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# Biomarkers and short-term prognosis in COVID-19

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**ABSTRACT**

**Purpose:** The aim of our study was to analyse the short-term prognostic value of different biomarkers in patients with COVID-19.

**Methods:** We included patients admitted to emergency department with COVID-19 and available concentrations of cardiac troponin I (cTnI), D-dimer, C-reactive protein (CRP) and lactate dehydrogenase (LDH). Patients were classified for each biomarker into two groups (low vs. high concentrations) according to their best cut-off point, and 30-day all-cause death was evaluated.

**Results:** After multivariate adjustment, cTnI ≥21 ng/L, D-dimer ≥1112 ng/mL, CRP ≥10 mg/dL and LDH ≥334 U/L at admission were associated with an increased risk of 30-day all-cause death (hazard ratio (HR) 4.30; 95% CI 1.74–10.58; $p = 0.002$; HR 3.35; 95% CI 1.58–7.13; $p = 0.002$; HR 2.25; 95% CI 1.13–4.50; $p = 0.021$; HR 2.00; 95% CI 1.04–3.84; $p = 0.039$, respectively). The area under the curve for cTnI was 0.825 (95% CI 0.759–0.892) and, in comparison, was significantly better than CRP (0.685; 95% CI 0.600–0.770; $p = 0.009$) and LDH (0.643; 95% CI 0.534–0.753; $p = 0.006$) but non-significantly better than D-dimer (0.756; 95% CI 0.674–0.837; $p = 0.115$).

**Conclusions:** In patients with COVID-19, increased concentrations of cTnI, D-dimer, CRP and LDH are associated with short-term mortality. Of these, cTnI provides better mortality risk prediction. However, differences with D-dimer were non-significant.

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Biomarkers; COVID-19; short-term prognosis; troponin; D-dimer; C-reactive protein; lactate dehydrogenase

## Introduction

Since the outbreak of coronavirus disease 2019 (COVID-19) in Wuhan, China, the disease caused by SARS-CoV-2, has become a global pandemic. It is currently one of the greatest concerns of humanity due to its high morbimortality and economic impact. COVID-19 is predominantly a respiratory disease and its range of presentation can vary from asymptomatic or barely symptomatic disease to severe respiratory failure and critical condition (Huang C *et al.* 2020). SARS-CoV-2 is known to enter human cells through angiotensin-converting enzyme 2, which is predominantly expressed not only in the lungs but also in other organs, such as the cardiovascular system, thus leading to a wide range of symptoms (Hoffmann *et al.* 2020).

Several biomarkers have been related to COVID-19 progression and short-term mortality. In fact, patients with cardiac troponin (cTn) elevation as a reflection of myocardial injury have been associated with a higher burden of cardiovascular disease and worse prognosis (Chen *et al.* 2020, Shi *et al.* 2020a). High D-dimer concentration has also been reported as a predictor of in-hospital mortality and higher risk of procoagulant state (Huang I *et al.* 2020). Likewise, C-reactive protein (CRP) as an inflammatory marker and lactate dehydrogenase (LDH) as a marker of cell damage have been related with the severity of COVID-19 (Ponti *et al.* 2020). With all this, there is evidence that biomarkers can be an efficient tool for prognostic stratification of COVID-19 patients. However, there is limited information about which one of those biomarkers can provide better prognostic value. Therefore, the aim of our study was to analyse the short-term prognostic value of different biomarkers and compare its predictive value in patients admitted with COVID-19.

## Clinical significance

- Increased concentrations of cTnI, D-dimer, CRP and LDH are associated with short-term mortality.
- cTnI provides better mortality risk prediction than CRP, LDH and D-dimer. However, differences with D-dimer were non-significant.

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Supplemental data for this article can be accessed here.

- Higher D-dimer values and especially higher cTnI concentrations were consistently related to an increased prevalence of older age, cardiovascular risk factors and medical history of cardiovascular diseases. Higher CRP concentrations were also associated with an increased prevalence of older age and cardiovascular risk factors but not with previous cardiovascular diseases (but LDH was not associated with any).

## Materials and methods

### *Study population*

This is a retrospective observational study that included consecutive patients admitted to our emergency department from 16 March 2020 to 15 May 2020 with symptoms and confirmed laboratory test of COVID-19 and available concentrations of cardiac troponin I (cTnI), D-dimer, CRP and LDH. At admission, patients were evaluated for their clinical status and risk factors. Those with mild symptoms and lack of risk factors were discharged and followed remotely, whereas those with moderate to severe clinical status or risk factors were admitted to the Internal Medicine Department or Intensive Care Unit as appropriate. We excluded patients without biomarkers information (Figure 1). During hospital admission, baseline demographics, medical history, admission symptoms and exploratory findings were registered. We also collected treatment information and need for intensive care or mechanical ventilatory support.

### *Laboratory analysis*

A confirmed COVID-19 case was defined as a positive result on polymerase chain reaction assay or antigen determination of nasal and pharyngeal swab specimens or plasma determination of antibodies. Viral RNA purification was performed by the RNeasy Mini Kit in the Qiacube Connect (QIAGEN, Hilden, Germany). The reverse transcription polymerase chain reaction was performed with the thermocycler CFX96 Touch System (Bio-Rad Laboratories Inc., Hercules, CA) with a commercial kit intended to amplify regions of the E, N and RdRP genes (Allplex™ 2019-nCoV Assay, Seegene Inc., Seoul, South Korea). Antigen determination was performed by immunochromatography (Fluorescence Ag Rapid Test®, BIOEASY Biotechnology Co., Ltd., Shenzhen, China), while antibodies were assessed by indirect chemiluminescent immunoassay (COVID-19 VIRCLIA Monotest, Vircell S.L., Granada, Spain).

Blood samples were obtained upon each patient's admission in our Emergency Department and transferred immediately to our clinical laboratory for testing our biomarkers. All samples were processed in the same way.

cTnI concentrations were measured with an automated immunoassay (High-Sensitivity Troponin I Assay, Advia Centaur, Siemens Healthineers, Erlangen, Germany). As described by the manufacturer, the detection limit of the assay is 2.5 ng/L and the upper limit of detection is 25,000 ng/L (measured with a coefficient of variation $<$10%). Measurement of D-dimers was performed by ACL TOP 500 CTS® using HemosIL D-Dimer HS-500 (HemosIL, Instrumentation Laboratory, Bedford, MA). Upper reference level was established at 500 ng/mL by the manufacturer testing blood donor samples. Measurement of CRP was performed by an immunoturbidimetric assay using ADVIA Chemistry XPT analyser (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). The assay efficiency at low concentrations was analysed as described in the EP17-A2 protocol of the Clinical and Laboratory Standards Institute, and the limit of quantification was established at 0.4 mg/dL, with a linear range until 91.2 mg/dL. The reference interval of CRP is below 1.0 mg/dL, as established by the manufacturer and assessed in our laboratory. LDH was assessed by an enzyme reaction (pyruvate/NADH) using the ADVIA Chemistry XPT analyser (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). The assay efficiency at low concentrations was analysed as described in the EP17-A2 protocol of the Clinical and Laboratory Standards Institute, and the limit of detection was established at 13 U/L, with a linear range until 700 U/L. The reference interval of LDH is between 120 and 246 U/L as established by the manufacturer and assessed in our laboratory. The estimated glomerular filtration rate (eGFR) was calculated by using the value of creatinine

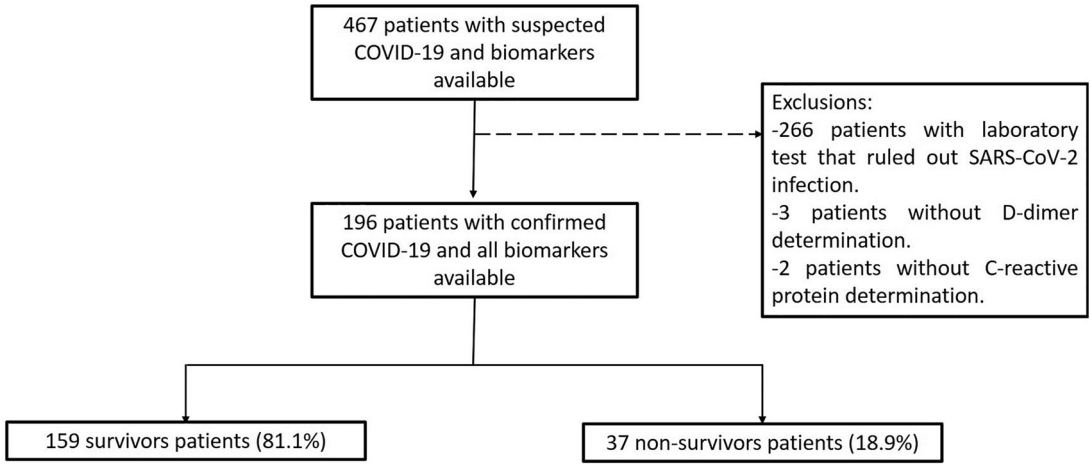


Figure 1. Flow diagram of patients.

at admission with the Chronic Kidney Disease Epidemiology Collaboration formula. Renal impairment at admission was defined as an eGFR <60 mL/min/1.73 $m^2$.

### Follow-up and outcomes

Patients were followed-up for 30-day all-cause death events. Deaths were identified by review of electronic medical records. Follow-up adjudication was performed by investigators who were blinded to biomarker measurements.

### Statistical analysis

Categorical variables are expressed as numbers and percentages, whereas continuous variables are expressed as medians and interquartile ranges (IQRs). Comparisons of categorical data (variables and also grouping variable: female sex, renal impairment at admission, cTnI ≥21 ng/L, D-dimer ≥1112 ng/mL, CRP ≥10 mg/dL, LDH ≥334 U/L and the variables included in cardiovascular risk factors, medical history, symptoms, electrocardiogram, radiology, clinical characteristics and treatment) were performed with the chi-squared test or Fisher's exact test when expected frequencies were <5, while numerical data (variables: age, time from onset of symptoms to admission, systolic arterial pressure, heart rate, oxygen saturation, glucose, eGFR, haemoglobin, leucocytes, lymphocytes, platelets, cTnI, D-dimer, CRP, LDH. Grouping variable were survivor/non-survivor status and biomarker concentration group in complementary tables) were analysed with the Mann–Whitney *U* test. A non-parametric test was used because variables were not normally distributed. The optimal biomarker cut-off points for short-term all-cause death were defined by a receiver operating characteristic (ROC) curve (Youden's index, Martínez-Camblor and Pardo-Fernández 2019). To study the relationship between biomarkers and outcomes, patients were categorized into two groups based on the concentration of their biomarkers. Survival probabilities were estimated by the Kaplan–Meier method and compared with the log-rank test. To determine if biomarker groups were associated with short-term all-cause death, univariable and multivariable Cox regressions were performed with the backward stepwise procedure. In the multivariable analysis, clinically relevant and significant variables in the univariable analysis were included. The number of variables included was limited to avoid overfitting. Therefore, multivariable Cox regression analyses were adjusted by the following variables: age, hypertension, medical history of chronic pulmonary disease and renal impairment at admission. The proportional hazards assumption was analysed by the Schoenfeld residuals (In and Lee 2019). Multicollinearity was searched by calculating the variance inflation factor. Finally, to compare the ability of each biomarker to predict short-term all-cause death, we performed ROC curve analyses (DeLong *et al.* 1988). Differences were considered statistically significant at $p < 0.05$. STATA 14.2 (StataCorp, College Station, TX) was used for statistical analysis.

### Ethics

The study was approved by the local ethical committee and complies with the Declaration of Helsinki.

## Results

### Baseline characteristics

A total of 196 patients were included in the study. The median (IQR) age was 67.5 (53.5–78.0) years, and 79 (40.3%) patients were female. Cardiovascular risk factors, medical history and clinical characteristics during admission are represented in Tables 1 and 2. Respectively, the median (IQR) cTnI, D-dimer, CRP and LDH concentrations were 14 (4–37) ng/L, 771 (445–1812) ng/mL, 8 (3–16) mg/dL and 276 (216–384) U/L. The best cTnI, D-dimer, CRP and LDH cut-off point and its sensitivity (Se) and specificity (Sp) for the prediction of all-cause death was 21 ng/L (Se 81% Sp 70%), 1112 ng/mL (Se 73% Sp 69%), 10 mg/dL (Se 68% Sp 62%) and 334 U/L (Se 62% Sp 62%), respectively. Patients were classified for each biomarker into two groups (low vs. high concentrations) according to their best cut-off point. Patients with higher D-dimer concentrations and especially those with higher cTnI concentrations were associated with older

Table 1. Demographics, cardiovascular risk factors and medical history.

| Variable | Overall (*N* = 196) | Survivors (*N* = 159) | Non-survivors (*N* = 37) | *p* Value |
|---|---|---|---|---|
| Demographics | | | | |
| Age, years | 67.5 (53.5–78.0) | 61.5 (51.5–75.5) | 76.5 (68.5–82.5) | <0.001 |
| Female sex | 79 (40.3) | 65 (40.9) | 14 (37.8) | 0.734 |
| Cardiovascular risk factors | | | | |
| Current or past smoker | 42 (21.4) | 29 (18.2) | 13 (35.1) | 0.024 |
| Hypertension | 87 (44.4) | 63 (39.6) | 24 (64.9) | 0.005 |
| Diabetes mellitus | 46 (23.5) | 33 (20.8) | 13 (35.1) | 0.063 |
| Hypercholesterolemia | 50 (25.5) | 37 (23.3) | 13 (35.1) | 0.136 |
| Medical history | | | | |
| Myocardial infarction | 19 (9.7) | 12 (7.6) | 7 (18.9) | 0.035 |
| Heart failure | 14 (7.1) | 11 (6.9) | 3 (8.1) | 0.731 |
| Cerebrovascular disease | 13 (6.6) | 8 (5.0) | 5 (13.5) | 0.074 |
| Peripheral arterial disease | 12 (6.1) | 8 (5.0) | 4 (10.8) | 0.245 |
| Chronic kidney disease | 23 (11.7) | 10 (6.3) | 13 (35.1) | <0.001 |
| Chronic pulmonary disease | 33 (16.8) | 20 (12.6) | 13 (35.1) | 0.001 |

cTnI: cardiac troponin I.
Data represent the number (percentage) or median (interquartile range). Comparisons of categorical data were performed with the chi-squared test or Fisher's exact test, while numerical data were analysed with the Mann–Whitney *U* test.

Table 2. Clinical characteristics.

| Variable | Overall ($N = 196$) | Survivors ($N = 159$) | Non-survivors ($N = 37$) | $p$ Value |
|---|---|---|---|---|
| Symptoms | | | | |
| Dyspnoea | 115 (58.7) | 92 (57.9) | 23 (62.2) | 0.632 |
| Fever | 139 (72.0) | 112 (70.9) | 27 (77.1) | 0.456 |
| Cough | 100 (51.8) | 87 (55.1) | 13 (37.1) | 0.055 |
| Myalgias | 11 (5.7) | 10 (6.4) | 1 (2.9) | 0.692 |
| Diarrhoea | 28 (14.5) | 25 (15.8) | 3 (8.6) | 0.270 |
| Chest pain | 17 (8.7) | 16 (10.1) | 1 (2.7) | 0.205 |
| Other symptoms | 93 (47.5) | 77 (48.4) | 16 (43.2) | 0.569 |
| Time from onset of symptoms to admission (days) | 4 (2–7) | 5 (2–7) | 4 (1–7) | 0.404 |
| Physical examination | | | | |
| Systolic arterial pressure (mmHg) | 124 (110–138) | 121 (109–137) | 130 (114–144) | 0.123 |
| Heart rate (bpm) | 87 (74–104) | 87 (74–101) | 87 (79–111) | 0.389 |
| Oxygen saturation (%) | 96 (92–99) | 97 (93–99) | 93 (88–96) | 0.001 |
| Electrocardiogram | | | | |
| Atrial fibrillation | 18 (10.1) | 13 (9.2) | 5 (13.5) | 0.432 |
| LBBB or RBBB | 8 (4.5) | 5 (3.5) | 3 (8.1) | 0.365 |
| Radiology | | | | |
| Consolidation | 38 (19.4) | 32 (20.1) | 6 (16.2) | 0.588 |
| Ground-glass opacity | 18 (9.2) | 12 (7.6) | 6 (16.2) | 0.116 |
| Bilateral pulmonary infiltration | 122 (62.9) | 93 (58.9) | 29 (80.6) | 0.015 |
| Laboratory findings | | | | |
| Glucose (mg/dL) | 106 (90–138) | 102 (90–124) | 138 (100–162) | 0.002 |
| eGFR (mL/min per 1.73 $m^2$) | 87 (59–103) | 91 (73–104) | 48 (19–80) | <0.001 |
| Renal impairment at admission | 52 (26.5) | 28 (17.6) | 24 (64.9) | <0.001 |
| Haemoglobin (g/dL) | 12.6 (11.3–13.9) | 12.7 (11.6–13.9) | 11.4 (9.5–13.6) | 0.016 |
| Leucocytes ($\times 10^9$/L) | 6.3 (4.7–8.9) | 6.2 (4.5–8.4) | 7.9 (5.1–10.2) | 0.065 |
| Lymphocytes ($\times 10^9$/L) | 0.8 (0.5–1.4) | 0.9 (0.5–1.4) | 0.6 (0.3–0.9) | 0.001 |
| Platelets ($\times 10^9$/L) | 208 (157–282) | 204 (161–282) | 219 (153–307) | 0.986 |
| Cardiac troponin I (ng/L) | 14 (4–37) | 11 (3–24) | 54 (22–153) | <0.001 |
| Cardiac troponin I $\geq$ 21 ng/L | 77 (39.3) | 47 (29.6) | 30 (81.1) | <0.001 |
| D-dimer (ng/mL) | 771 (445–1812) | 618 (387–1351) | 1834 (1056–2806) | <0.001 |
| D-dimer $\geq$ 1112 ng/mL | 77 (39.3) | 50 (31.5) | 27 (73.0) | <0.001 |
| C-reactive protein (mg/dL) | 8 (3–16) | 7 (2–14) | 12 (8–21) | <0.001 |
| C-reactive protein $\geq$ 10 mg/dL | 86 (43.9) | 61 (38.4) | 25 (67.6) | 0.001 |
| Lactate dehydrogenase (U/L) | 276 (216–384) | 266 (212–354) | 345 (251–455) | 0.007 |
| Lactate dehydrogenase $\geq$ 334 U/L | 65 (33.2) | 46 (28.9) | 19 (51.4) | 0.009 |
| Clinical characteristics | | | | |
| Hospital admission | 165 (84.2) | 130 (81.8) | 35 (94.6) | 0.054 |
| ICU admission | 34 (17.4) | 29 (18.2) | 5 (13.5) | 0.494 |
| Days at ICU | 17 (7–34) | 25 (10–38) | 9 (1–11) | 0.064 |
| Invasive mechanical ventilation | 29 (14.8) | 24 (15.1) | 5 (13.5) | 0.807 |
| Pulmonary embolism | 1 (0.5) | 1 (0.6) | 0 (0.0) | 1.000 |
| Treatment | | | | |
| Antibiotics[a] | 143 (73.3) | 114 (71.7) | 29 (80.6) | 0.278 |
| Hydroxychloroquine | 122 (62.9) | 104 (65.8) | 18 (50.0) | 0.076 |
| Lopinavir/ritonavir | 87 (45.1) | 73 (46.5) | 14 (38.9) | 0.408 |
| Azithromycin | 75 (39.1) | 62 (39.5) | 13 (37.1) | 0.797 |
| Corticoids | 17 (8.8) | 13 (8.2) | 4 (11.4) | 0.516 |
| ACE inhibitors or ARBs | 21 (10.7) | 17 (10.7) | 4 (10.8) | 1.000 |

LBBB: left bundle branch block; RBBB: right bundle branch block; eGFR: estimated glomerular filtration rate; ICU: intensive care unit; ACE: angiotensin-converting enzyme; ARBs: angiotensin II receptor blockers; cTnI: cardiac troponin I.
aAzithromycin not included. Data represent the number (percentage) or median (interquartile range).
Comparisons of categorical data were performed with the chi-squared test or Fisher's exact test, while numerical data were analysed with the Mann–Whitney $U$ test.

age, cardiovascular risk factors and medical history of cardiovascular diseases (Supplementary Tables 1 and 2), higher CRP concentrations were also associated with an increased prevalence of older age and cardiovascular risk factors but not with previous cardiovascular diseases (but LDH was not associated with any) (Supplementary Tables 3 and 4). In general, biomarkers were not correlated with admission symptoms; however, physical examination showed lower oxygen saturation in those with higher biomarker values. A decline of eGFR was seen among higher cTnI, D-dimer and CRP concentrations but not in LDH. There were associations of higher concentrations of biomarkers with each other (Supplementary Tables 5–8). Hospital admission was more prevalent in higher values of all biomarkers, yet intensive care unit admission and use of invasive mechanical ventilation was only observed more frequently among higher CRP and LDH concentrations. Finally, antibiotics were used more in patients with higher values of cTnI, D-dimer and CRP and the combination of lopinavir and ritonavir in those patients with higher concentrations of CRP and LDH (Supplementary Tables 5–8).

## 30-Day all-cause death endpoint

### cTnI

During 30-day follow-up, 37 patients died. Of those, 7 (5.9%) patients had cTnI concentrations <21 ng/L, and 30 (39.0%) patients had cTnI concentrations ≥21 ng/L (Figure 2). Higher values of cTnI were associated with an increased risk of all-cause death (unadjusted hazard ratio (HR) 7.95; 95% confidence interval (CI) 3.49–18.10; $p < 0.001$). After adjustment

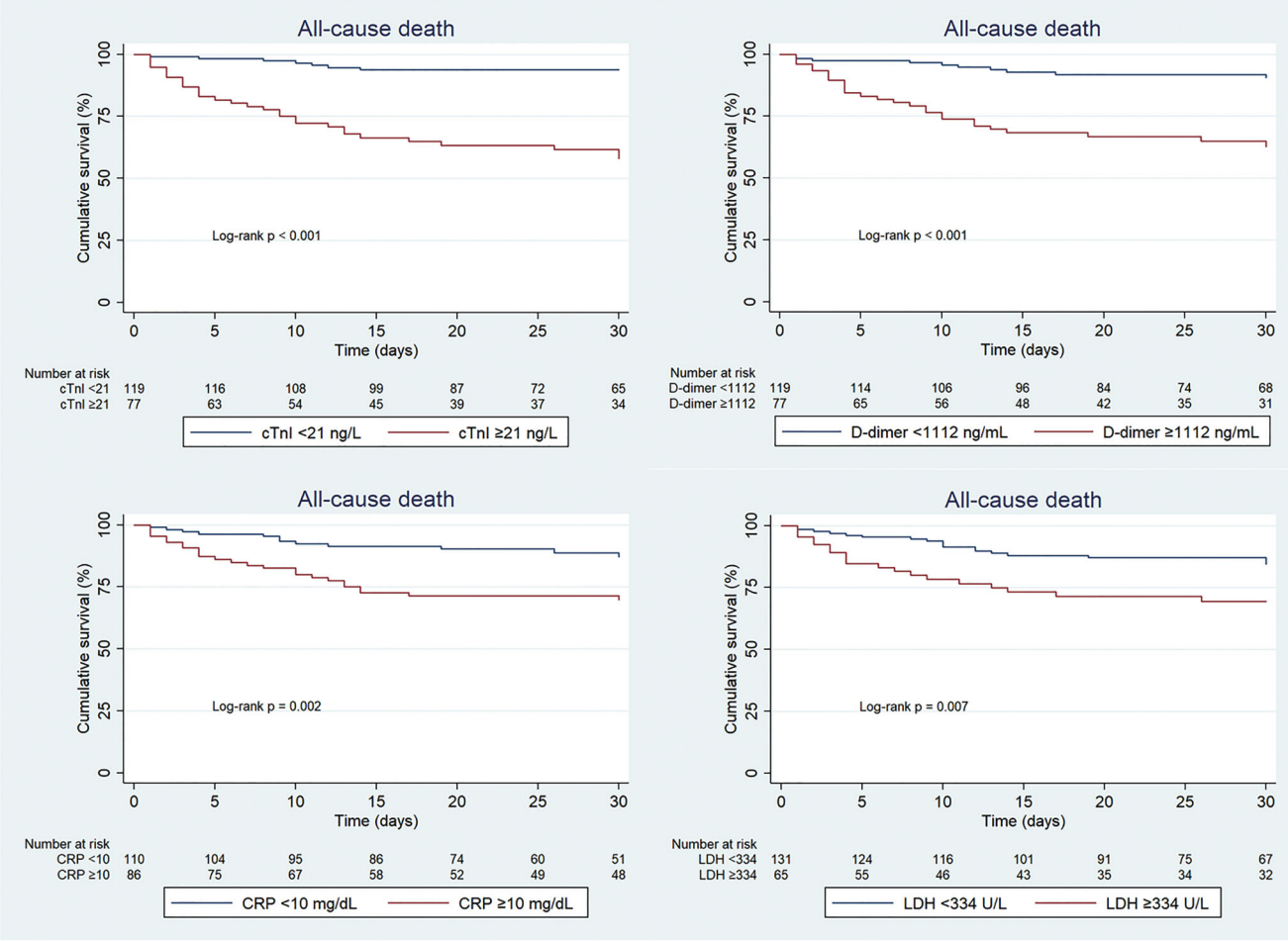


Figure 2. All-cause death cumulative survival by cTnI, D-dimer, CRP and LDH.

Table 3. All-cause death events during 30-day follow-up and adjusted hazard ratio.

| Biomarker | Low concentration | High concentration | *p* Value |
|---|---|---|---|
| Cardiac troponin I | <21 ng/L | ≥21 ng/L | |
| 30-day all-cause death, no. (%) | 7 (18.9) | 30 (81.1) | <0.001 |
| Adjusted HR for high vs. low cTnI concentration | | 4.30 (1.74–10.58) | 0.002 |
| D-dimer | <1112 ng/mL | ≥1112 ng/mL | |
| 30-day all-cause death, no. (%) | 10 (27.0) | 27 (73.0) | <0.001 |
| Adjusted HR for high vs. low D-dimer concentration | | 3.35 (1.58–7.13) | 0.002 |
| C-reactive protein | <10 mg/dL | ≥10 mg/dL | |
| 30-day all-cause death, no (%) | 12 (32.4) | 25 (67.6) | 0.001 |
| Adjusted HR for high vs. low CRP concentration | | 2.25 (1.13–4.50) | 0.021 |
| Lactate dehydrogenase | <334 U/L | ≥334 U/L | |
| 30-day all-cause death, no. (%) | 18 (48.6) | 19 (51.4) | 0.009 |
| Adjusted HR for high vs. low LDH concentration | | 2.00 (1.04–3.84) | 0.039 |

HR: hazard ratio; vs.: versus; cTnI: cardiac troponin I; CRP: C-reactive protein; LDH: lactate dehydrogenase.
Events are presented as number (percentage). Multivariable Cox regressions were performed with the backward stepwise procedure and adjusted for age, hypertension, medical history of chronic pulmonary disease and renal impairment at admission. Hazard ratios are presented with their 95% confidence intervals.

for potential confounders, cTnI concentrations ≥21 ng/L were independently associated with a higher risk of all-cause death (adjusted HR 4.30; 95% CI 1.74–10.58; $p = 0.002$) (Table 3 and Supplementary Table 9).

### *D-dimer*

Among patients with D-dimer concentrations <1112 ng/mL, 10 (8.4%) patients died, while 27 (35.1%) patients died with D-dimer concentrations ≥1112 ng/mL (Figure 2). An unadjusted analysis showed that patients with D-dimer ≥1112 ng/mL had an increased risk of all-cause death (unadjusted HR 4.80; 95% CI 2.32–9.92; $p < 0.001$). This excess risk was still significant after adjustment for potential confounders (adjusted HR 3.35; 95% CI 1.58–7.13; $p = 0.002$) (Table 3 and Supplementary Table 9).

### *CRP*

Of the 37 patients who died during follow-up, 12 (10.9%) patients had CRP concentrations <10 mg/dL and 25 (29.1%) patients had CRP concentrations ≥10 mg/dL (Figure 2).

Higher concentrations of CRP were associated with an increased risk of death (unadjusted HR 2.81; 95% CI 1.41–5.60; $p = 0.003$), even after adjustment (adjusted HR 2.25; 95% CI 1.13–4.50; $p = 0.021$) (Table 3 and Supplementary Table 9).

### *LDH*

During follow-up, 18 (13.7%) patients died with LDH concentrations <334 U/L, and 19 (29.2%) patients died with LDH concentrations ≥334 U/L (Figure 2). Higher LDH values were associated with all-cause death (unadjusted HR 2.36; 95% CI 1.24–4.50; $p = 0.009$), even after multivariate analysis (adjusted HR 2.00; 95% CI 1.04–3.84; $p = 0.039$) (Table 3 and Supplementary Table 9).

In our population, COVID-19 diagnosis approach and treatment were not associated with the four biomarkers. Even more, we did an exploratory combination and there was no significant improvement in the prediction of short-term mortality.

### *Analysis of ROC curves*

ROC curves were performed to determine which biomarker provided better prediction of 30-day all-cause death. AUCs were as follows: cTnI 0.825 (95% CI 0.759–0.892); D-dimer 0.756 (95% CI 0.674–0.837); CRP 0.685 (95% CI 0.600–0.770); LDH 0.643 (95% CI 0.534–0.753) (Figure 3). ROC curve analyses showed non-significant differences when cTnI vs. D-dimer ($p = 0.115$) were compared, but significant differences when cTnI vs. CRP ($p = 0.009$) and cTnI vs. LDH ($p = 0.006$) were compared. Non-significant differences were observed when the D-dimer was compared against CRP ($p = 0.269$) and LDH ($p = 0.118$) and also when CRP was compared against LDH ($p = 0497$).

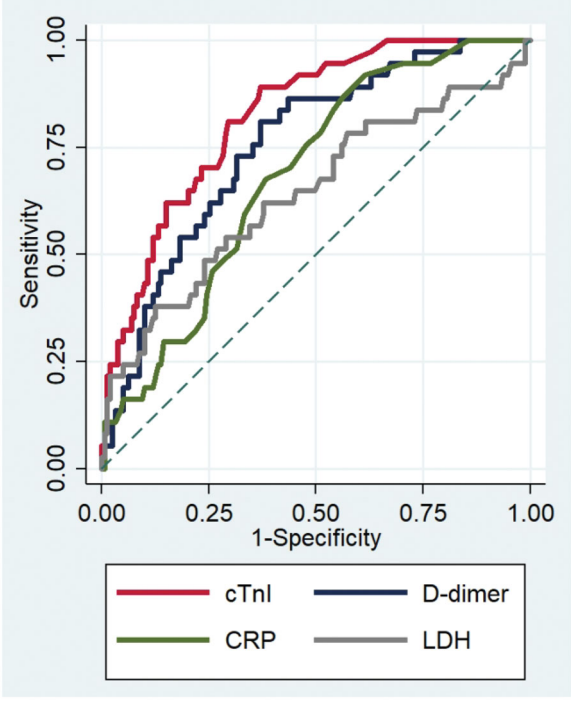


Figure 3. ROC curves for predicting all-cause death.

## Discussion

In our study, we analysed the prognostic value of cTnI, D-dimer, CRP and LDH to stratify the risk of 30-day all-cause death in patients admitted with COVID-19. We found that cTnI ≥21 ng/L, D-dimer ≥1112 ng/mL, CRP ≥10 mg/dL and LDH ≥334 U/L at admission were associated with an increased risk of short-term mortality. Even more, we compared the prognostic value of all four biomarkers, and we observed that cTnI provided better prediction of 30-day all-cause death than CRP, LDH and D-dimer. However, differences with D-dimer were non-significant. Finally, higher D-dimer values and especially higher cTnI concentrations were consistently related with an increased prevalence of older age, cardiovascular risk factors and medical history of cardiovascular diseases. Higher CRP concentrations were also associated with an increased prevalence of older age and cardiovascular risk factors but not with previous cardiovascular diseases and LDH was not associated with any.

CRP is a routinely used inflammatory biomarker produced and released by the liver in response to intereukin-6 stimulation. In situations of an acute inflammatory state, CRP increases its serum concentration, and in most cases, it increases according to the disease severity and decreases when inflammation is resolved. SARS-CoV-2 infection produces an inflammatory response that, in some patients, can develop a hyperinflammatory state characterized by cytokine storm, septic shock, coagulation disorders, metabolic dysregulation and multiorgan dysfunction (Potempa *et al.* 2020, Siddiqi *et al.* 2020). For that reason, in COVID-19, CRP increases progressively at the beginning of the infection and has been associated with disease severity and mortality (Liu *et al.* 2020, Ponti *et al.* 2020, Sahu *et al.* 2020, Shang *et al.* 2020, Wang *et al.* 2020). In addition, CRP correlates with computed tomography findings (Tan *et al.* 2020) and respiratory failure (Poggiali *et al.* 2020). Those previous reports are like our findings where CRP was significantly associated with 30-day all-cause death. We found an optimal cut-off point of 10 mg/dL, which was higher than the majority of previous studies, and we also found an area under the curve (AUC) lower than previous reports (Huang I *et al.* 2020, Soraya and Ulhaq 2020). Our different findings could be explained by a CRP determination at an early stage of the disease compared to other studies.

LDH is an enzyme involved in carbohydrate metabolism by conversion of lactate and pyruvate. It is widely present in human cells, and its plasma concentration increases in various diseases that cause cellular damage. In COVID-19, LDH has been reported as a prognostic biomarker. Higher LDH concentrations at admission have been associated with severe COVID-19 (Deng *et al.* 2020, Kermali *et al.* 2020, Ponti *et al.* 2020). In fact, high LDH values have been found among non-survivors (Chen *et al.* 2020), patients admitted to the intensive care unit (Huang C *et al.* 2020), and patients with respiratory failure (Poggiali *et al.* 2020), and they correlate

with the severity of pneumonia assessed by computed tomography (Xiong *et al.* 2020). After multivariable analysis, Mo *et al.* found that LDH was not associated with refractory COVID-19 (Mo *et al.* 2020); however, Li *et al.*, in a larger cohort, reported a significant association between LDH >445 U/L and severe cases (Li *et al.* 2020). In our study, we found LDH ≥334 U/L associated with an increased risk of 30-day all-cause death. Therefore, those previous reports are essentially in line with our findings and suggest that LDH concentration increases with the extent of tissue damage and disease severity. However, we found that LDH provides worse prediction capacity than cTnI.

D-dimer concentration increases after the degradation of fibrin by plasmin and, therefore, D-dimer can be used as a biomarker of fibrinolytic activity. In critically ill patients, especially those with sepsis, an activation of the coagulation cascade by proinflammatory cytokines has been reported (Shorr *et al.* 2002). Similarly, COVID-19 can produce a procoagulant state by multiple factors that are not yet fully understood (Yu *et al.* 2020). What is known in COVID-19 is that higher D-dimer concentrations are frequently observed in patients with adverse outcomes. High D-dimer levels have been associated with mortality, severe disease, admission to the intensive care unit and an increased risk of pulmonary embolism (Aboughdir *et al.* 2020, Huang I *et al.* 2020, Mestre-Gómez *et al.* 2020, Ponti *et al.* 2020, Zhang *et al.* 2020). Even more, an upward trend of D-dimer within the course of COVID-19 has been related with deceased patients (Ye *et al.* 2020). However, there is not a standardized cut-off value (Huang I *et al.* 2020). In our study, we found D-dimer ≥1112 ng/mL as the optimal cut-off point for short-term mortality prediction with an AUC lower, but not significantly, than cTnI.

Elevation of cTn as a reflection of myocardial injury in the absence of an acute coronary syndrome can be detected in several diseases (Bardaji *et al.* 2015). In fact, it is common in critically ill patients with community acquired pneumonia (Frencken *et al.* 2019), and it is also the cause of severe COVID-19 disease (Guo *et al.* 2020, Lala *et al.* 2020, Shi *et al.* 2020a, 2020b, Zhou *et al.* 2020). Although variable prevalence has been reported, one of the latest studies found myocardial injury in 36% of hospitalized COVID-19 patients (Lala *et al.* 2020). Those patients with an increased concentration of cTn have been consistently related with higher risk of mortality and severe course of the disease (Guo *et al.* 2020, Lala *et al.* 2020, Shi *et al.* 2020a, 2020b, Zhou *et al.* 2020). In our study, we demonstrate that even very small amounts of myocardial injury (cTnI ≥ 21 ng/L) can be associated with short-term mortality and provide an excellent prediction capacity, even more accurate than other biomarkers. Of note, Shi (2020b) reported a similar cTnI cut-off value (26 ng/L). However, greater amounts of cTnI (>90 ng/L) correlate with higher risk of death than small concentrations (>30–90 ng/L) as Lala (2020) reported. We have shown myocardial injury is associated with higher prevalence of cardiovascular risk factors and prior myocardial diseases. The mechanism of acute myocardial injury caused in COVID-19 disease is now under study; however, systemic inflammation, sepsis and severe hypoxia may have a potential role in it. Other reported causes are stress cardiomyopathy, myocarditis, pulmonary embolism and also type 1 myocardial infarction (Imazio *et al.* 2020).

Altogether, cTnI, D-dimer, LDH and CRP are interesting biomarkers that could be used for short-term risk stratification of patients admitted with COVID-19. Our study determines D-dimer and especially cTnI as the best prognostic biomarkers and provides cut-off values to facilitate their clinical use. We hypothesized that D-dimer and cTnI superiority could be explained by the strong association of these biomarkers with cardiovascular risk factors and previous cardiovascular diseases. On the other hand, CRP and LDH correlated more with the activity of the disease. As we tested the biomarkers at admission, some patients could be in an early stage of the disease and, therefore, have lower concentrations of LDH and CRP, which could limit their prognostic value.

### *Limitations*

The study has the following limitations. It is a unicentric retrospective observational study with a relatively small sample size. Biomarkers were measured only once at the time of admission, so we are unaware if the kinetics of biomarkers could improve or worsen the observed results. We provide several variables in our cohort; however, some variables may be missing, such as lung computed tomography scan information. Although viral presence was confirmed mainly by polymerase chain reaction assay, false positives and false negatives could be present. Finally, although a multivariable analysis was performed, a potential impact of residual confounding may be present due to the nature of a retrospective observational study.

## Conclusions

Our study demonstrates that cTnI, D-dimer, CRP and LDH can be used for short-term mortality risk stratification in patients admitted with COVID-19. Even more, we demonstrate that cTnI provides better prediction of 30-day all-cause death than CRP, LDH and D-dimer. However, differences with D-dimer were non-significant. Therefore, these biomarkers should be used routinely to stratify risk in COVID-19 patients presenting in the Emergency Department and can be an excellent tool to facilitate the decision to hospitalize.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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