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1 **The Spectrum of Biochemical Alterations Associated with Organ Dysfunction and**  
2 **Inflammatory Status and Their Association with Disease Outcomes in Severe COVID-**  
3 **19: A Longitudinal Cohort and Time-Series Design Study.**

4  
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1 **ABSTRACT**

2 **BACKGROUND:** In patients with severe COVID-19, no data are available on the  
3 longitudinal evolution of biochemical abnormalities and their ability to predict disease  
4 outcomes.

5 **METHODS:** Using a retrospective, longitudinal cohort study design on consecutive patients  
6 with severe COVID-19, we used an extensive biochemical dataset of serial data and time-  
7 series design to estimate the occurrence of organ dysfunction and the severity of the  
8 inflammatory reaction and their association with acute respiratory failure (ARF) and death.

9 **FINDINGS:** On the 162 studied patients, 1151 biochemical explorations were carried out for  
10 up to 59 biochemical markers, totaling 15,260 biochemical values. The spectrum of  
11 biochemical abnormalities and their kinetics were consistent with a multi-organ involvement,  
12 including lung, kidney, heart, liver, muscle, and pancreas, along with a severe inflammatory  
13 syndrome. The proportion of patients who developed an acute kidney injury (AKI) stage 3,  
14 increased significantly during follow-up (0.9%, day 0; 21.4%, day 14;  $P < 0.001$ ). On the 20  
15 more representative biochemical markers (>250 iterations), only CRP >90 mg/L (odds ratio  
16 [OR] 6.87, 95% CI, 2.36–20.01) and urea nitrogen >0.36 g/L (OR 3.91, 95% CI, 1.15–  
17 13.29) were independently associated with the risk of ARF. Urea nitrogen >0.42 g/L was the  
18 only marker associated with the risk of COVID-19 related death.

19 **INTERPRETATION:** Our results point out the lack of the association between the  
20 inflammatory markers and the risk of death but rather highlight a significant association  
21 between renal dysfunction and the risk of COVID-19 related acute respiratory failure and  
22 death.

23 **FUNDING:** No funding.

## 1 **Research in context**

### 2 **Evidence before this study**

3 We performed a literature search to assess the available data regarding the follow-up of multi-  
4 organ dysfunction and inflammatory status using biochemical biomarkers kinetics in patients  
5 with severe COVID-19. We searched PubMed from inception to April 4, 2020, for studies  
6 that have reported clinical and biological data, and disease outcomes in patients with COVID-  
7 19. In patients with severe COVID-19, no data are available on the follow-up of multi-organ  
8 dysfunction and inflammatory status by assessing the kinetics of biochemical biomarkers and  
9 its association with disease-related complications.

10

### 11 **Added value of this study**

12 In this retrospective cohort study on 162 consecutive patients with newly diagnosed severe  
13 COVID-19, we report the first longitudinal analysis using an extensive biochemical dataset of  
14 serial data (59 biochemical markers; 15,260 biochemical measures) and time-series design to  
15 estimate the occurrence of organ dysfunction and the severity of the inflammatory reaction  
16 and their association with acute respiratory failure (ARF) and death. Among all the studied  
17 biomarkers, only urea nitrogen and C-reactive protein (CRP) were independently associated  
18 with acute respiratory failure.

19

### 20 **Implications of all the available evidence**

21 In patients with newly diagnosed severe COVID-19, the follow-up of biochemical biomarkers  
22 kinetics was consistent with a severe multi-organ involvement along with a severe acute  
23 inflammatory response. Our study pointed out new biochemical abnormalities targeting other  
24 organs than lungs and kidneys, including the liver and biliary tract with marked cholestatic

- 1 syndromes. High levels of CRP and urea nitrogen were potential predictors of acute
- 2 respiratory failure among patients with severe COVID-19.

## 1 INTRODUCTION

2 A novel human coronavirus, called severe acute respiratory syndrome coronavirus 2  
3 (SARS-CoV-2), causing the disease COVID-19 (according to the WHO nomenclature, for  
4 coronavirus disease 2019) was identified in China in December 2019.<sup>1</sup> The pandemic has  
5 rapidly progressed from China to all continents with more than 180 countries concerned in  
6 mid-April 2020, with 1,777,666 identified cases and 108,867 COVID-19 related deaths.<sup>2</sup>  
7 During January 2020, three cases of COVID-19 were confirmed in France as the first cases in  
8 Europe.<sup>3</sup> During March 2020, the COVID-19 epidemic has rapidly spread in France, notably  
9 from an epidemic cluster in the North East of France with 130,730 identified cases and 13,851  
10 deaths by mid-April 2020.<sup>2</sup> Several papers reported the baseline characteristics, the clinical  
11 findings, and outcomes of patients with COVID-19 from China<sup>4-9</sup>, Singapore<sup>10</sup>, and Italy.<sup>11</sup>  
12 These studies assessed the clinical and demographic factors of patients with the COVID-19  
13 and pointed out an increased risk of mortality among older males and patients with co-  
14 morbidities.<sup>4-11</sup> Pulmonary, systemic inflammation and subsequent multi-organ dysfunction  
15 have been reported in association with severe COVID-19.<sup>8</sup> Acute respiratory distress  
16 syndrome with respiratory failure requiring mechanical ventilation and acute cardiac injury  
17 with heart failure were the most common critical complications potentially leading to death in  
18 patients with severe COVID-19.<sup>8</sup>

19 In patients with severe COVID-19, no data are available on the longitudinal follow-up  
20 of organ dysfunction and inflammation by assessing the kinetics of biochemical biomarkers  
21 using a time-series design. Furthermore, even if some studies have evaluated the risk of  
22 COVID-19 related complications using a small set of biological markers at baseline, no study  
23 has evaluated, on a wide range of biochemical markers, the spectrum and the magnitude of  
24 biochemical disturbances, as well as their kinetics over time and their potential association  
25 with disease outcomes in patients with severe COVID-19. Using a big-data approach and

1 multilevel modeling adapted for repeated measures on a well-phenotyped retrospective cohort  
2 of consecutive patients with newly diagnosed severe COVID-19, we were able to assess the  
3 occurrence of organ dysfunction (kidney, lung, heart, liver, muscle) and the severity of the  
4 inflammatory reaction as evaluated by biomarker kinetics in hospitalized patients. We  
5 assessed the association of the more representative biochemical markers with the risk of  
6 occurrence of ARF and death.

7

## 8 **METHODS**

### 9 **Study design, setting, and patients' inclusion criteria**

10 We carried out a retrospective, longitudinal cohort study, and time-series design on all  
11 newly diagnosed consecutive patients among the first cases of severe COVID-19 that required  
12 hospitalization at the University Hospital of Nancy. Inclusion in the cohort began on the day  
13 of hospital admission, and each patient was then followed until discharge from hospital or  
14 death if it occurred during hospitalization. The inclusion criteria were: i) diagnosis of COVID-  
15 19 based on the detection of SARS-CoV-2 ribonucleic acids (RNA) from nasopharyngeal  
16 swabs; ii) severe COVID-19 defined by an oxygen saturation of 94% or less while the patient  
17 was breathing ambient air or a need for oxygen support<sup>12,13</sup>; iii) hospitalization in one of the  
18 healthcare departments of the University Hospital of Nancy from March 1, 2020, to March 25,  
19 2020. The final date of follow-up was March 31, 2020. The cohort was observational, i.e., all  
20 clinical assessments, biochemical explorations, imaging examinations, and clinical diagnoses  
21 were carried out at the discretion of the treating physicians as part of the standards of care of  
22 patients with suspected COVID-19 during the study period at the University Hospital of  
23 Nancy (see Supplementary Methods). The Ethics committee of the University Hospital of  
24 Nancy approved the study.

25

## 1 **Description of the Nancy Biochemical Database**

2 As previously reported<sup>14</sup>, the “Nancy Biochemical Database” is a prospectively  
3 maintained electronic database that increments the biochemical results of consecutive patients  
4 hospitalized in 67 healthcare departments at the University Hospital of Nancy  
5 (**Supplementary Figure 1**). The biochemical data are connected to clinical data at the patient  
6 level through the electronic health record system of the University Hospital of Nancy. All  
7 biochemical data that were obtained in patients with COVID-19 were extracted for the  
8 purposes of the study using the GLIMS laboratory information management system v9 (MIPS  
9 France S.a.r.l., Paris, France). Clinical data were retrieved through electronic chart review  
10 using DxCare® software (Dedalus France, Le Plessis Robinson, France). All biochemical  
11 investigations were prescribed at the discretion of the treating physicians from each healthcare  
12 department. The “Nancy Biochemical Database” is registered at the French National  
13 Commission on Informatics and Liberty, CNIL, under the record N°1763197v0. The Ethics  
14 committee of the University Hospital of Nancy approved the study (ID: 2020/264).

15

## 16 **Data collected for the study**

17 The following data were available in the Nancy Biochemical Database: patient  
18 identification number, patient’s age at hospital admission, date and time of blood sampling,  
19 patient health care department. A total of 59 biochemical markers were available, with 46 in  
20 the blood and 13 in the urine. The biochemical markers available in blood were: **electrolytes**,  
21 **renal markers**: sodium (mmol/L), potassium (mmol/L), chloride (mmol/L), creatinine  
22 (mg/L), urea nitrogen (g/L), phosphorus (mg/L), calcium (mg/L), magnesium (mg/L),  
23 calculated and measured (mOsmol/kg), uric acid (mg/L), phosphorus reabsorption rate (%),  
24 fractional excretion of sodium (%), fractional excretion of potassium (%), fractional excretion  
25 of chloride (%), and anionic gap (mmol/L); **blood gas**: hemoglobin (g/dL), partial pressure of

1 oxygen (PO<sub>2</sub>) (mmHg), partial pressure of carbon dioxide (PCO<sub>2</sub>) (mmHg), pH, bicarbonate  
2 (HCO<sub>3</sub><sup>-</sup>) (mmol/L), and lactate (mmol/L); **liver, pancreas:** aspartate aminotransferases  
3 (ASAT) (U/L), alanine aminotransferases (ALAT) (U/L), total bilirubin (mg/L), conjugated  
4 bilirubin (mg/L), alkaline phosphatase (UI/L),  $\gamma$ -glutamyltransferase (U/L), and lipase (U/L);  
5 **inflammatory markers:** C-reactive protein (CRP) (mg/L), procalcitonin (ng/ml), and  
6 interleukin 6 (pg/mL); **iron markers:** ferritin ( $\mu$ g/L), iron-binding capacity saturation (%),  
7 iron (mg/L), and transferrin (g/L); **cardiac markers:** high-sensitivity cardiac troponin I (hs-c  
8 Troponin I) (pg/mL), N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) (pg/mL);  
9 **muscle markers:** lactate dehydrogenase (U/L), creatine kinase (U/L); **nutritional markers:**  
10 total protein (g/L), albumin (g/L), prealbumin (g/L), glucose (g/L), triglycerides (g/L), and  
11 cholesterol (g/L). The biochemical markers available in the urine were: sodium (mmol/L),  
12 potassium (mmol/L), chloride, urine (mmol/L), creatinine (mg/L), urea nitrogen (g/L),  
13 glucose (g/L), proteins (g/L), anion gap (mmol/L), uric acid (mg/L), osmolality (mOsmol/kg),  
14 phosphorus (mg/L), calcium (mg/L), and microalbumin (mg/L). The following clinical data  
15 were available: date of hospital admission; patient's medical history (hypertension, type 2  
16 diabetes, cardiovascular disease, vascular disease, dyslipidemia, chronic obstructive  
17 pulmonary disease, obstructive sleep apnea syndrome, asthma, cancer, and chronic kidney  
18 disease); patient's outcome during the hospitalization for the management of COVID-19  
19 (acute respiratory failure [ARF] as defined by the treating physicians <sup>15-17</sup>, intubation with  
20 mechanical ventilation, pulmonary embolism, and in-hospital mortality related to the COVID-  
21 19, defined as the occurrence of death in relation with a complication of the COVID-19 <sup>18</sup>).

22

### 23 **Molecular detection of SARS-CoV-2**

24 After viral RNA extraction from nasopharyngeal swabs, the procedure applied for the  
25 detection of SARS-CoV-2 genome was based on primers and probes designed to target the

1 RdRp gene spanning nucleotides 12,621–12,727 and 14,010–14,116 (positions according to  
2 SARS-CoV, NC\_004718, National reference Center for Respiratory Viruses, Pasteur Institute,  
3 Paris, France; sequences available on request). Each real-time reverse transcriptase-  
4 polymerase chain reaction (RT-qPCR) included negative and positive samples (in vitro  
5 synthesized RNA transcripts). The RT-PCR assay sensitivity was around 100 copies of RNA  
6 genome equivalent. Regarding the specificity of the test, none of the tested respiratory viruses  
7 showed reactivity to influenza A-B, respiratory syncytial virus, bocavirus (hBoV),  
8 metapneumovirus (hMPV), HRV/enterovirus, adenovirus, and other coronaviruses (HCoV:  
9 KU1, OC43, 229E, and NL63). All the virological diagnoses of SARS-CoV-2 infection were  
10 carried out at the Division of Virology of the University Hospital of Nancy, which served as  
11 one of the two regional reference centers for the SARS-CoV-2 diagnosis in North East of  
12 France.

13

#### 14 **Biochemistry assays**

15 Biochemistry analyses were performed on Atellica immunoassay (IM) and clinical  
16 chemistry (CH) multiparametric analyzers (Siemens Healthcare SAS, France). Blood gas  
17 analyses were performed on ABL800 FLEX analyzers (Radiometer France SAS, France).

18

#### 19 **Standards of care of patients with suspected COVID-19 during the study period at the** 20 **University Hospital of Nancy**

21 At the University Hospital of Nancy, during March 2020, the recommended best  
22 standard of care for suspected COVID-19 patients requiring hospital admission for non-  
23 critical respiratory failure included a systematic diagnostic workup with a clinical and  
24 biological assessment, molecular detection of SARS-CoV-2 by real-time reverse  
25 transcriptase-polymerase chain reaction (RT-qPCR), blood and sputum cultures, and chest

1 CT-scan. Patients were managed according to National Health Authority guidelines, including  
2 the following items: i) oxygen delivery to maintain peripheral capillary oxygen saturation  
3 ( $\text{SpO}_2$ )  $>90\%$ , ii) 48 hours of probabilistic antibiotic therapy combining cefotaxime with  
4 spiramycin awaiting SARS-CoV-2 RT-qPCR result and bacteriological assessment; iii)  
5 therapeutic dose of antithrombotic therapy using low molecular weight heparin or  
6 unfractionated heparin in case of renal failure. All other aspects related to the standards of  
7 care and patients' monitoring are detailed in Supplementary Methods).

8

### 9 **Study aims and endpoints**

10 The primary aim was to assess the occurrence of organ dysfunction (kidney, lung,  
11 heart, liver, and muscle) and the severity of the inflammatory reaction as evaluated by  
12 biomarker kinetics in patients with severe COVID-19. The secondary aims were to assess the  
13 biochemical markers potentially associated with: i) COVID-19 related ARF, and ii) in-  
14 hospital mortality related to the COVID-19. For each studied biochemical marker, the primary  
15 endpoint was the percentage of time above or below the upper or the lower reference limit,  
16 respectively, during the hospital stay. The most frequently observed biochemical  
17 abnormalities were defined as those that were observed more than 25% of time above or  
18 below the upper or the lower reference limit, respectively. For the prognostic objective  
19 (secondary aim), the endpoints were: i) the occurrence of COVID-19 related ARF as defined  
20 by the treating physicians<sup>15-17</sup> and ii) in-hospital mortality related to the COVID-19, defined  
21 as the occurrence of death in relation with a complication of the COVID-19.<sup>18</sup> The diagnosis  
22 and severity of acute kidney injury (AKI) were classified according to the AKI network  
23 criteria, based on the results of serum creatinine.<sup>19</sup>

24

25

## 1 **Statistical analyses**

2 All quantitative variables are shown as the median and interquartile range (IQR, 25–  
3 75th percentile) and qualitative variables as percentages and 95% confidence interval (95%  
4 CI). The kinetics over time of the studied biochemical markers was modeled using the  
5 isotonic regression method, which was estimated using the pool adjacent violators  
6 algorithm.<sup>20,21</sup> The pool adjacent violators algorithm is a linear time algorithm for linear  
7 ordering isotonic regression.<sup>21</sup> Using a multistep approach, we evaluated a set of 20  
8 biochemical markers with a sufficiently high number of iterations (n >250, study power  
9 analysis reported in Supplementary Methods) to assess the relationship between their  
10 variation over time and the occurrence of the endpoint (ARF, death). In **step #1**, for each  
11 biochemical variable, we assessed the optimal threshold using receiver operating  
12 characteristic (ROC) analysis, according to DeLong *et al.*<sup>22</sup> The classification variable used in  
13 the ROC analysis was the studied endpoint. The optimal diagnostic cut-off was defined using  
14 the Youden index J. Bias-corrected and accelerated (BC<sub>a</sub>)-bootstrap interval after 10,000  
15 iterations for the Youden index and its associated values were performed.<sup>23</sup> We used  
16 Bonferroni's correction to account for multiple comparisons. In **step #2**, all the variables that  
17 were significantly associated with the studied endpoint in ROC analyses were assessed using  
18 time-series analysis.<sup>24</sup> The evolution times were calculated from the first day of biochemical  
19 assessment and were expressed in days. The time-series analysis aimed to compare the  
20 percentage of time below or above the ROC-defined threshold between two patients'  
21 subgroups according to the presence or absence of the studied endpoint. The calculated  
22 summary effects were reported as percentages of the total time of observation with the 95%  
23 confidence interval (95% CI). Normality testing was performed using the D'Agostino-Pearson  
24 test. Subgroups comparison regarding the percentage of time below or above the prespecified  
25 threshold was carried out using the Student's *t*-test or the Mann-Whitney *U* test according to

1 the parametric or nonparametric distribution of the variables, respectively. In **step #3**, we  
2 looked for independent predictors using multivariable multilevel analysis. All the predictors  
3 that were still significant at the step #2 were assessed using a two-level hierarchical logistic  
4 model (HLM), which enabled to take into account the correlation between all the studied  
5 biochemical markers, which correspond to the level 1 of the HLM, and the patient-level  
6 characteristics, which correspond to the level 2 of the HLM (i.e., age, gender, patient's  
7 medical history).<sup>25</sup> For each endpoint of interest, a multivariable model was then constructed  
8 following the modeling strategy recommended for HLM.<sup>26</sup> We estimated the empty model  
9 without level 1 or level 2 variables to confirm the relevance of using the HLM assumption,  
10 which is based on a median odds ratio  $\gg 1$ , considering a variable number of iterations  
11 between each patient.<sup>27</sup> Each biochemical variable (level 1, HLM) was tested for the  
12 association with the endpoint using bivariate analyses at a 0.2 threshold. A stepwise backward  
13 multivariable selection procedure was used to retain the variables associated with the endpoint  
14 at a 0.05 threshold, without adjustment for patient-level characteristics (level 2, HLM).  
15 Random slopes on the significant biochemical variables retained in the HLM model were  
16 tested for their association with the endpoint, considering the between-patient variability.  
17 While alternative procedures exist to build models, the procedure we applied has been shown  
18 to be the most suitable one for HLM specification (data not shown).<sup>28</sup> At the final step of the  
19 HLM, eligible patient characteristics (level 2) on collinearity testing were incremented in the  
20 validated HLM model, and cross-level interactions, i.e., interactions between time  
21 measurement and patients variables, were tested in the full model (level 1 and level 2). The  
22 HLM model was estimated using the predictive quasi-likelihood method. For each variable  
23 retained in the full HLM model, summary measures were reported as odds ratio (OR), 95%  
24 CI, and the associated *P*-value. Statistical analyses other than the multivariate model analysis  
25 were performed using MedCalc for Windows, version 19.1 (MedCalc Software, Ostend,

1 Belgium) based on a two-sided type I error with an alpha level of 0.05. The multivariate  
2 model analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

3

#### 4 **Role of funding source**

5 This research received no specific grant from any funding agency.

6

## 7 **RESULTS**

### 8 **Study population, database structure, and main biochemical abnormalities observed in** 9 **severe COVID-19**

10 Between March 1, 2020, and March 25, 2020, 162 patients were admitted to one of the  
11 healthcare departments of the University Hospital of Nancy for severe COVID-19  
12 (**Supplementary Table 1**). The median age was 66 (IQR, 56 to 77) years, and the proportion  
13 of males was 59% (**Table 1**). No patient received hydroxychloroquine or remdesivir. During  
14 the study period, 1151 biochemical explorations were carried out for up to 59 biochemical  
15 markers (46 in the blood and 13 in the urine), totaling 15,260 biochemical values. The  
16 distribution of the 59 biochemical markers is reported in **Table 2** (blood) and **Supplementary**  
17 **Table 2** (urine). The kinetics over time of the biochemical abnormalities is reported in  
18 **Figures 1-3 and Supplementary Figures 2-4** for blood parameters and in **Supplementary**  
19 **Figure 5** for urine parameters. The most frequently observed biochemical abnormalities  
20 during the follow-up were ( $\geq 25\%$  of the observed time): increased urea nitrogen,  
21 hyperosmolality, hypocalcemia, low hemoglobin, hypoxemia; major cytolysis with severe  
22 cholestatic syndromes and conjugated hyperbilirubinemia; increased C-reactive protein  
23 (CRP); increased cardiac (high-sensitivity cardiac troponin I, NT-proBNP: N-Terminal pro-  
24 Brain Natriuretic Peptide) and muscle (lactate dehydrogenase, creatine kinase) markers; and  
25 hypoalbuminemia with hypertriglyceridemia (**Table 2 and Figures 1-3**). The evolution of

1 phosphorus followed a biphasic curve with a decrease during the first four days, followed by a  
2 progressive increase in parallel with urea nitrogen and creatinine. The proportion of patients  
3 who developed an acute kidney injury (AKI) stage 3, according to the AKI classification,  
4 increased significantly over time (0.9%, day 0; 13.6%, day 7; and 21.4%, day 14;  $P < 0.001$ )  
5 (**Supplementary Figure 6** and **Supplementary Table 3**). Other biochemical abnormalities  
6 (10–24% of the observed time) included hypernatremia, hyperkalemia, hyperchloremia,  
7 increased creatinine, hypoproteinemia, hyperlactatemia, hyperlipasemia, and increased  
8 procalcitonin (**Table 2** and **Supplementary Figures 2-4**).

9

#### 10 **Factors associated with an occurrence of acute respiratory failure related to severe** 11 **COVID-19**

12 On the 20 biochemical variables assessed in ROC analysis, 15 were significantly  
13 associated with the risk of ARF after Bonferroni correction (**Table 3** and **Supplementary**  
14 **Figure 7**). All the 15 dichotomized variables differed significantly in time-series analyses  
15 between patients with or without ARF, with a significantly higher proportion of time with the  
16 biochemical marker above or under the ROC-defined threshold among patients with ARF  
17 when compared to patients without ARF (**Table 3** and **Figure 4**). These markers included  
18 electrolyte and blood gas disturbances, increased renal markers, liver and muscle cytolysis,  
19 severe inflammatory syndrome, and hypoproteinemia. In the multivariable multilevel analysis,  
20 three variables were independently associated with the risk of ARF, namely: CRP  $>90$  mg/L  
21 (OR, 6.87 [95% CI, 2.36–20.01];  $P < 0.001$ ), urea nitrogen  $>0.36$  g/L (OR, 3.91 [95% CI,  
22 1.15–13.29];  $P = 0.03$ ), and the medical history of type 2 diabetes (OR, 4.49 [95% CI, 1.07–  
23 18.89];  $P = 0.04$ ) (**Table 4**). In the alternative model, that used creatinine instead of urea  
24 nitrogen to avoid collinearity, CRP  $>90$  mg/L, and the medical history of type 2 diabetes were  
25 independently associated with the risk of ARF (**Table 4**).

## 1 **Factors associated with in-hospital mortality related to severe COVID-19**

2 On the 20 biochemical variables assessed in ROC analysis, five were significantly  
3 associated with the risk of death after Bonferroni correction (**Table 5**). In time-series analyses,  
4 among the five dichotomized variables, only one differed significantly between patients who  
5 died or not: urea nitrogen  $>0.42$  g/L (**Table 5** and **Figure 5**). In the multivariable multilevel  
6 analysis, only age (OR, 1.12 [95% CI, 1.03–1.22];  $P = 0.006$ ) and medical history of chronic  
7 obstructive pulmonary disease (OR, 14.06 [95% CI, 1.08–182.74];  $P = 0.04$ ) were  
8 significantly associated with the risk of COVID-19 related death. Urea nitrogen  $>0.42$  g/L had  
9 an OR of 3.64 (95% CI, 0.71–18.71;  $P = 0.12$ ) for the association with in-hospital mortality  
10 (**Table 4**).

11

## 12 **DISCUSSION**

13 Using a big-data approach and time-series design on a well-phenotyped retrospective  
14 cohort of consecutive patients with newly diagnosed SARS-CoV-2 infection, we were able to  
15 describe the most frequently observed biochemical abnormalities over time in patients with  
16 severe COVID-19. In these patients, the kinetics of biochemical abnormalities was consistent  
17 with a multi-organ involvement (**Figure 6**). The results of our study point out the lack of the  
18 association between the inflammatory markers and the risk of death but rather highlight a  
19 significant association between renal dysfunction and the risk of COVID-19 related ARF or  
20 death.

21 A cohort study from China reported a baseline prevalence of acute kidney injury  
22 among 5.1% of patients with COVID-19 that were admitted to a tertiary hospital.<sup>29</sup> Patients  
23 with an AKI stage 1 to 3 were at a higher risk of in-hospital mortality.<sup>29</sup> One of the  
24 hypotheses for the occurrence of acute renal damage is the inadequacy of the inflammatory  
25 response linked to the cytokine storm observed in severe forms of COVID-19 and their effect

1 on hypoperfusion of renal tubules.<sup>30</sup> A recent study reported that patients with severe COVID-  
2 19 have a high risk of developing Fanconi syndrome, leading to potentially life-threatening  
3 plasma disturbances.<sup>31</sup> SARS-CoV-2 infects the cells through its binding to the membrane-  
4 bound form of receptor-angiotensin converting enzyme II (ACE2) and subsequent  
5 internalization of the complex by the host cell.<sup>1,32-34</sup> There is emerging evidence about the  
6 direct cytopathic effect of SARS-CoV-2 since the cell entry receptor ACE2 is expressed on  
7 podocytes and tubule epithelial cells.<sup>30</sup> In line with these data, we observed a biphasic  
8 evolution of the phosphorus level in the blood of patients with severe COVID-19 with an  
9 initial decrease during the first four days, followed by a progressive increase that mirrored the  
10 degradation of kidney function. This evolution may reflect a direct cytotoxic effect of the  
11 virus with initial involvement of tubule epithelial cells, which is responsible for a phosphorus  
12 leakage in the urine and, in a second step, a glomerular attack, explaining the progressive  
13 increase in urea nitrogen and creatinine as well as phosphorus. These data are corroborated by  
14 the progressive increase in the proportion of patients with stage 3 AKI during the follow-up.  
15 In our study, patients with ARF and those who die from COVID-19 share the same pattern of  
16 urea nitrogen alteration. In addition to pulmonary involvement, renal involvement leads to  
17 life-threatening alterations in homeostatic regulatory processes. Further studies should address  
18 the significance of acute kidney injury in the prediction of COVID-19 related death and plead  
19 for long-term follow-up by a nephrologist of patients with severe COVID-19.

20 The ACE2 protein is highly expressed in the gastrointestinal tract, notably in the  
21 duodenum and the small intestine, gallbladder, testis, seminal vesicle, and adrenal gland.<sup>35</sup> A  
22 recent study also confirmed the expression of the ACE2 protein in human myocardial  
23 pericytes.<sup>36</sup> We can hypothesize that the direct cytopathic effect of SARS-CoV-2 on pericytes  
24 may result in endothelial cell and microvascular dysfunction.<sup>36</sup> A retrospective single-center

1 case series of 187 patients with COVID-19 from China showed that myocardial injury was  
2 significantly associated with the risk of death.<sup>37</sup>

3 To date, descriptive data on COVID-19 did not report obvious cholestasis in patients  
4 with severe forms.<sup>38,39</sup> In our series, we have reported cholestatic involvement with  
5 conjugated hyperbilirubinemia and cytotoxicity over 30% of the observation time. These results  
6 raise the possibility of a direct toxic effect of the virus on the biliary epithelium, especially  
7 given the high level of expression of the ACE2 protein in the biliary tissue.<sup>35</sup> Long term  
8 evolution of these cholestatic syndromes deserves further studies, notably regarding the risk  
9 of cholestatic liver disease. The present study provides new data on the elevation of lipase  
10 levels among patients with severe COVID-19. This elevation could be related to several  
11 mechanisms, other than direct pancreatic involvement, and include intestinal disease, acidosis,  
12 or the presence of shock. We have observed elevations in CK and LDH that have also been  
13 reported in the literature.<sup>8,38,40-42</sup>

14 We acknowledge several potential limitations of the study that should be considered in  
15 the interpretation of our findings. Although we have studied an extensive follow-up dataset,  
16 our study is based on a relatively small number of patients, and our results need to be  
17 confirmed in independent studies. Nevertheless, we have systematically included all  
18 consecutive patients with newly diagnosed severe COVID-19, which allowed us to describe  
19 the biochemical abnormalities on a homogeneous population. The study design is  
20 retrospective; nevertheless, we used data from a structured and prospectively maintained  
21 database.<sup>14</sup> In our study, the follow-up of patients was relatively short, which could lead to an  
22 underestimation of the risk of COVID-19 related mortality. Patients with severe COVID-19  
23 can exhibit immunological determinants of a cytokine storm<sup>42-45</sup>, notably with high levels of  
24 CXCL10, CCL7, and IL-1 receptor antagonist, and which could influence the risk of lung  
25 injury and fatal outcome.<sup>45</sup> In our study, IL-6 and procalcitonin —an indirect marker of innate

1 immunity— were not tested enough in patients to be assessed in the analysis. Our study has  
2 several strengths. First, we report an exhaustive description of the biochemical abnormalities  
3 and their kinetics over time among patients with severe COVID-19. Second, we assessed the  
4 relationship between the variation over time in biochemical markers and the occurrence of  
5 disease-related complications through a multilevel modeling approach adapted for repeated  
6 measures. Third, we studied a wide range of biochemical markers relating to both blood and  
7 urine and targeting several organs and vital functions. We notably highlighted a potentially  
8 strong association between acute kidney injury, the development of severe kidney damage,  
9 and their association with COVID-19 related in-hospital mortality.

10 In conclusion, in this retrospective, longitudinal cohort study, using an extensive  
11 biochemical dataset of serial data and time-series design on consecutive patients with newly  
12 diagnosed severe COVID-19, the follow-up of biochemical biomarkers kinetics was  
13 consistent with a severe multi-organ involvement along with a severe acute inflammatory  
14 response. High levels of CRP and urea nitrogen were potential predictors of ARF among  
15 patients with severe COVID-19. Further studies should address the significance of acute  
16 kidney failure in the prediction of COVID-19 related death. Our results open new perspectives  
17 on the understanding of the pathophysiological mechanisms related to disease  
18 decompensation and multi-organ dysfunction that targets kidney function in the setting of  
19 severe COVID-19.

20

## 1 **DATA SHARING STATEMENT**

2 Anonymized patient data are available for use in collaborative studies to researchers upon  
3 reasonable request (abderrahim.oussalah@univ-lorraine.fr). Data will be provided following  
4 the review and approval of a research proposal (including a statistical analysis plan) and  
5 completion of a data-sharing agreement. Responses to the request for the raw data will be  
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7

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9

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## 11 **AUTHORS' CONTRIBUTIONS**

12 **AO:** has full access to all the data in this study and take full responsibility as the guarantor for  
13 the integrity of the data and the accuracy of the data analysis, study concept, laboratory  
14 diagnostic assessment, literature review, data synthesis and statistical analysis,  
15 drafting/revision of the manuscript, analysis and interpretation of data, approved the final  
16 draft, final responsibility for the decision to submit for publication; **SG:** laboratory diagnostic  
17 assessment, data extraction, analysis and interpretation of data, approved the final draft; **ICU:**  
18 data synthesis and statistical analysis and interpretation of data, drafting/revision of the  
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1 assessment, analysis and interpretation of data, approved the final draft; **MM**: analysis and  
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3 and interpretation of data, approved the final draft; **RK**: management of patients, analysis and  
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1 **DECLARATION OF INTERESTS**

2 The authors who have taken part in this study declare that they do not have anything to  
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4

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4

## 1 TABLES

2 Table 1. Characteristics of the patients with severe COVID-19

<b>Demographics</b>		
Age (years) — N, median (IQR)	162	66 (56–77)
Male gender — n/N, % (95% CI)	96/162	59% (52–67)
<b>Patients' medical history — n/N, % (95% CI)</b>		
Hypertension	68/135	50% (42–59)
Type 2 diabetes	39/135	29% (21–37)
Cardiovascular disease	38/135	28% (21–36)
Vascular disease	37/135	27% (20–35)
Dyslipidemia	32/135	24% (16–31)
Chronic obstructive pulmonary disease	17/135	13% (7–18)
Obstructive sleep apnea syndrome	17/135	13% (7–18)
Asthma	8/135	6% (2–10)
Cancer	8/135	6% (2–10)
Chronic kidney disease	8/135	6% (2–10)
<b>Outcomes — n/N, % (95% CI)</b>		
Acute respiratory failure	76/149	51% (43–59)
Intubation and mechanical ventilation	54/149	36% (28–44)
COVID-19 related death	21/149	14% (8–20)
Pulmonary embolism	2/149	1% (0–3)

3 Note. IQR: interquartile range 25<sup>th</sup> – 75<sup>th</sup>; N: number of patients; n: number of observations;  
 4 %: percentage; 95% CI: 95% confidence interval.

1 **Table 2. Distribution of the 46 biochemical markers in the blood in patients with severe COVID-19**

<b>Electrolytes, renal markers</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference Values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
Sodium (mmol/L)	848	141	138–144	136–145	8.7 (5.2–12.1)	12.9 (9.2–16.7)
Potassium (mmol/L)	854	4.00	3.68–4.38	3.40–4.50	8.6 (5.7–11.5)	11.0 (7.9–14.2)
Chloride (mmol/L)	799	104	101–107	98–107	5.7 (3.0–8.4)	21.5 (16.3–26.8)
Creatinine (mg/L)	845	8.0	6.0–11.9	M: 7–13 F: 5.5–10.2	M: 20.6 (13.7–27.5) F: 22.7 (13.8–31.5)	M: 19.0 (12.2–26.0) F: 10.7 (3.7–17.7)
Urea nitrogen (g/L)	844	0.43	0.26–0.75	0.19–0.49	7.7 (4.3–11.3)	28.7 (22.5–34.9)
Phosphorus (mg/L)	299	30.96	25.08–38.39	24–51	12.6 (7.4–17.7)	5.6 (1.8–9.5)
Calcium (mg/L)	266	83.6	79.6–88.0	87–104	38.7 (29.7–47.7)	1.3 (0–3.3)
Magnesium (mg/L)	164	20.4	18.0–23.7	16–26	4.8 (0.5–9.1)	8.4 (3.0–13.7)
Osmolality, calculated (mOsmol/kg)	240	297	289–307	282–290	2.6 (0–7.8)	26.9 (18.9–35.0)
Osmolality, measured (mOsmol/kg)	40	295.5	289–317	282–290	Low sample size	Low sample size
Uric acid (mg/L)	82	44	30–62	M: 37–92 F: 31–78	Low sample size	Low sample size
Phosphorus reabsorption rate (%)	27	82.4	71–88	>85%	Low sample size	Not applicable
FENa (%)	28	0.3	0.15–0.75	1–2	Low sample size	Low sample size
FEK (%)	29	7.2	5.38–13.10	4–16	Low sample size	Low sample size
FECl (%)	23	0.7	0.22–1.45	—	Low sample size	Low sample size
Anionic gap (mmol/L)	33	14.5	13.3–15.7	10 – 16	Low sample size	Low sample size
<b>Blood gas</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
Hemoglobin (g/dL)	787	11.8	10.4–13.6	M: 13.5–17.5 F: 12.0–16.0	M: 50.0 (40.8–59.1) F: 35.3 (22.6–48.0)	M: 4.1 (1.6–6.6) F: 3.9 (0.3–7.4)
PO <sub>2</sub> (mmHg)	784	77.7	66.8–94.6	80–100	41.3 (35.0–47.7)	14.0 (10.5–17.4)
PCO <sub>2</sub> (mmHg)	785	38.8	33.9–45.5	35–45	25.3 (19.1–31.5)	14.7 (10.3–19.1)
pH	785	7.43	7.39–7.47	7.37–7.43	10.5 (6.9–14.2)	43.9 (36.9–50.9)
Bicarbonate (mmol/L)	762	25.7	23.1–28.5	M: 22.2–28.3 F: 21.2–27.0	M: 14.6 (8.4–20.8) F: 7.3 (0.7–13.9)	M: 18.4 (12.3–24.5) F: 12.4 (4.0–20.8)
Lactate (mmol/L)	715	1.2	0.9–1.5	0.5–1.6	0.7 (0–1.5)	15.6 (11.0–20.2)

<b>Liver, pancreas</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
ASAT (U/L)	492	60.5	36–95	13–40	0.8 (0–2.0)	47.0 (39.3–54.6)
ALAT (U/L)	491	54	31–98	7–40	0 (—)	43.1 (35.4–50.8)
Bilirubin, total (mg/L)	466	6.4	4.7–9.9	2.0–11.0	1.3 (0–3.1)	12.4 (7.5–17.3)
Bilirubin, conjugated (mg/L)	108	11	6.0–18.0	1.0–3.0	0 (—)	60.9 (53.1–68.7)
Alkaline phosphatase (U/L)	196	69.5	51–102	46–116	7.1 (2.6–11.6)	9.1 (3.9–14.5)
γ-glutamyltransferase (U/L)	161	60	27–164	M: ≤73 F: ≤38	M: Not applicable F: Not applicable	M: 31.4 (18.0–44.7) F: 20.4 (7.4–33.3)
Lipase (U/L)	49	47	36–76	12–53	0 (—)	14.2 (3.0–25.4)
<b>Inflammatory markers</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
C-reactive protein (mg/L)	388	80.5	30.5–147.2	≤10	Not applicable	60.4 (52.6–68.2)
Procalcitonin (ng/ml)	94	0.2	0.09–0.91	≤0.05	Not applicable	20.7 (11.1–30.3)
Interleukin 6 (pg/mL)	16	95.47	59.8–223.9	≤6.4	Not applicable	Low sample size
<b>Iron markers</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
Ferritin (μg/L)	107	1358	685–2281	M: 22–322 F: 10–291	Low sample size	Low sample size
Iron-binding capacity, saturation	12	12.0	6.0–16.5	20–40	Low sample size	Low sample size
Iron (mg/L)	12	0.23	0.14–0.34	M: 0.65–1.75 F: 0.5–1.7	Low sample size	Low sample size
Transferrin (g/L)	12	1.40	1.20–1.75	M: 2.15–3.65 F: 2.5–3.8	Low sample size	Low sample size
<b>Cardiac markers</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
hs-c Troponin I (pg/mL)	255	21.0	7.5–80.7	M: ≤24 F: ≤12	Not applicable	M: 36.5 (25.9–47.2) F: 20.8 (7.2–34.4)
NT-proBNP (pg/mL)	232	589	143–2459	<75 y: <125 >75 y: <450	Not applicable	<75 y: 39.5 (28.0–51.1) >75 y: 50.5 (34.0–67.0)
<b>Muscle markers</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>

LDH (U/L)	155	373	285–444	120–246	0 (—)	33.0 (23.4–42.6)
Creatine kinase (U/L)	272	148	69–434	M: 46–171 F: 34–145	M: 6.3 (1.7–10.9) F: 12.9 (0.6–25.2)	M: 36.7 (26.2–47.2) F: 14.3 (2.8–25.8)
<b>Nutritional markers</b>	<b>N</b>	<b>Median</b>	<b>IQR, 25<sup>th</sup> – 75<sup>th</sup></b>	<b>Reference values*</b>	<b>% of time, &lt; lower limit</b>	<b>% of time, &gt; upper limit</b>
Proteins, total (g/L)	744	60	55–65	57–82	21.4 (16.0–26.7)	0 (—)
Albumin (g/L)	134	27.2	23.2–31.0	35–52	30.3 (21.2–39.5)	0 (—)
Prealbumin (g/L)	51	0.06	0.05–0.14	0.1–0.4	Low sample size	Low sample size
Glucose (g/L)	625	1.23	1.03–1.60	0.74–1.06	Not relevant	Not relevant
Triglycerides (g/L)	133	1.87	1.4–2.4	<1.5	Not applicable	42.8 (29.5–56.2)
Cholesterol (g/L)	16	1.26	1.00–1.60	Desirable, <2	Not applicable	Low sample size

1 Note. IQR: interquartile range; M: males; F: females; ASAT: aspartate aminotransferases; ALAT: alanine aminotransferases; FENa: fractional  
2 excretion of sodium; FEK: fractional excretion of potassium; FECl: fractional excretion of chloride; hs-c Troponin I: high-sensitivity cardiac  
3 troponin I; LDH: lactate dehydrogenase; NT-proBNP: N-Terminal pro-Brain Natriuretic Peptide; PO<sub>2</sub>: partial pressure of oxygen; PCO<sub>2</sub>: partial  
4 pressure of carbon dioxide.

5 \* The reference values are adapted to an adult population.

1 **Table 3. Biochemical markers associated with the occurrence of acute respiratory failure among patients with severe COVID-19 disease**  
 2 **in bivariate analyses**

Biological variable	n	ROC, <i>P</i> -value*	AUROC*	ROC- defined cut-off	Time- series analysis <i>P</i> -value†	Percentage of time according to the threshold (95% CI), No ARF	Percentage of time according to the threshold, (95% CI), ARF
Sodium (mmol/L)	854	0.68	0.509 (0.466–0.554)	—	—	—	—
Potassium (mmol/L)	854	<0.001‡	0.588 (0.544–0.632)	>3.7	<0.001	43.1 (32.9–53.4)	70.1 (62.6–77.6)
Chloride (mmol/L)	799	<0.001‡	0.589 (0.539–0.635)	≤104	<0.001	27.0 (17.2–36.9)	45.2 (36.3–54.2)
Urea nitrogen (g/L)	844	<0.001‡	0.729 (0.687–0.767)	>0.36	<0.001	25.1 (15.3–34.8)	62.3 (52.8–71.9)
Creatinine (mg/L)	845	<0.001‡	0.610 (0.566–0.650)	>9.7	0.002	18.8 (9.9–27.6)	34.7 (25.2–44.3)
Hemoglobin (g/dL)	787	<0.001‡	0.706 (0.644–0.758)	≤12.7	<0.001	18.5 (7.9–29.1)	56.6 (47.6–65.6)
pH	785	<0.001‡	0.643 (0.584–0.701)	≤7.41	<0.001	7.9 (0.1–15.7)	32.1 (24.6–39.5)
pO <sub>2</sub> (mm/Hg)	785	<0.001‡	0.643 (0.581–0.699)	>80.8	<0.001	14.5 (5.4–23.6)	43.7 (36.6–50.7)
pCO <sub>2</sub> (mmHg)	785	<0.001‡	0.701 (0.642–0.753)	>38.8	<0.001	9.0 (0.8–17.3)	43.9 (34.7–53.2)
Bicarbonate (HCO <sub>3</sub> <sup>-</sup> ) (mmol/L)	762	<0.001‡	0.640 (0.590–0.688)	>26.6	<0.001	7.2 (0–14.4)	34.4 (25.7–43)
Lactates (mmol/L)	715	0.58	0.521 (0.446–0.594)	—	—	—	—
ASAT (U/L)	492	<0.001‡	0.740 (0.682–0.791)	>48	<0.001	18.1 (8.6–27.5)	59.2 (49.2–69.1)
ALAT (U/L)	491	<0.001‡	0.676 (0.614–0.734)	>38	<0.001	21.8 (11.2–32.5)	63.2 (53–73.4)
Bilirubin, total (mg/L)	466	0.70	0.512 (0.453–0.571)	—	—	—	—
Total proteins (g/L)	744	<0.001‡	0.687 (0.639–0.733)	≤61	<0.001	15.9 (8.1–23.8)	57.8 (48.8–66.7)
C-reactive protein (mg/L)	388	<0.001‡	0.761 (0.709–0.807)	>90	<0.001	10.8 (4.2–17.5)	49.5 (37.9–61.1)
Calcium (mg/L)	266	<0.001‡	0.825 (0.755–0.876)	≤83.6	<0.001	4.3 (0–10.9)	46.2 (35.1–57.4)
Phosphorus (mg/L)	299	0.20	0.553 (0.471–0.635)	—	—	—	—
hs-c Troponin I (pg/mL)	255	0.14	0.572 (0.474–0.665)	—	—	—	—
CK (U/L)	272	<0.001‡	0.670 (0.591–0.741)	>331	<0.001	2.6 (0–6.8)	28.8 (18.9–38.6)

3 Note. ARF: acute respiratory failure; AUROC: area under the receiver operating characteristic curve; ASAT: aspartate aminotransferases; ALAT:  
 4 alanine aminotransferases; CK: creatine kinase; hs-c Troponin I: high-sensitivity cardiac troponin I; PO<sub>2</sub>: partial pressure of oxygen; PCO<sub>2</sub>:  
 5 partial pressure of carbon dioxide; ROC: receiver operating characteristics.

- 1 \* Receiver operating characteristic (ROC) analysis, according to DeLong *et al.* with Bias-corrected and accelerated (BCa)-bootstrap interval after
- 2 10,000 iterations for the Youden index.
- 3 † Time-series analysis using a non-parametric test.
- 4 ‡ All nominal  $P$ -values maintained their significance after Bonferroni correction.

1 **Table 4. Association between biochemical abnormalities and the occurrence of acute respiratory failure or in-hospital mortality among**  
 2 **patients with severe COVID-19 in the multivariable multilevel analysis**

<i>Acute respiratory failure, model #1: Urea nitrogen, C-reactive protein, age, gender, medical history*</i>				
Variables	Estimation	Standard error	Odds ratio, adjusted (95% CI)	P-value†
<b>C-reactive protein &gt; 90 mg/L</b>	<b>1.93</b>	<b>0.55</b>	<b>6.87 (2.36–20.01)</b>	<b>&lt;0.001</b>
<b>Medical history of type 2 diabetes</b>	<b>1.50</b>	<b>0.73</b>	<b>4.49 (1.07–18.89)</b>	<b>0.04</b>
<b>Urea nitrogen &gt; 0.36 g/L</b>	<b>1.36</b>	<b>0.63</b>	<b>3.91 (1.15–13.29)</b>	<b>0.03</b>
Age	0.03	0.02	1.03 (0.99–1.07)	0.18
Male gender	0.92	0.67	2.51 (0.68–9.24)	0.17
Medical history of dyslipidemia	0.86	0.82	2.36 (0.47–11.78)	0.30
<i>Acute respiratory failure, model #2: Creatinine, C-reactive protein, age, gender, medical history*</i>				
Variable	Estimation	Standard error	Odds ratio, adjusted (95% CI)	P-value†
<b>C-reactive protein &gt; 90 mg/L</b>	<b>1.93</b>	<b>0.54</b>	<b>6.90 (2.38–20.01)</b>	<b>&lt;0.001</b>
<b>Medical history of type 2 diabetes</b>	<b>1.45</b>	<b>0.73</b>	<b>4.26 (1.02–17.90)</b>	<b>0.049</b>
Male gender	1.15	0.67	3.16 (0.86–11.69)	0.09
Age	0.01	0.02	1.01 (0.98–1.05)	0.52
Creatinine >9.7 mg/L	0.33	0.60	1.39 (0.43–4.49)	0.58
Medical history of dyslipidemia	0.71	0.81	2.04 (0.41–10.00)	0.38
<i>In-hospital mortality, Model: Urea nitrogen, age, gender, medical history</i>				
Variable	Estimation	Standard error	Odds ratio, adjusted (95% CI)	P-value†
<b>Age</b>	<b>0.12</b>	<b>0.04</b>	<b>1.12 (1.03–1.22)</b>	<b>0.006</b>
<b>Medical history of COPD</b>	<b>2.64</b>	<b>1.31</b>	<b>14.06 (1.08–182.74)</b>	<b>0.04</b>
Male gender	1.90	1.06	6.70 (0.83–53.87)	0.07
Urea nitrogen >0.42 g/L	1.29	0.84	3.64 (0.71–18.71)	0.12
Medical history of type 2 diabetes	1.35	0.97	3.85 (0.57–25.97)	0.17
Medical history of cardiovascular disease	1.04	0.98	2.82 (0.41–19.31)	0.29

- 1 Note. COPD: chronic obstructive pulmonary disease; 95% CI: 95% confidence interval.
- 2 \* Patients' medical histories were selected to avoid multicollinearity regarding the endpoint.
- 3 † Two-level hierarchical logistic model (HLM), using the predictive quasi-likelihood method.

1 **Table 5. Biochemical markers associated with in-hospital mortality among patients with severe COVID-19 disease in bivariate analyses**

Biological variable	n	ROC, <i>P</i> -value*	AUROC*	ROC-defined cut-off	Time-series analysis <i>P</i> -value†	Percentage of time according to the threshold (95% CI), No death	Percentage of time according to the threshold (95% CI), Death
Sodium (mmol/L)	854	0.77	0.510 (0.444–0.577)	—	—	—	—
Potassium (mmol/L)	854	0.07‡	0.557 (0.494–0.618)	—	—	—	—
Chloride (mmol/L)	799	0.85	0.506 (0.444–0.569)	—	—	—	—
Urea nitrogen (g/L)	844	<0.001	0.597 (0.540–0.651)	>0.42	0.002	31.1 (23.8–38.3)	60.8 (41.2–80.4)
Creatinine (mg/L)	845	0.25	0.541 (0.468–0.611)	—	—	—	—
Hemoglobin (g/dL)	787	0.11	0.557 (0.488–0.626)	—	—	—	—
pH	785	<0.001	0.683 (0.618–0.743)	≤7.43	0.24	30.1 (23.2–36.9)	44.9 (23–66.8)
PO <sub>2</sub> (mm/Hg)	785	0.31	0.539 (0.464–0.615)	—	—	—	—
PCO <sub>2</sub> (mmHg)	785	0.04‡	0.585 (0.501–0.663)	—	—	—	—
Bicarbonate (HCO <sub>3</sub> <sup>-</sup> ) (mmol/L)	762	0.10	0.570 (0.483–0.652)	—	—	—	—
Lactates (mmol/L)	715	<0.001	0.677 (0.606–0.738)	>1.3	0.27	25.7 (18.6–32.8)	37.7 (16.2–59.1)
ASAT (U/L)	492	0.28	0.546 (0.461–0.630)	—	—	—	—
ALAT (U/L)	491	0.03‡	0.587 (0.504–0.663)	—	—	—	—
Bilirubin, total (mg/L)	466	0.53	0.527 (0.440–0.609)	—	—	—	—
Total proteins (g/L)	744	0.002	0.614 (0.541–0.686)	≤56	0.44	16.9 (11.5–22.2)	26.6 (6.8–46.4)
C-reactive protein (mg/L)	388	0.06‡	0.580 (0.491–0.658)	—	—	—	—
Calcium (mg/L)	266	0.54	0.537 (0.416–0.654)	—	—	—	—
Phosphorus (mg/L)	299	0.045‡	0.606 (0.497–0.705)	—	—	—	—
hs-c Troponin I (pg/mL)	255	<0.001	0.737 (0.613–0.833)	>40	0.90	18.2 (10.4–25.9)	18.3 (0–45.5)
CK (U/L)	272	0.03‡	0.617 (0.508–0.714)	—	—	—	—

2 Note. ALAT: alanine aminotransferases; ASAT: aspartate aminotransferases; AUROC: area under the receiver operating characteristic curve;  
3 CK: creatine kinase; hs-c Troponin I: high-sensitivity cardiac troponin I; PCO<sub>2</sub>: partial pressure of carbon dioxide; PO<sub>2</sub>: partial pressure of  
4 oxygen; ROC: receiver operating characteristics.

- 1 \* Receiver operating characteristic (ROC) analysis, according to DeLong *et al.* with Bias-corrected and accelerated (BCa)-bootstrap interval after
- 2 10,000 iterations for the Youden index.
- 3 † Time-series analysis using a non-parametric test.
- 4 ‡ Nominal  $P$ -values did not maintain their significance after Bonferroni correction.

1 **FIGURES LEGENDS**

2 **Figure 1. Kinetics over time of the most frequently observed biochemical abnormalities**  
3 **during the follow-up of patients with severe COVID-19 (part 1):** **A:** urea nitrogen (g/L);  
4 **B:** creatinine (mg/L); **C:** osmolality, calculated (mOsmol/kg); **D:** phosphorus (mg/L); **E:**  
5 calcium (mg/L); **F:** albumin (g/L); **G:** triglycerides (g/L); and **H:** C-reactive protein (mg/L).  
6 The dashed lines correspond to the upper (U) and lower (L) limits of the reference range. The  
7 red line indicates the evolution trend of the biomarker during follow-up according to the  
8 isotonic regression method.

9  
10 **Figure 2. Kinetics over time of the most frequently observed biochemical abnormalities**  
11 **during the follow-up of patients with severe COVID-19 (part 2):** **A:** hemoglobin (g/dL);  
12 **B:** partial pressure of oxygen (PO<sub>2</sub>) (mmHg); **C:** high-sensitivity cardiac troponin I (hs-c  
13 Troponin I) (pg/mL); **D:** hs-c Troponin I (view limited to hs-c Troponin I <400 pg/mL); **E:** N-  
14 Terminal pro-Brain Natriuretic Peptide (NT-proBNP) (pg/mL); **F:** NT-proBNP (view limited  
15 to NT-proBNP <1000 pg/mL); **G:** CK (view limited to CK < 2000 U/L); **H:** lactate  
16 dehydrogenase (LDH) (U/L). The dashed lines correspond to the upper (U) and lower (L)  
17 limits of the reference range. The red line indicates the evolution trend of the biomarker  
18 during follow-up according to the isotonic regression method.

19  
20 **Figure 3. Kinetics over time of the most frequently observed biochemical abnormalities**  
21 **during the follow-up of patients with severe COVID-19 (part 3):** **A:** aspartate  
22 aminotransferases (ASAT) (U/L); **B:** ASAT (U/L) (view limited to ASAT <400 U/L); **C:**  
23 alanine aminotransferases (ALAT) (U/L); **D:** ALAT (U/L) (view limited to ALAT <400 U/L);  
24 **E:** bilirubin, total (mg/L); **F:** bilirubin, conjugated (mg/L); **G:** alkaline phosphatase (UI/L); **H:**

1  $\gamma$ -glutamyltransferase (U/L). The red line indicates the evolution trend of the biomarker  
2 during follow-up according to the isotonic regression method.

3

4 **Figure 4.** Biochemical abnormalities associated with the occurrence of acute respiratory  
5 failure (ARF) among patients with severe COVID-19. A set of 20 biochemical markers with  
6 at least 250 iterations was systematically screened for the association with the occurrence of  
7 ARF using ROC analysis. All markers with a statistically significant ROC-defined threshold  
8 were assessed using time series analysis to calculate the percentage of time below or above  
9 the defined threshold. The calculated summary effects are reported as percentages of the total  
10 time of observation with the 95% CI, as noted in the Forest plot.

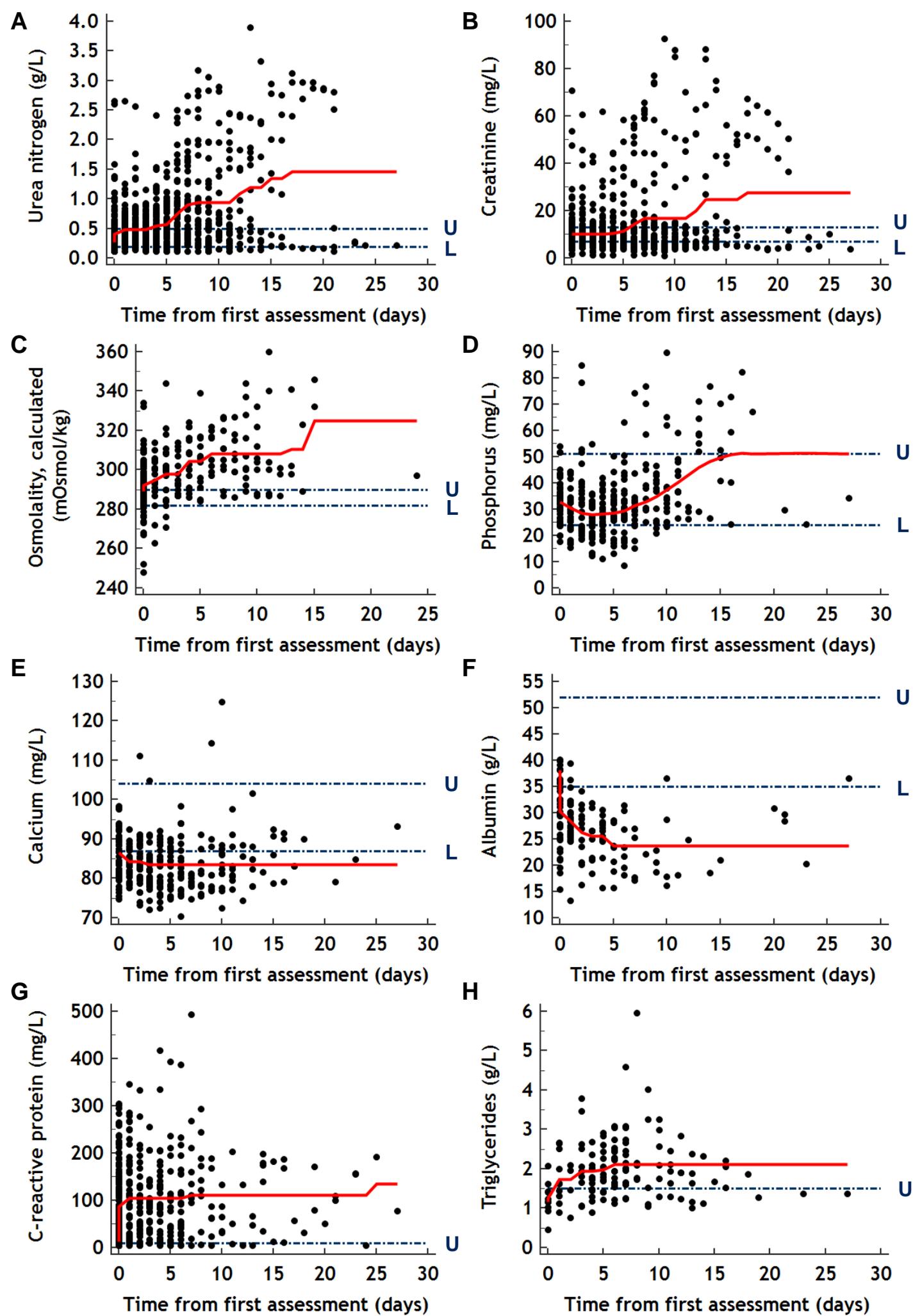
11

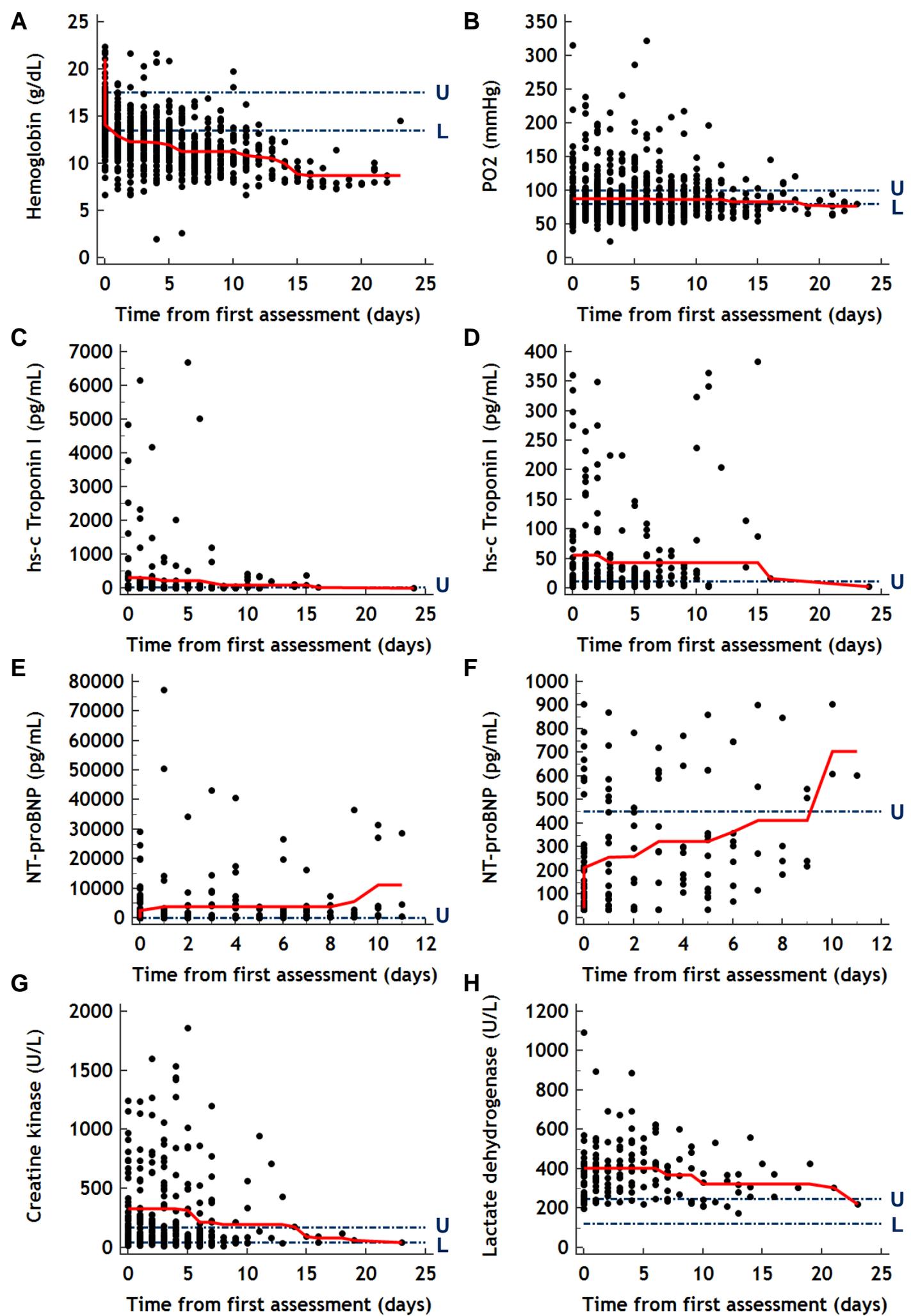
12 **Figure 5.** Biochemical abnormalities associated with the occurrence of in-hospital mortality  
13 among patients with severe COVID-19. A set of 20 biochemical markers with at least 250  
14 iterations was systematically screened for the association with the occurrence of in-hospital  
15 mortality using ROC analysis. All markers with a statistically significant ROC-defined  
16 threshold were assessed using time series analysis to calculate the percentage of time below or  
17 above the defined threshold. The calculated summary effects are reported as percentages of  
18 the total time of observation with the 95% CI, as noted in the Forest plot.

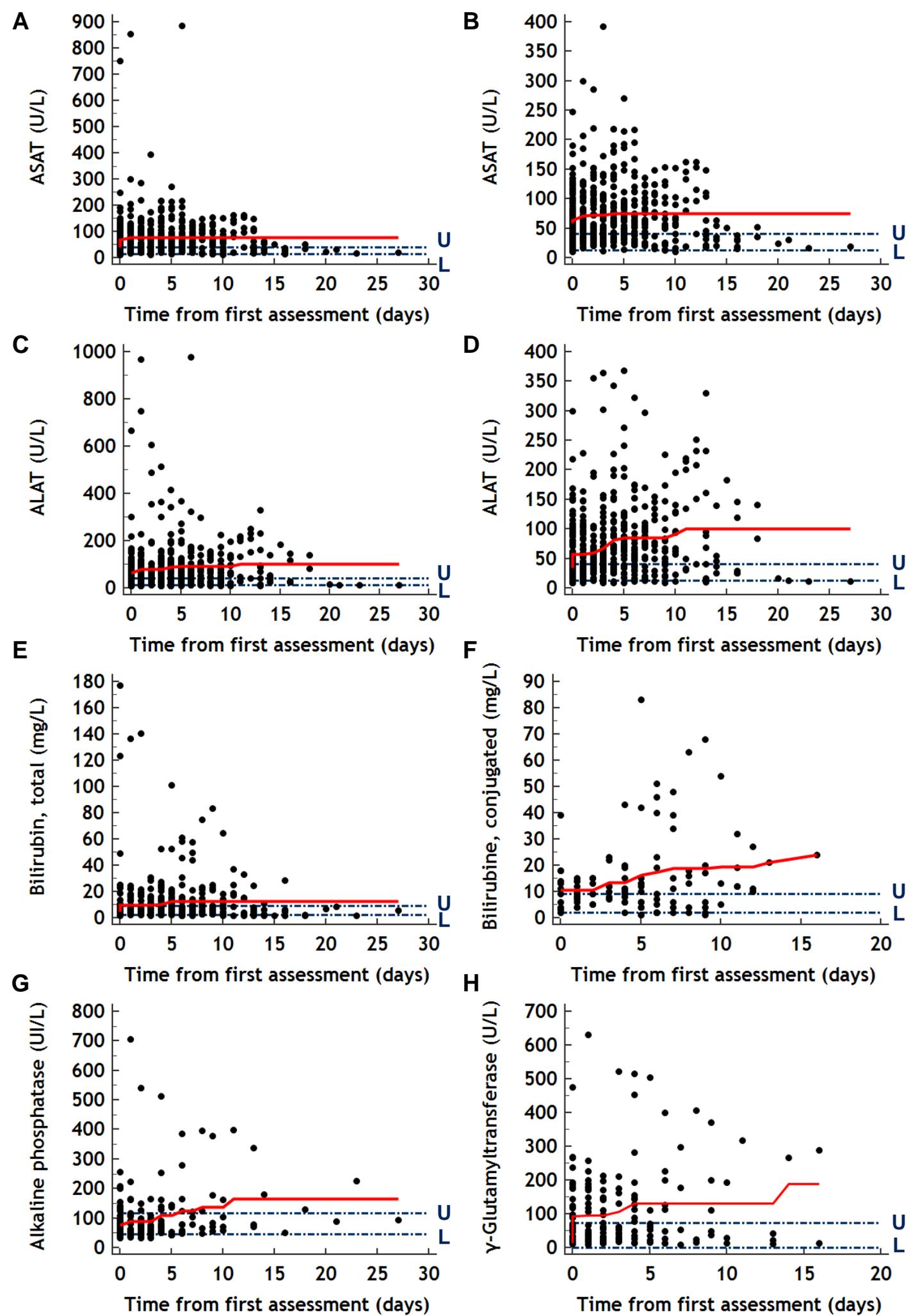
19

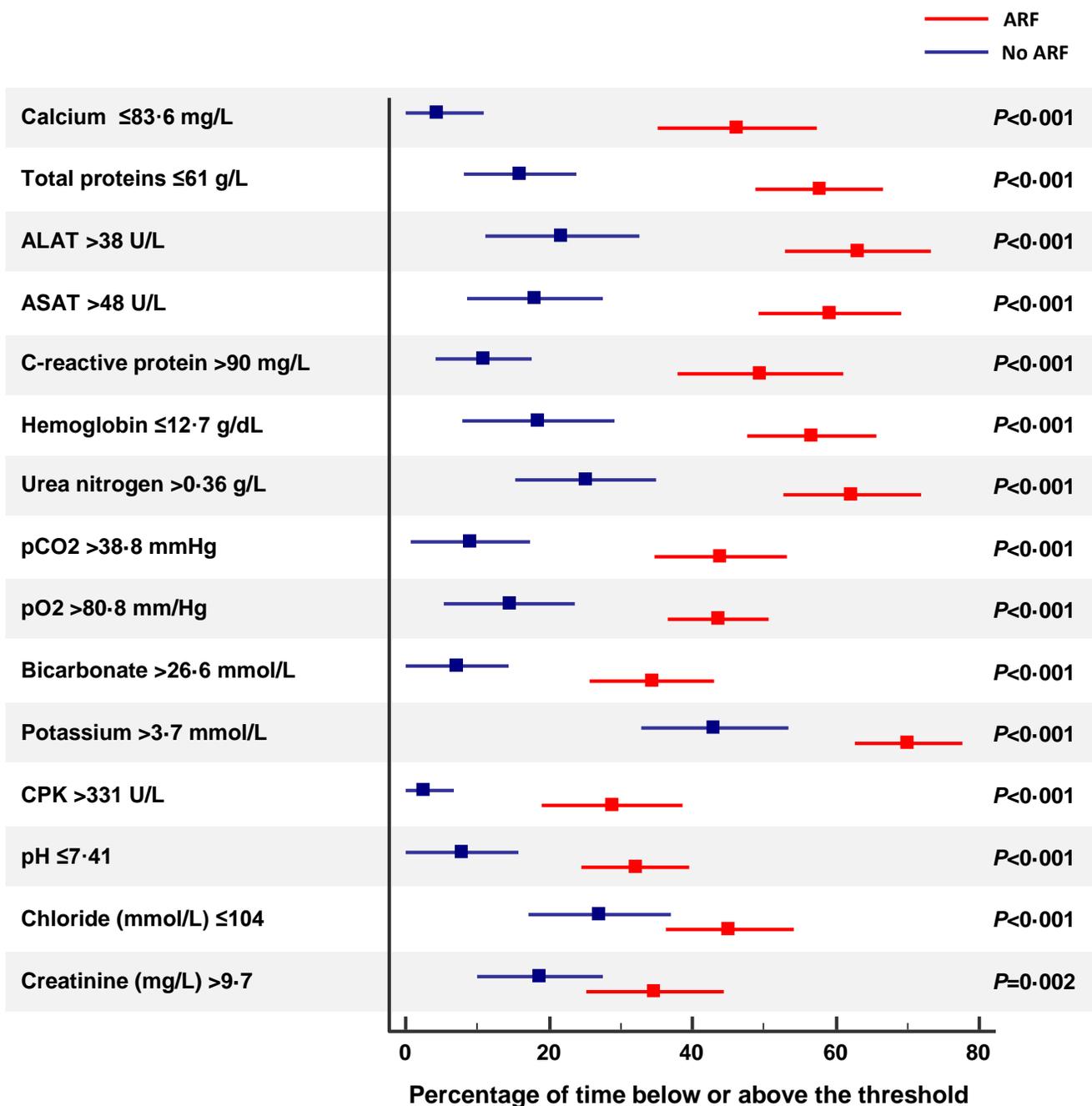
20 **Figure 6.** Overview of the evolution of the main biochemical abnormalities and potential  
21 predictors acute respiratory failure (ARF) or in-hospital mortality among patients with severe  
22 COVID-19. Predictors of ARF in bivariate analyses are highlighted in blue font. Predictors of  
23 COVID-19 related mortality in bivariate analyses are highlighted in red font. Independent  
24 predictors of ARF in the multivariable multilevel analysis are underlined. ALP: alkaline  
25 phosphatase; ASAT: aspartate aminotransferases; ALAT: alanine aminotransferases; CK:

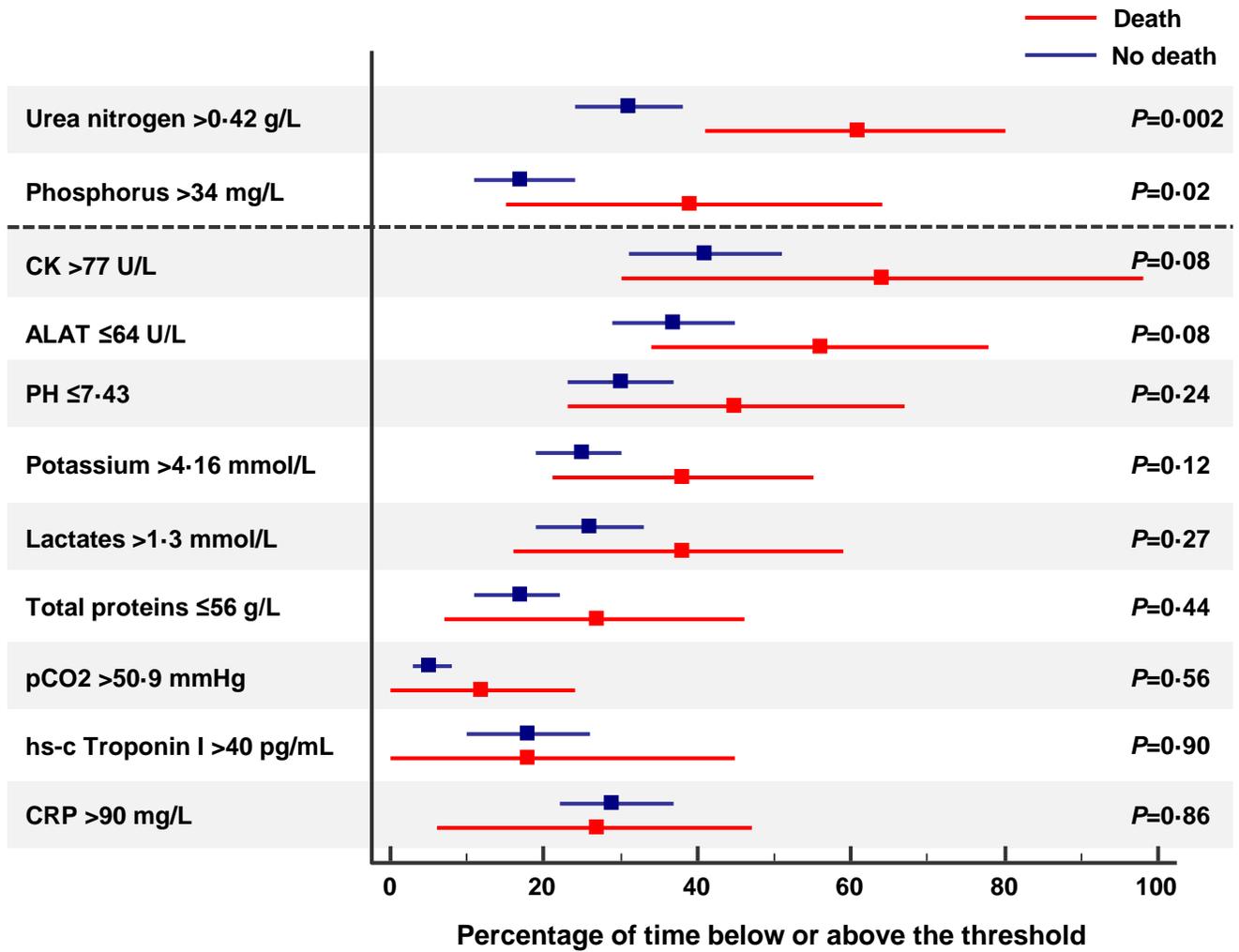
- 1 creatine kinase; CRP: C-reactive protein; GGT:  $\gamma$ -glutamyltransferase; hs-c Troponin I: high-
- 2 sensitivity cardiac troponin I; IL-6: interleukin 6; LDH: lactate dehydrogenase; NT-pro-BNP:
- 3 N-Terminal pro-Brain Natriuretic Peptide; PCT: procalcitonin; (icons made by flaticon,
- 4 flaticon.com; CC-BY-3.0).











# Severe COVID-19 disease: multi-organ involvement

Blue font: Factors associated with ARF  
 Red font: Factors associated with mortality

- Major increase in hs-c Troponin I
- Major increase in NT-proBNP

