The spectrum of biochemical alterations associated with organ dysfunction and inflammatory status and their association with disease outcomes in severe COVID-19: A longitudinal cohort and time-series design study

Abderrahim Oussalah, Stanislas Gleye, Isabelle Clerc Urmes, Elodie Laugel, Françoise Barbe, Sophie Orlowski, Catherine Malaplate, Isabelle Aimone-Gastin, Beatrice Maatem Cailliere, Marc Merten, Elise Jeannesson, Raphael Kormann, Jean-Luc Olivier, Rosa-Maria Rodriguez-Guéant, Farès Namour, Sybille Bevilacqua, Nathalie Thilly, Marie-Reine Losserh, Antoine Kimmoun, Luc Frimat, Raphaël Kormann, Jean-Louis Guéant.

ARTICLE INFO
Article History:
Received 9 June 2020
Revised 2 September 2020
Accepted 7 September 2020
Available online 20 September 2020

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

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

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

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

© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
In patients with severe COVID-19, no data are available on the longitudinal follow-up of organ dysfunction and inflammation by assessing the kinetics of biochemical biomarkers using a time-series design. Furthermore, even if some studies have evaluated the risk of COVID-19 related complications using a small set of biological markers at baseline, no study has evaluated, on a wide range of biochemical markers, the spectrum and the magnitude of biochemical disturbances, as well as their kinetics over time and their potential association with disease outcomes in patients with severe COVID-19. Using a big-data approach and multilevel modeling adapted for repeated measures on a well-phenotyped retrospective cohort of consecutive patients with newly diagnosed severe COVID-19, we were able to assess the occurrence of organ dysfunction (kidney, lung, heart, liver, muscle) and the severity of the inflammatory reaction as evaluated by biomarker kinetics in hospitalized patients. We assessed the association of the more representative biochemical markers with the risk of occurrence of ARF and death.

2. Methods

2.1. Study design, setting, and patients' inclusion criteria

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

2.2. Description of the Nancy biochemical database

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

2.3. Data collected for the study

The following data were available in the Nancy Biochemical Database: patient identification number, patient’s age at hospital...
admission, date and time of blood sampling, patient health care department. A total of 59 biochemical markers were available, with 46 in the blood and 13 in the urine. The biochemical markers available in blood were: electrolytes, renal markers: sodium (mmol/L), potassium (mmol/L), chloride (mmol/L), creatinine (mg/L), urea nitrogen (g/L), phosphorus (mg/L), calcium (mg/L), magnesium (mg/L), calculated and measured (mM/mL/kg), uric acid (mg/L), phosphorus reabsorption rate (%), fractional excretion of sodium (%), fractional excretion of potassium (%), fractional excretion of chloride (%), and anionic gap (mmol/L); blood gas: hemoglobin (g/dL), partial pressure of oxygen (PO₂) (mmHg), partial pressure of carbon dioxide (PCO₂) (mmHg), pH, bicarbonate (HCO₃⁻) (mM/mL), and lactate (mmol/L); liver, pancreas: aspartate aminotransferases (AST (U/L), alanine aminotransferases (ALT (U/L), total bilirubin (mg/L), conjugated bilirubin (mg/L), alkaline phosphatase (U/L), γ-glutamyltransferase (U/L), and lipase (U/L); inflammatory markers: C-reactive protein (CRP) (mg/L), procalcitonin (ng/mL), and interleukin 6 (pg/mL); iron markers: ferritin (μg/L), iron-binding capacity saturation (%), iron (mg/L), and transferrin (g/L); cardiac markers: high-sensitivity cardiac troponin 1 (hs-c Troponin 1) (pg/mL), N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) (pg/mL); muscle markers: lactate dehydrogenase (U/L), creatine kinase (U/L); nutritional markers: total protein (g/L), albumin (g/L), prealbumin (g/L), glucose (g/L), triglycerides (g/L), and cholesterol (g/L). The biochemical markers available in the urine were: sodium (mmol/L), potassium (mmol/L), chloride, uric acid (mg/L), creatinine (mg/L), urea nitrogen (g/L), glucose (g/L), proteins (g/L), anion gap (mmol/L), osmolality (mosmol/kg), phosphorus (mg/L), calcium (mg/L), and microalbumin (mg/L). The following clinical data were available: date of hospital admission; patient's medical history (hypertension, type 2 diabetes, cardiovascular disease, vascular disease, dyslipidemia, chronic obstructive pulmonary disease, obstructive sleep apnea syndrome, asthma, cancer, and chronic kidney disease); patient's outcome during the hospitalization for the management of COVID-19 (acute respiratory failure [ARF] as defined by the treating physicians [15-17], intubation with mechanical ventilation, pulmonary embolism, and in-hospital mortality related to the COVID-19, defined as the occurrence of death in relation with a complication of the COVID-19 [18]).

2.4. Molecular detection of SARS-CoV-2

After viral RNA extraction from nasopharyngeal swabs, the procedure applied for the detection of SARS-CoV-2 genome was based on primers and probes designed to target the RdRp gene spanning nucleotides 12,561–12,727 and 14,010–14,116 (positions according to SARS-CoV, NC_004718, National Reference Center for Respiratory Viruses, Pasteur Institute, Paris, France; sequences available on request). Each real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) included negative and positive samples (in vitro synthesized RNA transcripts). The RT-PCR assay sensitivity was around 100 copies of RNA genome equivalent. Regarding the specificity of the test, none of the tested respiratory viruses showed reactivity to influenza A-B, respiratory syncytial virus, bocavirus (hBoV), metapneumovirus (hMPPV), HRV/enterovirus, adenovirus, and other coronaviruses (hCoV: KU1, OC43, 229E, and NL63). All the virological diagnoses of SARS-CoV-2 infection were carried out at the Division of Virology of the University Hospital of Nancy, which served as one of the two regional reference centers for the SARS-CoV-2 diagnosis in North East of France.

2.5. Biochemistry assays

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

2.6. Standards of care of patients with suspected COVID-19 during the study period at the university hospital of Nancy

At the University Hospital of Nancy, during March 2020, the recommended best standard of care for suspected COVID-19 patients requiring hospital admission for non-critical respiratory failure included a systematic diagnostic workup with a clinical and biological assessment, molecular detection of SARS-CoV-2 by real-time reverse transcriptase-polymerase chain reaction (RT-qPCR), blood and sputum cultures, and chest CT-scan. Patients were managed according to National Health Authority guidelines, including the following items: i) oxygen delivery to maintain peripheral capillary oxygen saturation (SpO₂) > 90%, ii) 48 h of probabilistic antibiotic therapy combining cefotaxime with spiramycin awaiting SARS-CoV-2 RT-qPCR result and bacteriological assessment; iii) therapeutic dose of antithrombotic therapy using low molecular weight heparin or unfractionated heparin in case of renal failure. All other aspects related to the standards of care and patients’ monitoring are detailed in Supplementary Methods.

2.7. Study aims and endpoints

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

2.8. Statistical analyses

All quantitative variables are shown as the median and interquartile range (IQR, 25–75th percentile) and qualitative variables as percentages and 95% confidence interval (95% CI). The kinetics over time of the studied biochemical markers was modeled using the isotonic regression method, which was estimated using the pool adjacent violators algorithm [20,21]. The pool adjacent violators algorithm is a linear time algorithm for linear ordering isotonic regression [21]. Using a multistep approach, we evaluated a set of 20 biochemical markers with a sufficiently high number of iterations (n > 250, study power analysis reported in Supplementary Methods) to assess the relationship between their variation over time and the occurrence of the endpoint (ARF, death). In step #1, for each biochemical variable, we assessed the optimal threshold using receiver operating characteristic (ROC) analysis, according to DeLong et al. [22]. The classification variable used in the ROC analysis was the studied endpoint. The optimal diagnostic cut-off was defined using the Youden index J. Bias-corrected and accelerated (BCα)-bootstrap interval after 10,000 iterations for the Youden index and its associated values were performed [23]. We used Bonferroni’s correction to account for multiple comparisons. In step #2, all the variables that were significantly associated with the studied endpoint in ROC analyses were assessed using time-series analysis [24]. The evolution times were calculated from the first day of biochemical assessment and were expressed in days.
The time-series analysis aimed to compare the percentage of time below or above the ROC-defined threshold between two patients’ subgroups according to the presence or absence of the studied endpoint. The calculated summary effects were reported as percentages of the total time of observation with the 95% confidence interval (95% CI). Normality testing was performed using the D’Agostino-Pearson test. Subgroups comparison regarding the percentage of time below or above the prespecified threshold was carried out using the Student’s t-test or the Mann-Whitney U test according to the parametric or nonparametric distribution of the variables, respectively. In step #3, we looked for independent predictors using multivariable multilevel analysis. All the predictors that were still significant at the step #2 were assessed using a two-level hierarchical logistic model (HLM), which enabled to take into account the correlation between all the studied biochemical markers, which correspond to the level 1 of the HLM, and the patient-level characteristics, which correspond to the level 2 of the HLM (i.e., age, gender, patient’s medical history) [25]. For each endpoint of interest, a multivariable model was then constructed following the modeling strategy recommended for HLM [26]. We estimated the empty model without level 1 or level 2 variables to confirm the relevance of using the HLM assumption, which is based on a median odds ratio >> 1, considering a variable number of iterations between each patient [27]. Each biochemical variable (level 1, HLM) was tested for the association with the endpoint using bivariate analyses at a 0.2 threshold. A stepwise backward multivariable selection procedure was used to retain the variables associated with the endpoint at a 0.05 threshold, without adjustment for patient-level characteristics (level 2, HLM). Random slopes on the significant biochemical variables retained in the HLM model were tested for their association with the endpoint, considering the between-patient variability. While alternative procedures exist to build models, the HLM, eligible patient characteristics (level 2) on collinearity testing for HLM specification, and cross-level interactions, i.e., interactions between time measurement and patients variables, were tested in the full model (level 1 and level 2). The HLM model was estimated using the predictive quasi-likelihood method. For each variable retained in the full HLM model, summary measures were reported as odds ratio (OR), 95% CI, and the associated P-value. Statistical analyses other than the multivariable model analysis were performed using MedCalc for Windows, version 19.1 (MedCalc Software, Ostend, Belgium) based on a two-sided type I error with an alpha level of 0.05. The multivariate model analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

2.9. Role of funding source

This research received no specific grant from any funding agency.

3. Results

3.1. Study population, database structure, and main biochemical abnormalities observed in severe COVID-19

Between March 1, 2020, and March 25, 2020, 162 patients were admitted to one of the healthcare departments of the University Hospital of Nancy for severe COVID-19 (Supplementary Table 1). The median age was 66 (IQR: 56 to 77) years, and the proportion of males was 59% (Table 1). No patient received hydroxychloroquine or remdesivir. During the study period, 1151 biochemical explorations were carried out for up to 59 biochemical markers (46 in the blood and 13 in the urine), totaling 15,260 biochemical values. The distribution of the 59 biochemical markers is reported in Table 2 (blood) and Supplementary Table 2 (urine). The kinetics over time of the biochemical abnormalities is reported in Figs. 1, 2 and 3 and Supplementary Figs. 2–4 for blood parameters and in Supplementary Fig. 5 for urine parameters. The most frequently observed biochemical abnormalities during the follow-up were (≥25% of the observed time): increased urea nitrogen, hyperosmolality, hypocalcemia, low hemoglobin, hypoxemia; major cytology with severe cholestatic syndromes and conjugated hyperbilirubinemia; increased C-reactive protein (CRP); increased cardiac (high-sensitivity cardiac troponin I, NT-proBNP: N-Terminal pro-Brain Natriuretic Peptide) and muscle (lactate dehydrogenase, creatine kinase) markers; and hypalbuminemia with hypertriglyceridemia (Table 2 and Figs. 1, 2 and 3). The evolution of phosphorus followed a biphasic curve with a decrease during the first four days, followed by a progressive increase in parallel with urea nitrogen and creatinine. The proportion of patients who developed an acute kidney injury (AKI) stage 3, according to the AKI classification, increased significantly over time (0.9%, day 0; 13.6%, day 7; and 21.4%, day 14; P = 0.001) (Supplementary Fig. 6 and Supplementary Table 3). Other biochemical abnormalities (10–24% of the observed time) included hypernatremia, hyperkalemia, hypercholesterolemia, increased creatinine, hypoproteinemia, hyperlactatemia, hyperlipasemia, and increased procalcitonin (Table 2 and Supplementary Figs. 2–4).

3.2. Factors associated with an occurrence of acute respiratory failure related to severe COVID-19

On the 20 biochemical variables assessed in ROC analysis, 15 were significantly associated with the risk of ARF after Bonferroni correction (Table 3 and Supplementary Fig. 7). All the 15 dichotomized variables differed significantly in time-series analyses between patients with or without ARF, with a significantly higher proportion of time with the biochemical marker above or under the ROC-defined threshold among patients with ARF when compared to patients without ARF (Table 3 and Fig. 4). These markers included electrolyte and blood gas disturbances, increased renal markers, liver and muscle cytology, severe inflammatory syndrome, and hypoproteinemia. In the multivariable multilevel analysis, three variables were independently associated with the risk of ARF, namely: CRP > 50 mg/L (OR, 6.87 [95% CI, 2.36–20.01]; P < 0.001), urea nitrogen > 0.36 g/L (OR, 3.91 [95% CI, 1.15–13.29]; P = 0.03), and the medical history of type

### Table 1

Characteristics of the patients with severe COVID-19.

| Demographics | | |
| --- | --- | --- |
| Age (years) – N, median (IQR) | 162 | 66 (56–77) |
| Male gender – n/N, (%95% CI) | 96/162 | 59% (52–67) |

| Patients’ medical history – n/N, (%95% CI) | |
| --- | --- |
| 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) |

### Table 2

Demographics

| Outcomes – n/N, (%95% CI) | |
| --- | --- |
| Acute respiratory failure | 76/149 | 51% (43–59) |
| Intubation and mechanical ventilation | 54/149 | 36% (28–44) |
| COVID-19 related death | 23/149 | 14% (8–20) |
| Pulmonary embolism | 2/149 | 1% (0–3) |

Note. IQR: interquartile range 25th – 75th; N: number of patients; n: number of observations; %: percentage; 95% CI: 95% confidence interval.

### Supplementary Table 1

Demographics

| Characteristics of the patients with severe COVID-19. |
| --- |
| Age (years) | 162 |
| Male gender | 96/162 |

### Supplementary Figs. 2–4

For blood parameters and in Supplementary Fig. 5 for urine parameters. The most frequently observed biochemical abnormalities during the follow-up were (≥25% of the observed time): increased urea nitrogen, hyperosmolality, hypocalcemia, low hemoglobin, hypoxemia; major cytology with severe cholestatic syndromes and conjugated hyperbilirubinemia; increased C-reactive protein (CRP); increased cardiac (high-sensitivity cardiac troponin I, NT-proBNP: N-Terminal pro-Brain Natriuretic Peptide) and muscle (lactate dehydrogenase, creatine kinase) markers; and hypalbuminemia with hypertriglyceridemia (Table 2 and Figs. 1, 2 and 3). The evolution of phosphorus followed a biphasic curve with a decrease during the first four days, followed by a progressive increase in parallel with urea nitrogen and creatinine. The proportion of patients who developed an acute kidney injury (AKI) stage 3, according to the AKI classification, increased significantly over time (0.9%, day 0; 13.6%, day 7; and 21.4%, day 14; P = 0.001) (Supplementary Fig. 6 and Supplementary Table 3). Other biochemical abnormalities (10–24% of the observed time) included hypernatremia, hyperkalemia, hypercholesterolemia, increased creatinine, hypoproteinemia, hyperlactatemia, hyperlipasemia, and increased procalcitonin (Table 2 and Supplementary Figs. 2–4).
Table 2

Distribution of the 46 biochemical markers in patients with severe COVID-19.

| Electrolytes, renal markers | N   | Median | IQR, 25th – 75th | Reference Values$^a$ | % of time, < lower limit | % of time, > upper limit |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| 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             | M: 20.6 (13.7 – 27.5)     | M: 19.0 (12.2 – 26.0)    |
| 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 – 21.7      | 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           | Low sample size          |
| Uric acid (mg/L)            | 82  | 44     | 30 – 62          | M: 37 – 92            | Low sample size           | Low sample size          |

| Blood gas                  |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| Hemoglobin (g/dL)           | 787 | 11.8   | 10.4 – 13.6      | M: 13.5 – 17.5        | M: 50.0 (40.8 – 59.1)    | M: 4.1 (1.6 – 6.6)       |
| PO$_2$ (mmHg)               | 784 | 77.7   | 66.8 – 94.6      | 80 – 100              | 41.3 (35.0 – 47.7)        | 14.0 (10.5 – 17.4)       |
| PCO$_2$ (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 – 28.5        | 22 – 28.3             | M: 16.4 (8.4 – 20.8)      | M: 18.4 (12.3 – 24.5)    |
| 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)       |

| Liver, pancreas             |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| 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            | 13 (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               | M: Not applicable         | M: 31.4 (18.0 – 44.7)    |
| Lipase (U/L)                | 49  | 47     | 36 – 76          | 12 – 53               | 0 (–)                    | 14.2 (3.0 – 25.4)        |

| Inflammatory markers        |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| 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          |

| Iron markers                |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| Ferritin (µg/L)             | 107 | 1358   | 685 – 2281       | M: 22 – 322           | Low sample size           | Low sample size          |
| Iron-binding capacity, saturation (mg/L) | 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          |
| Transferrin (g/L)           | 12  | 2.0    | 1.20 – 1.75      | M: 2.15 – 3.65        | F: 2.5 – 3.8             | Low sample size          |

| Cardiac markers             |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| hs-c Troponin I (pg/mL)     | 255 | 21.0   | 7.5 – 80.7       | M: ≤ 24               | Not applicable            | M: 36.5 (25.9 – 47.2)    |
| NT-proBNP (pg/mL)           | 232 | 589    | 143 – 2459       | > 75 yrs: < 125       | > 75 yrs: Not applicable  | > 75 yrs: Not applicable |

| Muscle markers              |
|-----------------------------|-----|--------|------------------|-----------------------|--------------------------|--------------------------|
| 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           | M: 6.3 (1.7 – 10.9)       | M: 36.7 (26.2 – 47.2)    |

Note: ASAT, Alanine aminotransferase; ALAT, Aspartate aminotransferase; M, Male; F, Female; IQR, Interquartile range; Median, Median value; % of time, percentage of time the value is below the lower limit or above the upper limit.
2 diabetes (OR, 4.49 [95% CI, 1.07–18.89]; P = 0.04) (Table 4). In the alternative model, that used creatinine instead of urea nitrogen to avoid collinearity, CRP > 90 mg/L, and the medical history of type 2 diabetes were independently associated with the risk of ARF (Table 4).

3.3. Factors associated with in-hospital mortality related to severe COVID-19

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

4. Discussion

Using a big-data approach and time-series design on a well-phenotyped retrospective cohort of consecutive patients with newly diagnosed SARS-CoV-2 infection, we were able to describe the most frequently observed biochemical abnormalities over time in patients with severe COVID-19. In these patients, the kinetics of biochemical abnormalities was consistent with a multi-organ involvement (Fig. 6). The results of our study point out the lack of the association between the inflammatory markers and the risk of death but rather highlight a significant association between renal dysfunction and the risk of COVID-19 related ARF or death. A cohort study from China reported a baseline prevalence of acute kidney injury among 5-1% of patients with COVID-19 that were admitted to a tertiary hospital [29]. Patients with an AKI stage 1 to 3 were at a higher risk of in-hospital mortality [29]. One of the
Fig. 1. Kinetics over time of the most frequently observed biochemical abnormalities during the follow-up of patients with severe COVID-19 (part 1); A: urea nitrogen (g/L); B: creatinine (mg/L); C: osmolality, calculated (mOsmol/kg); D: phosphorus (mg/L); E: calcium (mg/L); F: albumin (g/L); G: triglycerides (g/L); and H: C-reactive protein (mg/L). The dashed lines correspond to the upper (U) and lower (L) limits of the reference range. The red line indicates the evolution trend of the biomarker during follow-up according to the isotonic regression method.
Fig. 2. Kinetics over time of the most frequently observed biochemical abnormalities during the follow-up of patients with severe COVID-19 (part 2): A: hemoglobin (g/dL); B: partial pressure of oxygen (PO2) (mmHg); C: high-sensitivity cardiac troponin I (hs-c Troponin I) (pg/mL); D: hs-c Troponin I (view limited to hs-c Troponin I < 400 pg/mL); E: N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) (pg/mL); F: NT-proBNP (view limited to NT-proBNP < 1000 pg/mL); G: CK (view limited to CK < 2000 U/L); H: lactate dehydrogenase (LDH) (U/L). The dashed lines correspond to the upper (U) and lower (L) limits of the reference range. The red line indicates the evolution trend of the biomarker during follow-up according to the isotonic regression method.
Fig. 3. Kinetics over time of the most frequently observed biochemical abnormalities during the follow-up of patients with severe COVID-19 (part 3): A: aspartate aminotransferases (ASAT) (U/L); B: ASAT (U/L) (view limited to ASAT <400 U/L); C: alanine aminotransferases (ALAT) (U/L); D: ALAT (U/L) (view limited to ALAT <400 U/L); E: bilirubin, total (mg/L); F: bilirubin, conjugated (mg/L); G: alkaline phosphatase (UI/L); H: γ-glutamyltransferase (U/L). The red line indicates the evolution trend of the biomarker during follow-up according to the isotonic regression method.
Table 4
Association between biochemical abnormalities and the occurrence of acute respiratory failure or in-hospital mortality among patients with severe COVID-19 in the multivariable multilevel analysis.

### Acute respiratory failure, model #1: Urea nitrogen, C-reactive protein, age, gender, medical history

| Variables                                      | Estimation | Standard error | Odds ratio, adjusted (95% CI) | P-valuea |
|------------------------------------------------|------------|----------------|--------------------------------|----------|
| C-reactive protein > 90 mg/L                   | 1.93       | 0.55           | 6.87 (2.36 – 20.01)            | <0.001   |
| Medical history of type 2 diabetes             | 1.50       | 0.73           | 4.49 (1.07 – 18.89)            | 0.04     |
| Urea nitrogen > 0.36 g/L                       | 1.16       | 0.63           | 3.81 (1.15 – 13.29)            | 0.03     |
| 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     |

### Acute respiratory failure, model #2: Creatinine, C-reactive protein, age, gender, medical history

| Variable                                      | Estimation | Standard error | Odds ratio, adjusted (95% CI) | P-valuea |
|------------------------------------------------|------------|----------------|--------------------------------|----------|
| C-reactive protein > 90 mg/L                   | 1.93       | 0.54           | 6.90 (2.38 – 20.01)            | <0.001   |
| Medical history of type 2 diabetes             | 1.45       | 0.73           | 4.26 (1.02 – 17.90)            | 0.04     |
| Male gender                                    | 1.15       | 0.67           | 3.16 (0.86 – 11.69)            | 0.09     |
| 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     |

### In-hospital mortality, Model: Urea nitrogen, age, gender, medical history

| Variable                                      | Estimation | Standard error | Odds ratio, adjusted (95% CI) | P-valuea |
|------------------------------------------------|------------|----------------|--------------------------------|----------|
| Age                                            | 0.12       | 0.04           | 1.12 (1.03 – 1.22)             | 0.006    |
| Medical history of COPD                        | 2.64       | 1.31           | 14.06 (1.08 – 182.74)          | 0.04     |
| 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     |

Note. COPD: chronic obstructive pulmonary disease; 95% CI: 95% confidence interval.

a Patients’ medical histories were selected to avoid multicollinearity regarding the endpoint.

b Two-level hierarchical logistic model (HLM), using the predictive quasi-likelihood method.

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

| Biological variable | n   | ROC, P-valueb | AUROCc | ROC-defined cut-off | Time-series analysis P-valued | 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 | 0.07e        | 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 (23.6 – 66.8)                                           |
| PO2 (mm/Hg)         | 785 | 0.31         | 0.539  (0.464 – 0.615) | –     | –                | –                                                            | –                                                            |
| PCO2 (mm/Hg)        | 785 | 0.04e        | 0.583  (0.501 – 0.663) | –     | –                | –                                                            | –                                                            |
| Bicarbonate (HCO3−) | 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 | 0.03e        | 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  | 0.06e      | 0.580  (0.491 – 0.658) | –     | –                | –                                                            | –                                                            |
| Calcium (mg/L)      | 266 | 0.54         | 0.537  (0.416 – 0.654) | –     | –                | –                                                            | –                                                            |
| Phosphorus (mg/L)   | 299 | 0.045e       | 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 | 0.03e        | 0.617  (0.508 – 0.714) | –     | –                | –                                                            | –                                                            |

Note. ALAT: alanine aminotransferases; ASAT: aspartate aminotransferases; AUROC: area under the receiver operating characteristic curve; CK: creatine kinase; hs-c Troponin I: high-sensitivity cardiac troponin I; PCO2: partial pressure of carbon dioxide; PO2: partial pressure of oxygen; ROC: receiver operating characteristics.

a Receiver operating characteristic (ROC) analysis, according to DeLong et al. with Bias-corrected and accelerated (BCa)-bootstrap interval after 10,000 iterations for the Youden index.

b Time-series analysis using a non-parametric test.

c Nominal P-values did not maintain their significance after Bonferroni correction.
hypotheses for the occurrence of acute renal damage is the inade-
quacy of the inflammatory response linked to the cytokine storm
observed in severe forms of COVID-19 and their effect on hypoperfu-
sion of renal tubules [30]. A recent study reported that patients with
severe COVID-19 have a high risk of developing Fanconi syndrome,
leading to potentially life-threatening plasma disturbances [31].
SARS-CoV-2 infects the cells through its binding to the membrane-
bound form of receptor-angiotensin converting enzyme II (ACE2) and
subsequent internalization of the complex by the host cell [1,32-34].
There is emerging evidence about the direct cytopathic effect of
SARS-CoV-2 since the cell entry receptor ACE2 is expressed on podo-
cytes and tubule epithelial cells [30]. In line with these data, we
observed a biphasic evolution of the phosphorus level in the blood of
patients with severe COVID-19 with an initial decrease during the
first four days, followed by a progressive increase that mirrored the
degradation of kidney function. This evolution may reflect a direct
cytotoxic effect of the virus with initial involvement of tubule
epithelial cells, which is responsible for a phosphorus leakage in the
urine and, in a second step, a glomerular attack, explaining the pro-
gressive increase in urea nitrogen and creatinine as well as phospho-
rus. These data are corroborated by the progressive increase in the
proportion of patients with stage 3 AKI during the follow-up. In our
study, patients with ARF and those who die form COVID-19 share the
same pattern of urea nitrogen alteration. In addition to pulmonary
involvement, renal involvement leads to life-threatening alterations
in homeostatic regulatory processes. Further studies should address
the significance of acute kidney injury in the prediction of COVID-19
related death and plead for long-term follow-up by a nephrologist of
patients with severe COVID-19.

The ACE2 protein is highly expressed in the gastrointestinal tract,
notably in the duodenum and the small intestine, gallbladder, testis,
seminal vesicle, and adrenal gland [35]. A recent study also confirmed
the expression of the ACE2 protein in human myocardial pericytes
[36]. We can hypothesize that the direct cytopathic effect of SARS-
CoV-2 on pericytes may result in endothelial cell and microvascular
dysfunction [36]. A retrospective single-center case series of 187
patients with COVID-19 from China showed that myocardial injury
was significantly associated with the risk of death [37].

To date, descriptive data on COVID-19 did not report obvious cho-
lestasis in patients with severe forms [38,39]. In our series, we have
reported cholestatic involvement with conjugated hyperbilirubine-
mia and cytolysis over 30% of the observation time. These results
raise the possibility of a direct toxic effect of the virus on the biliary
epithelium, especially given the high level of expression of the ACE2
protein in the biliary tissue [35]. Long term evolution of these chole-
static syndromes deserves further studies, notably regarding the risk
of cholestatic liver disease. The present study provides new data on
the elevation of lipase levels among patients with severe COVID-19.
This elevation could be related to several mechanisms, other than
direct pancreatic involvement, and include intestinal disease, acido-
sis, or the presence of shock. We have observed elevations in CK and
LDH that have also been reported in the literature [8,38,40-42].

We acknowledge several potential limitations of the study that
should be considered in the interpretation of our findings. Although
we have studied an extensive follow-up dataset, our study is based
on a relatively small number of patients, and our results need to be
confirmed in independent studies. Nevertheless, we have systemati-
cally included all consecutive patients with newly diagnosed severe
COVID-19, which allowed us to describe the biochemical abnormali-
ties on a homogeneous population. The study design is retrospective;
nevertheless, we used data from a structured and prospectively
maintained database [14]. In our study, the follow-up of patients was
relatively short, which could lead to an underestimation of the risk of
COVID-19 related mortality. Patients with severe COVID-19 can exhibit immunological determinants of a cytokine storm [42-45],
notably with high levels of CXCL10, CCL7, and IL-1 receptor antago-
nist, and which could influence the risk of lung injury and fatal out-
come [45]. In our study, IL-6 and procalcitonin—an indirect marker
of innate immunity—were not tested enough in patients to be
assessed in the analysis. Our study has several strengths. First, we
report an exhaustive description of the biochemical abnormalities
and their kinetics over time among patients with severe COVID-19.
Second, we assessed the relationship between the variation over
time in biochemical markers and the occurrence of disease-related
complications through a multilevel modeling approach adapted for
repeated measures. Third, we studied a wide range of biochemical
markers relating to both blood and urine and targeting several organs
and vital functions. We notably highlighted a potentially strong asso-
ciation between acute kidney injury, the development of severe kid-
ney damage, and their association with COVID-19 related in-hospital
mortality.

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

Declaration of Interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflicts of interest concerning this manuscript.

Data sharing statement

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

Acknowledgments

We would like to warmly thank all the medical (Dr. Patricia Frank, Dr. Anne Debourgogne) and the technical staff of the Laboratory of Biology and Biopathology of the University Hospital of Nancy for their valuable contribution to the present work. Additionally, we would like to acknowledge the following collaborators. All collaborators approved the final draft. Matthieu Garcia (MSc), University Hospital of Nancy, for data management on the “Nancy Biochemical Database”. Isabelle Chouviac (PharmD), University Hospital of Nancy, for quality support on laboratory diagnostic assessment. Sibel Berger (PhD), University Hospital of Nancy, for quality support on laboratory diagnostic assessment. Audrey Jacquot (MD), University Hospital of Nancy, for management for patients. Matthieu Koszutski (MD), University Hospital of Nancy, for management for patients. Philippe Guerci (MD, PhD), University Hospital of Nancy, for management for patients. Ombeline Empis de Vendin (MD), University Hospital of Nancy, for management for patients. Matthieu Delannoy (MD), University Hospital of Nancy, for management for patients. Laura Chevard (MD), University Hospital of Nancy, for management for patients. Jean-Marc Lalot (MD), University Hospital of Nancy, for management for patients. Emmanuel Noy (MD), University Hospital of Nancy, for management for patients. Jean-Pierre Pertek (MD), University Hospital of Nancy, for management for patients. Asma Alla (MD), University Hospital of Nancy, for management for patients. Benjamin Lefèvre (MD), University Hospital of Nancy, for management for patients. Hélène Jeulin (PharmD), University Hospital of Nancy, for laboratory diagnostic assessment. Cédric Hartard (PharmD, PhD), University Hospital of Nancy, for laboratory diagnostic assessment.
diagnostic assessment. Zakia Aitidjafer (MD), University Hospital of Nancy, for laboratory diagnostic assessment. Véronique Venard (PharmD, PhD), University Hospital of Nancy, for laboratory diagnostic assessment. Alain Lzoñewski (PharmD, PhD), University Hospital of Nancy, for laboratory diagnostic assessment.

Authors’ contributions
AO: has full access to all the data in this study and take full responsibility as the guarantor for the integrity of the data and the accuracy of the data analysis, study concept, laboratory diagnostic assessment, literature review, data synthesis and statistical analysis, drafting/revision of the manuscript, analysis and interpretation of data, approved the final draft, final responsibility for the decision to submit for publication; SG: laboratory diagnostic assessment, data extraction, analysis and interpretation of data, approved the final draft; ICU: data synthesis and statistical analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; EL: data extraction, analysis and interpretation of data, approved the final draft; FB: laboratory diagnostic assessment, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; SO: laboratory diagnostic assessment, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; CM: laboratory diagnostic assessment, analysis and interpretation of data, approved the final draft; IAC: laboratory diagnostic assessment, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; BMC: laboratory diagnostic assessment, analysis and interpretation of data, approved the final draft; MM: analysis and interpretation of data, approved the final draft; EJ: laboratory diagnostic assessment, analysis and interpretation of data, approved the final draft; RK: management of patients, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; MLL: laboratory diagnostic assessment, analysis and interpretation of data, approved the final draft; AK: management of patients, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; LF: management of patients, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; BL: management of patients, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; SG: management of patients, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; ES: laboratory diagnostic assessment, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft; JLG: study concept, laboratory diagnostic assessment, analysis and interpretation of data, drafting/revision of the manuscript, approved the final draft.

Funding
No funding.

Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2020.100554.

References
[1] Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.
[2] Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:533–4.
[3] Bernard Stoocklin S, Rolland P, Silve Y, et al. First cases of coronavirus disease 2019 (COVID-19) in France: surveillance, investigations and control measures, January 2020. Euro Surveill 2020;25:2000094.
[4] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.
[5] Xu XW, Wu XX, Jiang XG, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-CoV-2) outside of Wuhan, China: retrospective case series. BMJ 2020;368:m906.
[6] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061–9.
[7] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[8] Chen T, Wu D, Chen H, et al. Critical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020;368:m1091.
[9] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507–13.
[10] Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 2020;323:1488–94.
[11] Grasselli G, Zangrillo A, Zanella A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. JAMA 2020;323:1574–81.
[12] Grein J, Ohmagari N, Shin D, et al. Compassionate use of Remdesivir for patients with severe Covid-19. N Engl J Med 2020;382:327–36.
[13] Organization WH. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: interim guidance, 13 March 2020. World Health Organization; 2020.
[14] Oussalah A, Ferrand J, Filicine-Trescuier P, et al. Diagnostic accuracy of procalcitonin for predicting blood culture results in patients with suspected bloodstream infection: an observational study of 35,343 consecutive patients (a stroke–compliant article). Medicine (Baltimore) 2015;94:e1774.
[15] Mcenery T, Gough C, Costello RW. COVID-19: respiratory support outside the intensive care unit. Lancet Respir Med 2020;8:538–9.
[16] Cheung TM, Yim LY, So UK, et al. Effectiveness of noninvasive positive pressure ventilation in the treatment of acute respiratory failure in severe acute respiratory syndrome. Chest 2004;126:845–50.
[17] Vitacca M, Nava S, Santus P, Harari S. Early consensus management for non-ICU ARF SARS-CoV-2 emergency in Italy: from ward to trenches. Eur Respir J 2020;55 pii:2000632.
[18] Vincent JL, Taccone FS. Understanding pathways to death in patients with COVID-19. Lancet Respir Med 2020;8:430–2.
[19] Mehta RL, Kellum JA, Shah SV, et al. Acute kidney injury network: report of an initiative to improve outcomes in kidney injury. Crit Care 2007;11:R31.
[20] Wu WB, Woodroofe M, Mezetti G. Isotonic regression: another look at the change-point problem. Biometrika 2001;88:793–804.
[21] Best MJ, Chakravarti N. Active set algorithms for isotonic regression; a unifying framework. Math Program 1990;47:425–29.
[22] DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
[23] Efros B, Tishberian R, Jafer R. An introduction to the bootstrap. Taylor & Francis; 1994.
[24] Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ 1990;300:230–5.
[25] Rice N, Jones A. Multilevel models and health economics. Health Econ 1997;6:561–75.
[26] Hox J. Multilevel analysis: techniques and applications. Quant Methodol Soc 2002:98.
[27] Larsen K, Petersen JH, Budtz –Jargensen E, Endahl L. Interpreting parameters in the logistic regression model with random effects. Biometrics 2000;56:903–14.
[28] Cheng J, Edwards LJ, Maldonado – Molina MM, Komro KA, Muller KE. Real longitudinal data analysis for real people: building a good enough mixed model. Stat Med 2010;29:504–20.
[29] Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:928–39.
[30] Durvasula R, Wellington T, McNamara E, Watnick S. COVID-19 and kidney failure in the acute care setting: our experience from Seattle. Am J Kidney Dis 2020;76:4–8.
[31] Kormann R, Jacquot A, Alla A, et al. Coronavirus disease 2019: acute Fanconi syndrome precedes acute kidney injury. Clin Kidney J 2020;13:362–70.
[32] South AM, Diz D, Chappell MC. COVID-19, ACE2 and the cardiovascular consequences. Am J Physiol Heart Circ Physiol 2020;318:H1084–90.
[33] Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395:565–74.
[34] Van R, Zhang Y, Li Y, Xia L, Guo Y, Zhao Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020;367:1444–8.
[35] Uhlen M, Fagerberg L, Hallstrom BM, et al. Tissue-based map of the human proteome. Science 2015;347:1260419.
Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res 2020;116:1097–100.

Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol 2020;5:811–8.

Jin X, Lian JS, Hu JH, et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. Gut 2020;69:1002–9.

Li J, Fan JG. Characteristics and mechanism of liver injury in 2019 coronavirus disease. J Clin Transl Hepatol 2020;8:13–7.

Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. Lancet Infect Dis 2020;20:689–96.

Ji D, Zhang D, Xu J, et al. Prediction for progression risk in patients with COVID-19 pneumonia: the CALL score. Clin Infect Dis 2020;71:1393–9.

Chen G, Wu D, Guo W, et al. Clinical and immunologic features in severe and moderate coronavirus disease 2019. J Clin Invest 2020;130:2620–9.

Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033–4.

Vaninov N. In the eye of the COVID-19 cytokine storm. Nat Rev Immunol 2020;20:277.

Yang Y, Shen C, Li J, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. J Allergy Clin Immunol; 2020;146:119–27.e4.