology and Hemostasis (SEHH) in November 2020.

Statistical analysis

Continuous variables were tested for normal distribution using the Kolmogorov–Smirnov's test. Data are summarized as numbers and frequencies for categorical variables and medians with interquartile ranges (IQRs) for continuous data. Comparisons between groups were performed with Chi-squared test for categorical data and Mann–Whitney's U tests for continuous data. For harmonized D-dimer, optimal cut-off value for mortality was calculated by Youden's index from a Receiver Operating Characteristic (ROC) curve analysis. For the survival analysis, time zero was defined as the time of admission to the ED. In order to assess survival probability by the Kaplan–Meier's method and log rank test, with the end-point being all-cause in-hospital mortality, the study population was divided according to the optimal cut-off value for D-dimer. Cox proportional hazards regression was performed for both univariate and multivariate analyses. Regression analyses were adjusted for age, gender, hypertension, CVD, diabetes mellitus, CKD and COPD. Statistical significance was set at 5%. SPSS software version 20 (IBM Corporation, USA) was used for all statistical analyses.

Results

Harmonization

Mean D-dimer concentrations (mg/L) in harmonization plasma pools for each assay were obtained. Slope and intercept of the linear regression analysis through the assay

specific values are shown in Table 2. The highest D-dimer levels were observed with Siemens Innovance® D-dimer assay.

For external validation, a total of 32 results were included. The means for the first and second levels measured by the reference method (HemosIL D-dimer HS-500) were 0.945 and 0.930 mg/L FEU, respectively. Results from the other three methods were harmonized, resulting in 0.735 and 0.703 mg/L FEU (HemosIL D-Dimer HS, Werfen), 0.752 and 0.554 mg/L FEU (Stago STA Liatest D-Di) and 0.638 and 0.705 mg/L FEU (Siemens Innovance® D-dimer). A non-parametric linear correlation of the measured-harmonized values and harmonized-reference method values was performed, yielding a coefficient of $r=0.846$ ($p<0.01$), with a mean difference between values of -21.2% (95% CI -45.3 to 12.6%); these differences were non-significant ($p=0.09$). A measure of quality control level 2 was excluded for the calculation of the adjusted Cohen's Kappa index, resulting in $\kappa=0.636$, with a degree of concordance of 94.4% for a total of 16 pairs of values.

According to Landis et al. [22], these data reveal substantial agreement, with a significant correlation between measured values and those harmonized according to the reference method.

Characteristics of study subjects

During the study period, a total of 2981 COVID-19 patients admitted to 32 Spanish hospitals were recruited. One-hundred and eight patients who were still hospitalized on May 20th, 2020, were excluded from analyses. D-dimer levels on admission were not available in 210 patients. Thus, the study population finally included 2663 hospitalized COVID-19 patients (Fig. 1). Median age was 65 years (IQR 54–76), ranging from 15 to 98 years, and 1560 patients were male (58.6%). The most common comorbidity was hypertension (45.3%), followed by diabetes mellitus (23.8%) and prior CVD (22.4%).

In-hospital mortality rate was 14.3% (364/2663). Characteristics of the study population according to in-hospital mortality are summarized in Table 3. Median harmonized D-dimer in the study population was 0.690 mg/L FEU (IQR 0.438–1.200), and 1787 (67.1%) presented with a level greater than 0.500 mg/L FEU, usually defined as the upper reference limit for identification of venous thromboembolic disease. Compared to survivors, non-survivors were older and commonly male and presented an increased prevalence of all the comorbidities; regarding laboratory tests, creatinine, lactate dehydrogenase (LDH), C-reactive protein (CRP) and D-dimer levels were higher and lymphocyte and platelet counts were lower in patients who died.

Harmonized D-dimer levels were significantly higher in patients who died (1.019 mg/L FEU [IQR 0.564–2.155] vs.

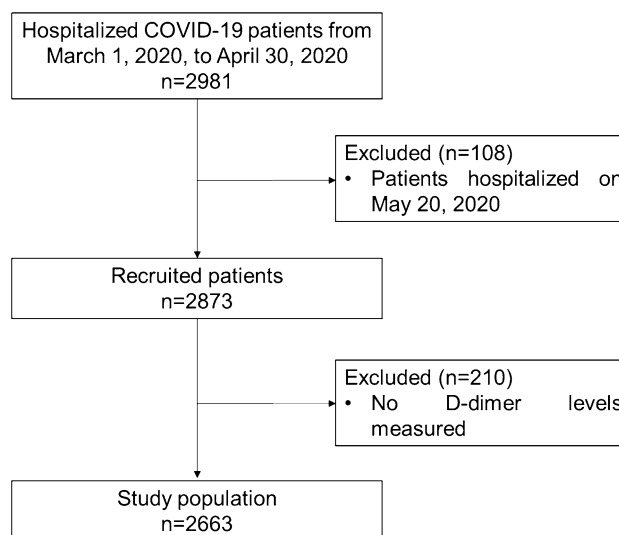


Fig. 1 Flowchart of patient recruitment

0.645 mg/L FEU [IQR 0.422–1.108]; $p<0.001$). AUC for baseline D-dimer levels as a predictor of death was 0.66 (95% Confidence interval (CI) 0.63–0.69; $p<0.001$), consistent with relatively poor discriminating ability for prognosis of this endpoint. According to the Youden's index, the optimal cut-off value of harmonized D-dimer was 0.945 mg/L FEU (sensitivity: 55.8% (95% CI 50.5–60.9%); specificity: 69.3% (95% CI 67.4–71.2%); positive predictive value: 22.4% (95% CI 19.7–25.2%), negative predictive value: 90.8% (95% CI 89.4–92.1). According to regression equations for harmonization, this cut-off value was equal to 0.862, 1.083 and 1.136 mg/L FEU for HemosIL D-Dimer HS, Stago STA Liatest D-Di and Siemens Innovance® D-dimer assays, respectively.

Patients with a harmonized D-dimer ≥ 0.945 mg/L FEU were older and comorbidities were more frequent, including hypertension, diabetes mellitus, COPD, CVD and CKD. In terms of laboratory tests, patients with a harmonized D-dimer ≥ 0.945 mg/L FEU had higher creatinine, CRP levels and platelet count along with a lower lymphocyte count (Table 4).

Association of D-dimer and in-hospital mortality

In-hospital mortality was higher among patients with a harmonized D-dimer level ≥ 0.945 mg/L FEU (203 [22.4%] vs. 161 [9.2%]; $p<0.001$). Kaplan–Meier's survival analysis was used to evaluate in-hospital mortality in COVID-19 patients with different D-dimer levels, according to optimal cut-off value. A significant association between D-dimer and mortality was observed (log-rank test $p<0.001$) (Fig. 2).

In multivariate Cox regression analysis, the hazard ratio (HR) for patients with D-dimer levels ≥ 0.945 mg/L FEU

Table 3 Patients' characteristics of patients in total population grouped by survival status

Variable n (%)	Survivors 2299 (86.3)	Non-survivors 364 (13.7)	p-value
Demographics			
Age (years)	63 (52–74)	76 (68–83)	<0.001
Gender (male)	1317 (57.3)	243 (66.8)	0.001
Pre-existing comorbidities			
Hypertension [n (%)]	963 (41.9)	243 (66.8)	<0.001
Diabetes mellitus [n (%)]	508 (22.1)	126 (34.6)	<0.001
Cardiovascular disease [n (%)]	453 (19.7)	144 (39.6)	<0.001
COPD [n (%)]	164 (7.1)	56 (15.4)	<0.001
Chronic kidney injury [n (%)]	141 (6.1)	75 (20.6)	<0.001
Laboratory findings			
Harmonized D-dimer (mg/L FEU), n=2663	0.645 (0.422–1.108)	1.019 (0.564–2.155)	<0.001
CRP (mg/L), n=2609	68.3 (30.2–135.6)	136.4 (76.8–205.7)	<0.001
Creatinine (μmol/L), n=2654	76.9 (63.7–94.6)	101.7 (78.7–138.8)	<0.001
Alanine aminotransferase (U/L), n=2542	28 (18–47)	26 (17–45)	0.208
LDH (U/L), n=2370	295 (232–387)	394 (292–557)	<0.001
Lymphocyte count (*10 ⁹ /L), n=2661	1.00 (0.70–1.39)	0.80 (0.53–1.10)	<0.001
Platelet count (*10 ⁹ /L), n=2661	197 (155–261)	185 (136–243)	<0.001

COPD Chronic obstructive pulmonary disease, *LDH* Lactate dehydrogenase, *CRP* C-reactive protein

Table 4 Patients' characteristics according to harmonized D-dimer level on admission

	Total population n = 2663	Harmonized D-dimer level		p-value
		< 0.945 mg/L FEU n = 1755 (65.9%)	≥ 0.945 mg/L FEU n = 908 (34.1%)	
Age, years [Median (IQR)]	65 (54–76)	62 (50–73)	71 (62–80)	<0.001
Gender, male [n (%)]	1560 (58.6)	1039 (59.2)	521 (57.4)	0.365
In-hospital mortality [n (%)]	364 (13.7)	161 (9.2)	203 (22.4)	<0.001
Pre-existing comorbidities				
Hypertension [n (%)]	1206 (45.3)	715 (40.7)	491 (54.1)	<0.001
Diabetes mellitus [n (%)]	634 (23.8)	370 (21.1)	264 (29.1)	<0.001
COPD [n (%)]	220 (8.3)	125 (7.1)	95 (10.5)	0.003
Cardiovascular disease [n (%)]	597 (22.4)	353 (20.1)	244 (26.9)	<0.001
Chronic kidney disease [n (%)]	216 (8.1)	111 (6.3)	105 (11.6)	<0.001
Laboratory tests on admission (Median [IQR])				
Creatinine (μmol/L)	78.7 (64.5–101.7)	76.9 (63.7–93.7)	84.0 (67.2–118.5)	<0.001
Alanine aminotransferase (U/L)	28 (18–46)	28 (18–46)	28 (17–47)	0.602
LDH (U/L)	306 (237–407)	287 (228–372)	356 (264–491)	<0.001
CRP (mg/L)	77 (34–147)	63 (28–122)	110 (52–189)	<0.001
Lymphocyte count (*10 ⁹ /L)	1.00 (0.70–1.35)	1.00 (0.70–1.39)	0.92 (0.61–1.30)	<0.001
Platelet count (*10 ⁹ /L)	196 (152–256)	190 (152–242)	209 (157–286)	<0.001
Harmonized D-dimer (mg/L FEU)	0.697 (0.438–1.200)	-	-	-

COPD Chronic obstructive pulmonary disease, *LDH* Lactate dehydrogenase, *CRP* C-reactive protein

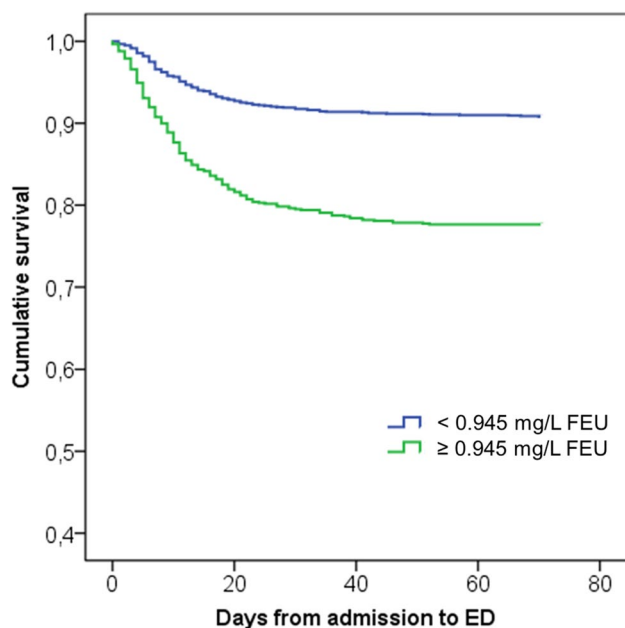


Fig. 2 Cumulative incidence of in-hospital mortality during hospitalization stratified by D-dimer on admission

remained significant after adjusting for age, gender, hypertension, diabetes, CVD, CKD and COPD (HR 1.709, 95% CI 1.380–2.115; $p < 0.001$) (Table 5).

Discussion

In this multicenter study, we retrospectively analyzed the prognostic role of harmonized D-dimer in a large population including hospitalized COVID-19 in 32 Spanish hospitals, with clinical and demographic characteristics similar to those recently described in other COVID-19 Spanish cohorts [23, 24]. Our study shows that elevated D-dimer levels upon admission, previously harmonized to guarantee the comparability among different assays,

were associated with a higher risk of in-hospital all-cause mortality. However, D-dimer levels upon admission, as an isolated measure, did not appear to be a reliable prognostic laboratory test for death among COVID-19 patients.

The potential prognostic role of D-dimer would be based in the fact that COVID-19 patients commonly experience a coagulopathy with different characteristics from those seen in bacterial sepsis-induced coagulopathy and disseminated intravascular coagulation; COVID-19 coagulopathy is also associated with a high incidence of thrombotic events leading to poor outcomes [4, 5, 25, 26]. Hence, biomarkers of coagulation, such as D-dimer, may be helpful in predicting clinical course and outcomes in these patients and its measurement upon admission to hospital has been recommended to identify early those COVID-19 patients at high-risk of thromboembolic events [27] and a poor evolution [12, 28].

Although the molecular mechanisms underlying the hypercoagulable state described in COVID-19 patients are still incompletely understood (with endotheliopathy, due to the direct endothelial infection with SARS-CoV-2 and the indirect damage caused by inflammation, playing a predominant role) [29], multiple studies, reviews and meta-analyses have reported higher D-dimer levels to be associated with poorer outcomes and may even help predict these outcomes [6–11]. However, this potential role is controversial. Although D-dimer levels might be attractive for the management of COVID-19, Gris et al. [30] reported that the cut-off points are variable and the clinical interpretation is still very uncertain. Importantly, no differential treatment exists that elevated D-dimer levels alone can promote. Therefore, this biomarker cannot currently be used to improve either management or prognosis. Furthermore, its utility remains beset with uncertainties by a lack of information in studies about D-dimer assays and their characteristics [13], which prevents the adoption of the cut-off points recommended in these studies [28].

Similar to previous reports [31], in ours, D-dimer elevation upon admission was frequently observed in our study (67%). This increase is one of the most consistent abnormal

Table 5 Cox regression model showing hazard ratios for the studied variables

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
Age	1.064 (1.055–1.073)	<0.001	1.055 (1.045–1.065)	<0.001
Male sex	1.454 (1.169–1.808)	0.001	1.762 (1.412–2.199)	<0.001
Hypertension	2.589 (2.089–3.232)	<0.001	1.286 (1.020–1.620)	0.033
Diabetes mellitus	1.773 (1.429–2.201)	<0.001	–	ns
COPD	2.155 (1.621–2.865)	<0.001	–	ns
Cardiovascular disease	2.473 (2.004–3.051)	<0.001	–	ns
Chronic kidney disease	3.357 (2.604–4.329)	<0.001	1.822 (1.401–2.370)	<0.001
D-dimer ≥ 0.945 mg/L FEU	2.636 (2.143–3.232)	<0.001	1.709 (1.380–2.115)	<0.001

HR Hazard ratio, CI Confidence interval, COPD Chronic obstructive pulmonary disease, ns non-significant

hemostatic laboratory tests in COVID-19 [31] and has been described in patients without confirmed pulmonary embolism/deep vein thrombosis or laboratory evidence compatible with disseminated intravascular coagulopathy (DIC). This supports its role not just a diagnostic tool for thromboembolism or DIC. Recently, the association of lung inflammation caused by SARS-CoV-2 infection with elevated fibrinogen levels has been hypothesized [32, 33]. Previous studies reported that early steps of viral invasion in the lung comprise airway inflammation and the leakage of various plasma proteins, including thrombin and fibrinogen [34]. Both proteins are leaked into the extravascular space and fibrinogen is converted into fibrin by thrombin and then degraded by the proteases released from neutrophils. In this line, high plasma fibrinogen levels and D-dimer are reported in patients with COVID-19 [35]. D-dimer formed in this manner may not imply generalised thrombus formation but could predict a poor prognosis, as they arise from lung exudates. Hunt et al. [36] have also recently suggested that the origin of elevated D-dimer levels is a direct consequence of an acute lung injury seen in COVID-19 pneumonia; hence, similar to other acute-phase proteins such as CRP, ferritin and fibrinogen, elevated D-dimer levels represent the degree of lung inflammation within the lungs in COVID-19. Moreover, being associated with the extent of this inflammation would explain why their plasma levels relate to clinical outcome.

Nevertheless, results from studies exploring the prognostic role of D-dimer are controversial. In addition, one of the main limitations in such literature is a lack of information about analytical methodologies used, which are known to be not interchangeable among them and require a previous harmonization, alongside with the different units for expression of D-dimer. These issues might lead to misinterpretations [13, 16], and hinder the translation of study findings into local laboratory practice [28].

D-dimer was one of the first biomarkers reported as useful for the prediction of a poor prognosis at an early stage in COVID-19 patients [3]. Subsequent studies confirmed the significant association of D-dimer levels with greater risk of all-cause mortality, with a wide variety of optimal cut-off values reported as predictors of death, including 1128 ng/mL [37], 2.00 µg/mL [38], 2.14 mg/L [39] and 2.38 µg/mL [40], although an association was not detected by Martín-Rojas et al. [4]. In our study, a higher harmonized D-dimer (≥ 0.945 mg/L FEU) on admission was also associated with greater risk of all-cause mortality. However, D-dimer, as an isolated measure evaluated by a ROC curve analysis, was not a reliable tool for predicting mortality among COVID-19 patients, showing a low AUC (0.66), similar to those recently reported by Chocron et al. [37] (0.65) and Naymagon et al. [40] (0.69), but significantly lower than reported by Yao et al. (0.85) [39] and Zhang et al. (0.89) [38]. According to Gris et al. [30], selection bias would be a major confounding factor

affecting the results in Chinese populations, with mortality rates (3.8% [38] and 6.9% [39], respectively) being lower than those found in western cohorts (14.3% in ours). These authors suggest that the patients enrolled in the study by Zhang et al. [38] were not initially affected by major severity criteria, likely by the non-representativeness of the patients analyzed by comparison with all the COVID-19 hospitalized into the recruiting medical centers, affecting the applicability of generalizing their results to all COVID-19 patients. Therefore, the value of using D-dimer for management of COVID-19 is strongly dependent on the true clinical representativeness and characteristics of the included patients [30].

Strengths and limitations

One of the main strengths of this work, in addition to the sample size, is the effort to harmonize D-dimer values, measured by different assays, to guarantee their comparability. To the best of our knowledge, no previous multicenter study has performed a similar approach.

Our study has some limitations. First, selection bias could have been introduced due to the retrospective design. Second, outcomes other than death, such as thrombosis, need of mechanical ventilation or critical illness were not included in this study [32]. Third, it should be noted that the differences between results of external validation were negative by more than 20% and the statistical significance below 10% suggests that they may become significant should the sample size be increased to enable a more extensive external validation. Fourth, in our study, only the prognostic role of D-dimer levels upon admission was explored; some studies have reported a high ability of D-dimer peak during hospital stay for prediction of multiple outcomes [41]. We also did not analyze the potential role of serial D-dimer levels to distinguish severe COVID-19 cases from the mild/moderate forms [42].

Conclusion

We performed a harmonization approach to ensure the comparability of D-dimer levels measured with different assays, and identified that higher D-dimer levels upon admission were associated with significantly greater risk of in-hospital mortality among hospitalized COVID-19 patients. Nevertheless, admission D-dimer had a limited usefulness as prognostic test.

Author contribution LGGR and DMG conceived, designed the study, supervised the conduct of the trial and analyzed the data. LGGR, DMG, EJF and JMB drafted the article. LGGR, DMG, IGG and PHC contributed to measure D-dimer levels for harmonization. All the authors contributed to data collection in the participating hospitals, contributed to its revision and gave approval before submission.

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Declarations

Conflicts of interest The authors state that there are no conflicts of interest to disclose.

Ethics approval The study was approved by the Ethics Committee of all participating hospitals. Because of the retrospective design, we received the approval for data collection with waiver of informed consent.

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