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Association between Biomarkers and COVID-19 Severity and Mortality: A Nationwide Danish Cohort Study

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Abstract

Objective: To evaluate the association between common biomarkers, death and ICU admission in patients with COVID-19.

Design: Retrospective cohort study. From electronic national registry data, we used Cox analysis and bootstrapping to evaluate associations between baseline levels of biomarkers and standardized absolute risks of death/ICU admission, adjusted for age and gender.

Setting: All hospitals in Denmark.

Participants: 1310 patients aged ≥18 years admitted to hospital with COVID-19 from February 27th to May 1st, 2020, with available biochemistry data.

Main outcome measures: A composite of death / ICU admission occurring within 30-days.

Results: Of the 1310 patients admitted to hospital (54.6% male; median age 73.6), 352 (26.9%) experienced the composite endpoint and 263 (20.1%) died. For the composite endpoint, the absolute risks for moderately and severely elevated C-reactive protein (CRP) were significantly higher, 21.5% and 39.2% respectively, compared to 5.0% for those with normal CRP. Moderately and severely elevated leucocytes were significantly higher, 34.5% and 46.6% risk respectively, compared to 23.2% for those with normal leucocytes. Moderately and severely decreased estimated glomerular filtration rate (eGFR) were significantly higher, 41.5% and 45.9% risk respectively, compared to 30.4% for those with normal/mildly decreased eGFR. Normal and elevated urea were significantly higher, 22.3% and 40.6% risk respectively, compared to 7.3% for those with low urea. Elevated D-dimer was significantly higher, 31.8% risk, compared to 17.5% for those with normal D-dimer. Moderately and severely elevated troponins were significantly higher, 27.7% and 57.3% risk respectively, compared to 9.4% for

those with normal troponin. Elevated procalcitonin was significantly higher, 52.1% risk, compared to 28.0% for those with normal procalcitonin.

Conclusion: In this nationwide study of patients admitted with COVID-19, elevated levels of CRP, leucocytes, procalcitonin, urea, troponins and D-dimer, and low levels of eGFR were associated with higher standardized absolute risk of death/ICU admission within 30 days.

Strengths and limitations of this study

- Much of the research concerning COVID-19 describes small case studies from China and Italy, without clearly defined outcomes.
- This study is the first to report the standardized absolute risk of individual laboratory tests on short-term mortality and ICU admission in a relatively large, European cohort of 1310 patients with COVID-19.
- Our study can help the clinician to understand which biomarkers are important in identifying patients with poor prognosis, which may be useful to assess disease severity or to enable early intervention.
- The main limitation of our study is its observational, non-randomized design.
- This study included only patients admitted to the hospital with COVID-19 and measured biochemical data, hence it is likely to represent symptomatic patients at the more severe end of the disease spectrum.

Introduction

Coronavirus disease 2019 (COVID-19) caused by the novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has spread rapidly to become a worldwide pandemic resulting in an enormous strain on healthcare systems globally. As of May 10th, 2020, the number of confirmed cases has surpassed 4.1 million, affecting 212 countries, although the actual number is likely to be much higher.[1] The clinical course of COVID-19 is variable, but is typically characterized by an initial phase with fever or mild upper respiratory symptoms (though many are asymptomatic). Among hospitalized patients, those with a poor prognosis tend to develop severe viral pneumonia requiring ventilatory support and intensive care unit (ICU) admission.[2] Despite supportive care, a high proportion of patients with COVID-19 suffer rapid deterioration with respiratory failure and death.[3–5] Identifying which patients are at risk of severe disease or death, may be useful in decision making, to determine whether hospitalization or ICU referral is required or to enable early intervention.[6] This is of particular importance given that the fast-pace of the pandemic has led to rationing of scarce resources, most notably mechanical ventilators.[7]

Several studies have demonstrated that older age and chronic diseases are associated with poor outcome in patients with COVID-19.[2,5] Although much of the early research describes small case studies without clearly defined outcomes, disease severity has been associated with more prominent laboratory abnormalities including markers of inflammation and organ damage including elevated troponins.[2,8–10] A recently published study showed that in multivariate analysis, older age, higher Sequential Organ Failure Assessment (SOFA) score, and elevated D-dimer on admission were independently associated with in-hospital death.[5] The present study

aims to expand on these findings and evaluate which biomarkers are associated with death and ICU admission in a large nationwide cohort.

Methods

Data sources

This study is based on four nationwide registers, The Danish National Patient Register, The Civil Registration System, The Danish Registry of Medicinal Product Statistics, and the database on blood samples (LABKA). These four nationwide registers were cross-linked on the individual level using the unique permanent identification number given to all Danish residents at birth or migration.

The Danish National Patient Register holds information on every hospital visit in Denmark, in which each visit is registered with a diagnosis according to the International Classification of Diseases, the 10th revision (ICD-10). The Civil Registration System holds information on the day of birth, sex, and vital status. The Danish Registry of Medicinal Product Statistics contains information on all prescriptions dispensed from Danish pharmacies and is coded according to the Anatomical Therapeutic Chemical (ATC) classification system.[11] The LABKA database holds information on blood samples from all hospital visits, including the emergency departments, outpatient consultations, and admissions to the hospital.

Study design and participants

This study included all laboratory-confirmed COVID-19 patients aged 18 years and older with available biochemistry data, admitted to hospital between February 27th, 2020, and May 1st, 2020 (the first Danish case was recorded on February 27th, 2020). Patients were included on the

first day of diagnosis with COVID-19 and followed for 30 days for the combined endpoint of all-cause mortality and ICU admission. A sub-analysis was also performed for the endpoint of all-cause mortality alone. Blood test results were obtained from electronic registries of laboratory data, with baseline values taken on admission (measured within 24 hours). We focused on readily available laboratory tests associated with inflammation or organ damage, including C-reactive protein (CRP), ferritin, procalcitonin, leucocyte count, estimated glomerular filtration rate (eGFR), urea, alanine aminotransferase (ALAT), D-dimer and troponin (both T and I). The eGFR was calculated using the Modification of Diet in Renal Disease (MDMD) equation, which includes creatinine level, age, race, and sex. In order to compare troponin values with different assays and reference values, a ratio between observed values and highest reference values was performed.

Statistical analysis

Categorical data were presented as counts with percentages, and the statistical difference was tested using Fisher's exact test. Continuous variables were presented as medians with the first and third quartile (Q1 and Q3), and the statistical difference was tested using the Wilcoxon rank-sum test. Cox analysis and bootstrapping with 100 bootstraps were used to derive age and gender adjusted standardized absolute risk and average treatment effects curves with 95% confidence intervals (CI) to evaluate the association between individual biomarkers and the 30-day risk for each endpoint. The above analysis was repeated with stratification by typical normal/elevated ranges used in the clinical setting. For the stratified analysis of troponin the cutoff values were defined as being moderately elevated (>1 to 2 times elevated) and severely

elevated (>two times elevated), relative to baseline troponin. A two-sided p-value ≤0.05 was considered statistically significant.

Data management and statistical analyses were conducted using R statistics (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.).

Results

A total of 4444 patients with COVID-19 were identified in the study period. We excluded 28 patients aged below 18 years old, 2653 patients who were not admitted to hospital and 453 with no available biochemistry data, leaving 1310 patients for inclusion in the study (54.6% male; median age 73.6). Of these, 352 (26.9%) patients experienced the composite endpoint and 263 (20.1%) died.

Baseline characteristics for the total cohort and stratified by the composite endpoint, are given in Table 1. Patients who experienced the composite endpoint of death/ICU admission within 30-days were more likely to be older, male, with a pre-existing comorbidity (diabetes, chronic obstructive pulmonary disease, atrial fibrillation, hypertension or heart failure); currently receiving treatment with aspirin, beta-blocker, angiotensin II receptor blockers, loop diuretics, calcium channel blockers, or spironolactone; with higher baseline values of leucocytes, urea, D-dimer, troponin or procalcitonin; or lower baseline values of eGFR (p≤0.044 for all). However, the proportion of patients who died or were admitted to ICU was not significantly different for those with prior ischemic stroke, ischemic heart disease, chronic kidney disease, cancer, or currently receiving treatment with nonsteroidal anti-inflammatory drugs or thiazides.

Biomarkers and Standardized Absolute Risk of Death and Intensive Care Unit Admission

C-reactive protein

Higher baseline CRP was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, moderately elevated (upper reference limit to 99 mmol/L) and severely elevated (100 to 400 mmol/L) baseline CRP were associated with an age and sex adjusted absolute risk of 21.5% (95%CI: 18.1-24.9) and 39.2% (95%CI: 35.6-43.0) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal CRP, absolute risk 5.0% (95%CI: 0.0-12.0) (Figure 3).

Similarly, moderately and severely elevated baseline CRP were associated with an age and sex adjusted absolute risk of 18.1% (95%CI: 15.1-21.3) and 28.8% (95%CI: 25.4-32.1) for 30-day mortality, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal CRP, absolute risk 6.6% (95%CI: 0.0-15.0) (Supplementals, Figure S2).

Leucocytes

Higher baseline leucocyte count was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, moderately elevated (upper reference limit to 15 x 10^9/L) and severely elevated (15 to 30 x 10^9/L) baseline leucocytes were associated with an age and sex adjusted absolute risk of 34.5% (95%CI: 29.5-39.4) and 46.6% (95%CI: 38.5-54.6) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal leucocytes, absolute risk 23.2% (95%CI: 20.4-22.2) (Figure 3).

In stratified analysis, moderately and severely elevated baseline leucocytes were associated with an age and sex adjusted absolute risk of 26.6% (95%CI: 22.0-31.3) and 37.0% (95%CI: 25.4-32.1) for 30 day-mortality, respectively, which was a significantly higher risk (both p<0.001) compared to those with normal leucocytes, absolute risk 17.6% (95%CI: 15.0-20.2) (Supplementals, Figure S2).

Estimated glomerular filtration rate

Lower baseline eGFR was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, moderately decreased (30 to 60 mmol/L) and severely decreased (0 to 30 mmol/L) baseline eGFR were associated with an age and sex adjusted risk risk of 41.5% (95%CI: 35.1-48.0) and 45.9% (95%CI: 34.9-56.8) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both p<0.001) compared to those with normal/mild decreased eGFR (>60 mmol/L), absolute risk 30.4% (95%CI: 26.7-34.1) (Figure 3).

In stratified analysis, moderately elevated and severely elevated baseline leucocytes were associated with an absolute risk of 34.8% (95%CI: 29.0-40.5) and 42.4% (95%CI: 32.9-52.0) for 30-day mortality, respectively, (Figure 3), which was a significantly higher risk (both p<0.001) compared to those with normal/mild decreased eGFR, absolute risk 22.0% (95%CI: 18.5-25.5) (Supplementals, Figure S2).

Urea

Higher baseline urea was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, normal (3.6 to 8.0 mmol/L) and elevated (>8 mmol/L) baseline urea were associated with an absolute risk of 22.3% (95%CI: 18.7-25.9) and 40.6% (95%CI: 35.5-45.7) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both p<0.001) compared to those with low urea, absolute risk 7.3% (95%CI: 1.6-12.9) (Figure 3).

In stratified analysis, normal and elevated baseline urea were associated with an absolute risk of 15.7% (95%CI: 11.2-17.4) and 31.9% (95%CI: 26.8-35.4) for 30-day mortality, respectively, which was a significantly higher risk (both p<0.001) compared to those with low urea, absolute risk 5.4% (95%CI: 0.0-11.0) (Supplementals, Figure S2).

Alanine aminotransferase

Higher baseline ALAT was associated with slightly higher absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, elevated (>upper reference limit) baseline ALAT was associated with an absolute risk of 36.9% (95%CI: 30.7-43.1) for death/ICU admission within 30 days, which was significantly higher than normal ALAT (10 U/L to upper reference limit), absolute risk 26.9% (95%CI: 23.6-29.3, p=0.002), however not significantly different to low ALAT (<10 U/L), absolute risk 43.8% (95%CI: 21.1-66.4) (Figure 3).

In stratified analysis, low baseline ALAT was associated with an absolute risk of 41.0% (95%CI: 22.7-59.4) for 30-day mortality, which was significantly higher than normal ALAT, absolute risk 20.8% (95%CI: 18.2-23.4, p=0.03), however not significantly different to high ALAT, absolute risk 25.8% (95%CI: 19.9-31.8) (Supplementals, Figure S2).

Ferritin

Higher baseline ferritin was associated with an inverted U shaped risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, elevated (> 300 mmol/L) baseline ferritin was associated with an age and sex adjusted absolute risk of 22.7% (95%CI: 15.1-30.3) for death/ICU admission within 30 days, which was not a significantly higher risk compared to those with normal ferritin (≤300 mmol/L), absolute risk 19.7% (95%CI: 12.5-32.9) (Figure 3).

In stratified analysis, elevated (>300 mmol/L) baseline ferritin was associated with an age and sex adjusted risk of 15.9% (95%CI: 0.09-22.8) for 30-day mortality, which was not a significantly higher risk compared to those with normal ferritin (≤300 mmol/L), absolute risk 19.8% (95%CI: 12.6-26.9) (Supplementals, Figure S2).

D-dimer

Higher baseline D-dimer was associated with an age and sex adjusted higher risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, elevated (>0.5 mg/L) baseline D-dimer was associated with an age and sex adjusted absolute risk of 31.8% (95%CI: 26.7-36.8) for death/ICU admission within 30 days, which was a significantly higher risk ($p<0.001$) compared to those with normal D-dimer, absolute risk 17.5% (95%CI: 10.9-24.1) (Figure 3).

In stratified analysis, elevated baseline D-dimer was associated with an age and sex adjusted risk of 19.1% (95%CI: 14.7-23.5) for 30-day mortality, which was a significantly higher risk ($p<0.001$) compared to those with normal D-dimer, absolute risk 13.5% (95%CI: 8.5-19.5) (Supplementals, Figure S2).

Troponin

Higher baseline troponin ratio was associated with age and sex adjusted higher risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, moderately elevated and severely elevated baseline troponin were associated with an age and sex adjusted absolute risk of 27.7% (95%CI: 16.5-38.9) and 57.3% (95%CI: 43.3-71.3) for death/ICU admission within 30 days, respectively, which was a significantly higher absolute risk ($p=0.003$ and $p<0.001$, respectively) compared to those with normal troponins, absolute risk 9.4% (95%CI: 4.2-14.5) (Figure 3).

In stratified analysis, moderately elevated and severely elevated baseline troponin were associated with an age and sex adjusted absolute risk of 10.8% (95%CI: 3.2-18.5) and 35.9% (95%CI: 23.2-48.6) for 30-day mortality, respectively. However, only the later was a significantly higher risk ($p<0.001$) compared to those with normal troponins, absolute risk 3.9% (95%CI: 0.0-8.7) (Supplementals, Figure S2).

Procalcitonin

Higher baseline procalcitonin was associated with age and sex adjusted higher risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, elevated (>0.5 mcg/L) baseline procalcitonin was associated with an age and sex adjusted risk of 52.1% (95%CI: 41.5-62.6) for death/ICU admission within 30 days, which was a significantly higher risk ($p<0.001$) compared to those with normal procalcitonin, absolute risk 28.0% (95%CI: 21.1-34.9) (Figure 3).

In stratified analysis, elevated baseline procalcitonin was associated with an age and sex adjusted absolute risk of 29.5% (95%CI: 19.9-39.0) for 30-day mortality, which was a significantly higher absolute risk (p=0.03) compared to those with normal procalcitonin, absolute risk 18.0% (95%CI: 12.0-24.0) (Supplementals, Figure S2).

Discussion

This nationwide study is the first of its kind to examine the association between common biomarkers and risk of early death and ICU admission in adult patients admitted to hospital with laboratory-confirmed COVID-19. In particular, the inflammatory markers CRP, leucocytes and procalcitonin, and markers of organ damage including eGFR, troponins and D-dimer are associated with higher risk of death/ICU admission within 30 days. However, the association between ferritin and ALAT was non-significant.

The novelty of the SARS-CoV-2, coupled with the rapid spread of the COVID-19 pandemic, has led to a tremendous burden on healthcare systems worldwide. To provide optimal care for patients, early diagnosis and identification of vulnerable patients who are at risk of severe disease is needed, as well as recognizing patients who may rapidly deteriorate and require ICU admission and mechanical ventilation. This relies on an accurate knowledge of the critical clinical predictors for disease progression in order to triage patients and allocate scarce resources efficiently. This is especially important when considering which patients should start treatment. For example, in the first trial to offer an effective treatment for COVID-19 disease, results showed that early administration of antiviral treatment with Remdesivir was superior to placebo in reducing time to recovery and in particular, the authors highlighted the need to start antiviral treatment before pulmonary disease progresses to require mechanical ventilation.[12]

In the present study, patients who died or were admitted to ICU were more likely to be older, male, and with a pre-existing comorbidity which is consistent with observed global trends. Our results are aligned with a recent large cohorts of patients hospitalized with COVID-19 in the UK and US, as well as meta-analysis of studies from China.[2,13,14] The median age of patients who died in our study was 81 years, similar to the values noted in the UK study (median 80 years) but much older compared to patients included in the early studies from the Wuhan region. Similarly, we reported a greater proportion death/ICU admission among males (64%) and patients with pre-existing comorbidity (prior diabetes, COPD, atrial fibrillation and hypertension). Differences are most likely due to a variety of factors including regional differences concerning demographics, preparedness and knowledge of COVID-19 that have drawn on experience from China and other countries as the outbreak has spread.

In terms of clinical laboratory biomarkers, a recent systematic review of the literature has highlighted many of the challenges inherent in the early studies of COVID-19, as many of the early studies are limited by the small size of the study populations with regional bias (most data is confined to China and Italy), and with limited or poorly defined outcomes.[6] Several studies have evaluated level of individual biomarkers in small and selected COVID cohorts. An Italian study including 239 patients, reported that several laboratory parameters including elevated lymphocytes, procalcitonin, interleukin-6, ferritin, CRP, and ALAT was associated with death and ICU admission in unadjusted Cox analysis. However, in adjusted analysis CRP was the only biomarker associated with increased risk of death/ICU admission.

An early meta-analysis identified a range of abnormal biomarkers that was elevated in COVID-19 patients who died, including elevated levels of inflammatory markers and acute phase reactants such as interleukin-6, CRP, ferritin, lymphopenia, as well as reduced CD4 and

CD8 counts and coagulation abnormalities including prolonged PT, increased D-dimer and thrombocytopenia.[11] We were able to expand on these findings, reporting age and gender adjusted absolute risk of death/ICU admission of individual biomarkers in a larger, European cohort. In the present study inflammatory markers such as CRP, leucocytes and procalcitonin, and markers of organ damage including eGFR, urea, troponins and D-dimer were associated with higher risk of death/ICU admission within 30 days. However, the association between ferritin and ALAT was non-significant. The association with inflammatory markers, and acute phase reactants likely reflects the cytokine storm associated with severe infection and subsequent end-organ damage from severe sepsis.[15] However, it is also essential to recognize that the acute phase reactants are non-specific markers of inflammation. For example, procalcitonin secretion is not induced by gamma-interferon (produced mainly in response to viral infections), making it primarily an attractive marker of bacterial infections.[16] Nevertheless, bacterial superinfection is an important consideration in COVID-19; for example, in the study by Wang et al., 81.7% of patients who died with COVID-19 had an associated bacterial infection.[17] Probably, patients with longer and more complex admissions or ICU treatment are more vulnerable to secondary infections e.g. ventilator acquired infection, with an expected rise in procalcitonin levels. Accordingly, Zhou et al. observed that half of the non-survivors experienced a secondary infection, with almost a third due to ventilator-associated pneumonia.[5] However, the association between higher absolute risk of death/ICU admission and elevated baseline procalcitonin in the present study suggests that early concomitant bacterial infection (not only secondary infection) is a significant factor in adverse prognosis for patients with COVID-19 that clinicians should be aware of.

In the study including 191 patients from Wuhan region, leucocytes, older age, higher Sequential Organ Failure Assessment (SOFA) score, and a higher D-dimer (greater than 1 µg/L on admission) were all associated with in-hospital mortality in patients with COVID-19.[5] Although the SOFA score is mainly a diagnostic marker of sepsis and septic shock, it may reflect the state and degree of multi-organ dysfunction due to infection in general.[18] In the present study, abnormal values of biomarkers reflecting organ damage including troponin, eGFR, urea, and D-dimer were all associated with higher absolute risk of 30-day mortality and ICU admission. Myocarditis is a known morbidity amongst patients with COVID-19.[19] Elevated troponin may reflect myocardial injury among COVID-19 patients, including direct damage to the cardiomyocytes, systemic inflammation, myocardial interstitial fibrosis, coronary plaque destabilization and hypoxia.[20] Furthermore, patients with cardiovascular disease are more likely to be admitted with COVID-19, with greater risk of cardiac involvement as their symptoms develop.[20] With respect to the abnormalities observed in D-dimer, research suggests that respiratory failure in COVID-19 is not only related to development of the acute respiratory distress syndrome (ARDS), but also due to microvascular thrombotic processes, which are associated with elevated D-dimer.[21] Finally, beyond the high mortality observed in those with organ dysfunction and respiratory failure, a generalized coagulopathy has also been noted in those with poor prognosis.[22]

Strengths and limitations:

The main strength of this study is the completeness of data from a nationwide, European cohort and the avoidance of selection bias resulting from race, age, sex, socioeconomic status, affiliation to selected hospitals, or healthcare systems. The Danish National Patient Registry and

the Danish Registry of Medicinal Product Statistics are known to be accurate.[23,24] The main limitation of our study is its observational non-randomized design. There is a lack of information about important clinical parameters, including blood pressure, body mass index, and smoking habits.

This study included only patients admitted to the hospital with COVID-19 and with measured baseline biochemical data. Therefore, it is likely to represent symptomatic patients, who are more likely to be elderly or with more comorbidities and at the more severe end of the disease spectrum. Furthermore, patients in the cohort may present to hospital at differing stages of their disease. A large proportion of patients with confirmed COVID-19 in Denmark had missing biochemistry data, which most likely represents patients who attended the emergency room with mild symptoms, which did not warrant admission or blood tests, and were not included in this study, thus leading to selection bias and limiting generalisability. Some biomarkers (particularly D-dimer, troponin and procalcitonin) are likely to be measured in those with the most severe disease (confounding by indication).

Conclusions:

In this nationwide study of patients admitted with COVID-19, elevated levels of CRP, leucocytes, procalcitonin, urea, troponins and D-dimer, and low levels of eGFR were associated with higher standardized absolute risk of death/ICU admission within 30 days.

Footnotes

Contributors: All authors approved the final manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Ethical approval: In Denmark, retrospective register studies do not require approval from the ethics committees or individual informed consent. The data responsible institute (The Capital Region of Denmark) approved this study (ref. no.: P-2019-191), in line with the General Data Protection Regulation (GDPR) and The Data Protection Act. Data were made available in a pseudonymized format such that specific individuals could not be identified. Dissemination to study patients is not possible/applicable.

Patient and Public Involvement: This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Transparency declaration: The lead authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Tables

Table 1: Characteristics of patients with coronavirus disease 2019 for total cohort, and stratified by death/ICU admission (within 30-days of diagnosis).

	Total	No Death/ICU admission	Death/ICU admission	p
Characteristics	1310	958 (73.1)	352 (26.9)	
Age, years (median [IQR])	73.60 [60.50, 81.90]	71.15 [56.52, 79.80]	77.50 [70.18, 84.53]	<0.001
Male sex, n (%)	715 (54.6)	489 (51.0)	226 (64.2)	<0.001
Prior ischemic stroke, n (%)	96 (7.3)	63 (6.6)	33 (9.4)	0.094
Prior diabetes, n (%)	221 (16.9)	145 (15.1)	76 (21.6)	0.008
Prior ischemic heart disease, n (%)	165 (12.6)	116 (12.1)	49 (13.9)	0.398
Prior COPD, n (%)	135 (10.3)	86 (9.0)	49 (13.9)	0.010
Prior atrial fibrillation, n (%)	212 (16.2)	132 (13.8)	80 (22.7)	<0.001
Prior chronic kidney disease, n (%)	131 (10.0)	88 (9.2)	43 (12.2)	0.119
Prior hypertension, n (%)	474 (36.2)	323 (33.7)	151 (42.9)	0.002
Prior cancer, n (%)	194 (14.8)	140 (14.6)	54 (15.3)	0.727
Heart failure, n (%)	95 (7.3)	60 (6.3)	35 (9.9)	0.030
Glimepiride, n (%)	245 (18.7)	164 (17.1)	81 (23.0)	0.017
Aspirin, n (%)	174 (13.3)	111 (11.6)	63 (17.9)	0.004
NSAID, n (%)	140 (10.7)	103 (10.8)	37 (10.5)	1.000
Beta blocker, n (%)	174 (13.3)	111 (11.6)	63 (17.9)	0.004
ACEi, n (%)	203 (15.5)	144 (15.0)	59 (16.8)	0.439
ARB, n (%)	463 (35.3)	323 (33.7)	140 (39.8)	0.044
Loop diuretic, n (%)	217 (16.6)	138 (14.4)	79 (22.4)	0.001
Thiazide diuretic, n (%)	116 (8.9)	87 (9.1)	29 (8.2)	0.742
CCB, n (%)	241 (18.4)	157 (16.4)	84 (23.9)	0.003
Spironolactones = 1 (%)	69 (5.3)	41 (4.3)	28 (8.0)	0.012
CRP, n (%)	1256 (95.9)	906 (94.6)	350 (99.4)	<0.001
Leucocytes, n (%)	1300 (99.2)	952 (99.4)	348 (98.9)	0.472
eGFR, n (%)	915 (69.8)	619 (64.6)	296 (84.1)	<0.001
Urea, n (%)	1067 (81.5)	781 (81.5)	286 (81.2)	0.936
ALAT, n (%)	1167 (89.1)	850 (88.7)	317 (90.1)	0.549
Ferritin, n (%)	252 (19.2)	205 (21.4)	47 (13.4)	0.001
D-dimer, n (%)	449 (34.3)	332 (34.7)	117 (33.2)	0.646
Troponin, n (%)	258 (19.7)	197 (20.6)	61 (17.3)	0.210
Procalcitonin, n (%)	249 (19.0)	164 (17.1)	85 (24.1)	0.005
CRP, mg/L (median [IQR])	88.00 [43.00, 160.00]	74.50 [35.25, 130.00]	131.00 [68.25, 218.00]	<0.001
Leucocytes, 10E9/L (median [IQR])	7.40 [5.50, 10.20]	7.10 [5.30, 9.60]	8.40 [6.12, 11.90]	<0.001
eGFR, mL/min/1.73m2 (median [IQR])	73.23 [56.39, 83.55]	74.80 [60.97, 84.17]	66.45 [48.43, 81.33]	<0.001
Urea, mmol/L (median [IQR])	6.50 [4.60, 9.60]	5.90 [4.20, 8.30]	9.05 [6.60, 12.47]	<0.001

ALAT, U/L (median [IQR])	31.00 [21.00, 52.00]	31.00 [21.00, 51.00]	32.00 [22.00, 53.00]	0.169
Ferritin, µg/L (median [IQR])	266.00 [143.00, 446.75]	264.00 [130.00, 439.00]	311.00 [189.50, 464.00]	0.110
D-dimer, mg/L (median [IQR])	0.94 [0.55, 1.80]	0.86 [0.51, 1.63]	1.40 [0.77, 2.40]	<0.001
Troponin ratio (median [IQR])	1.00 [0.57, 1.79]	0.92 [0.39, 1.34]	2.14 [1.29, 3.43]	<0.001
Procalcitonin, µg/L (median [IQR])	0.20 [0.11, 0.49]	0.15 [0.08, 0.28]	0.40 [0.20, 0.92]	<0.001

ACEi, angiotensin-converting enzyme inhibitor; ALAT, alanintransaminase; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, inter-quartile range; NSAID, nonsteroidal anti-inflammatory drug.

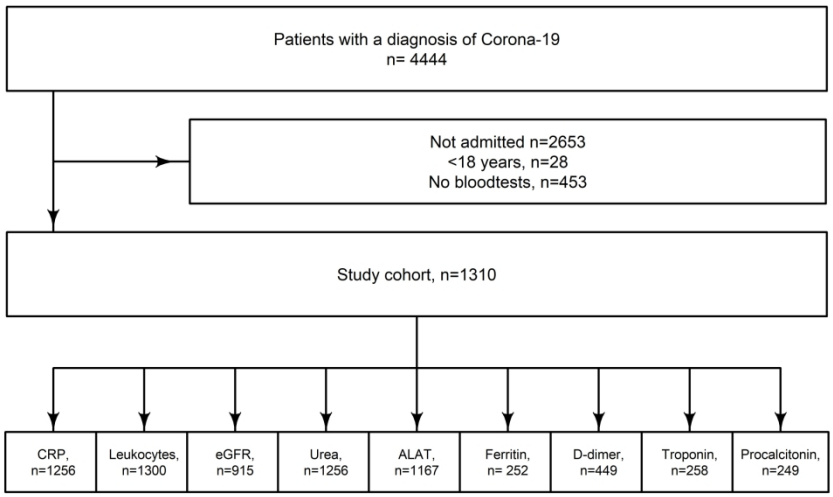

Figure 1: Flowchart of study cohort
Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

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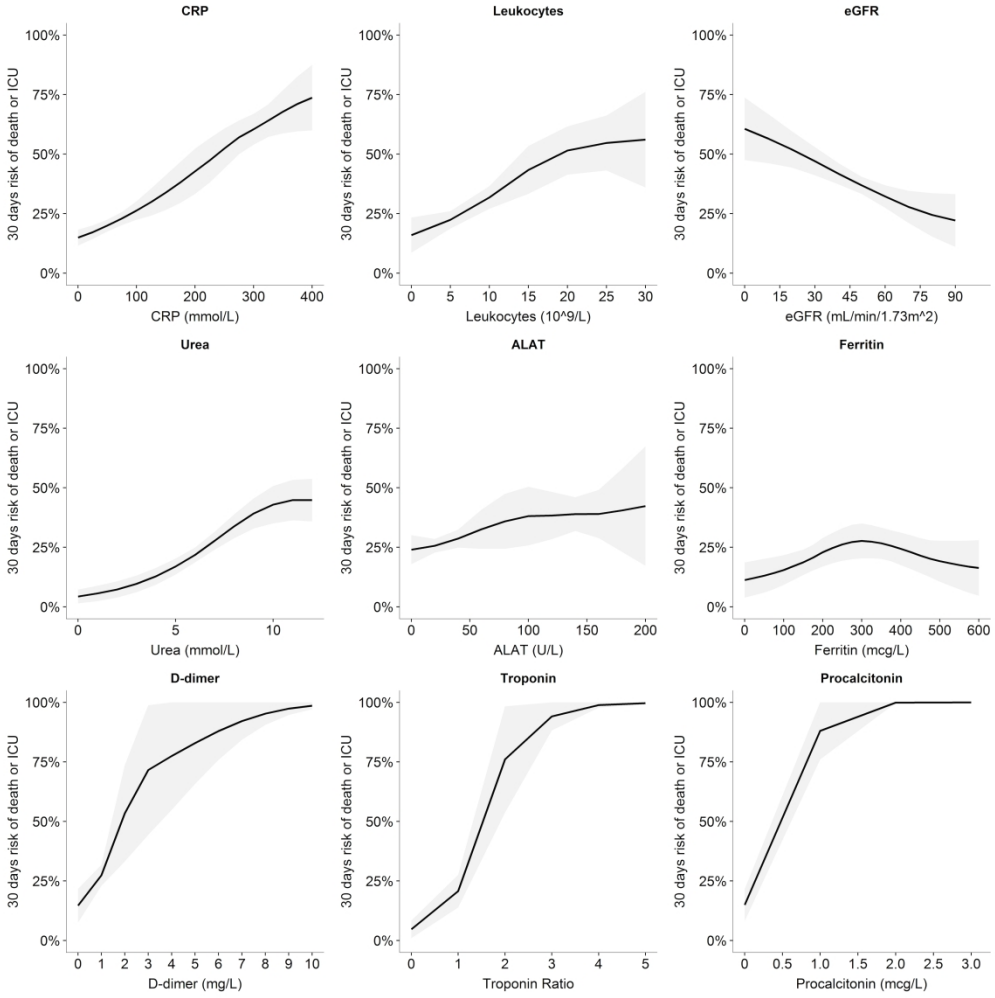

Figure 2: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for age and gender.Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit.

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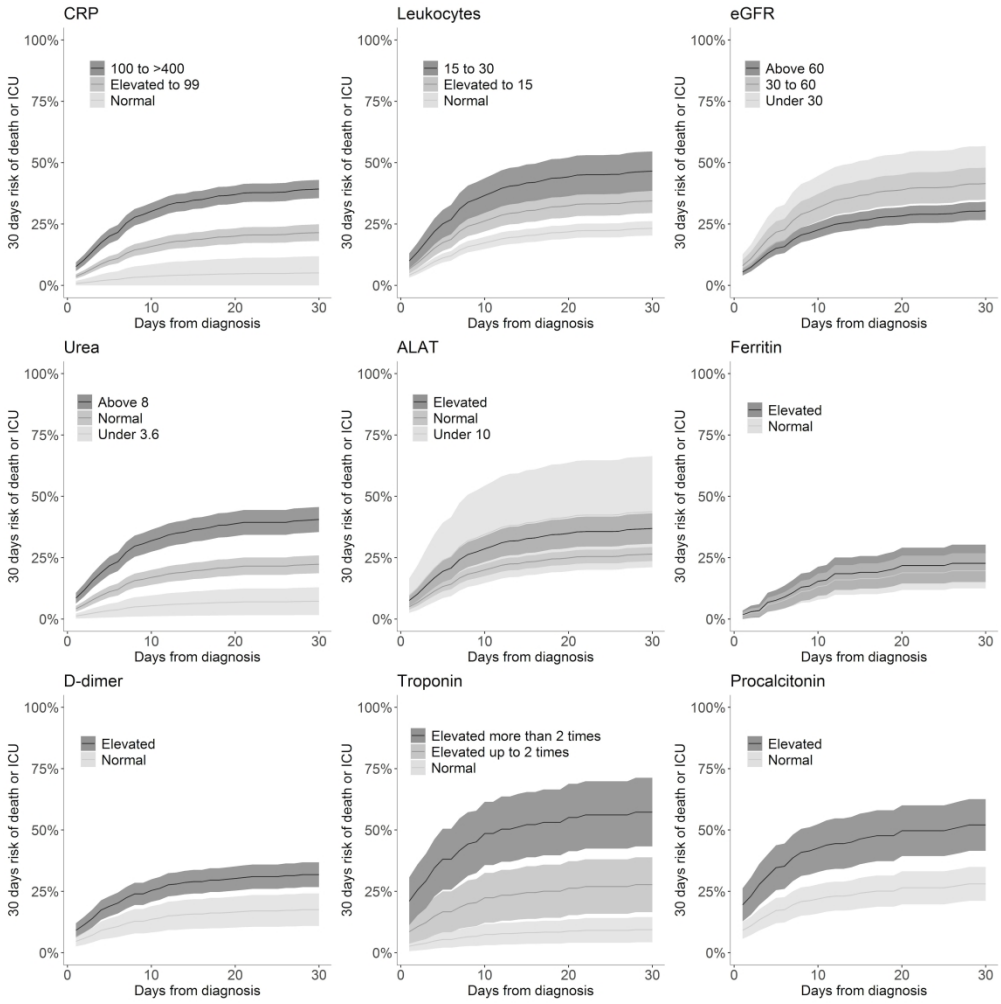


Figure 3: 30-day absolute risk for the composite outcome of death or ICU admission, stratified by normal/elevated ranges, and adjusted for age and gender.Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit.

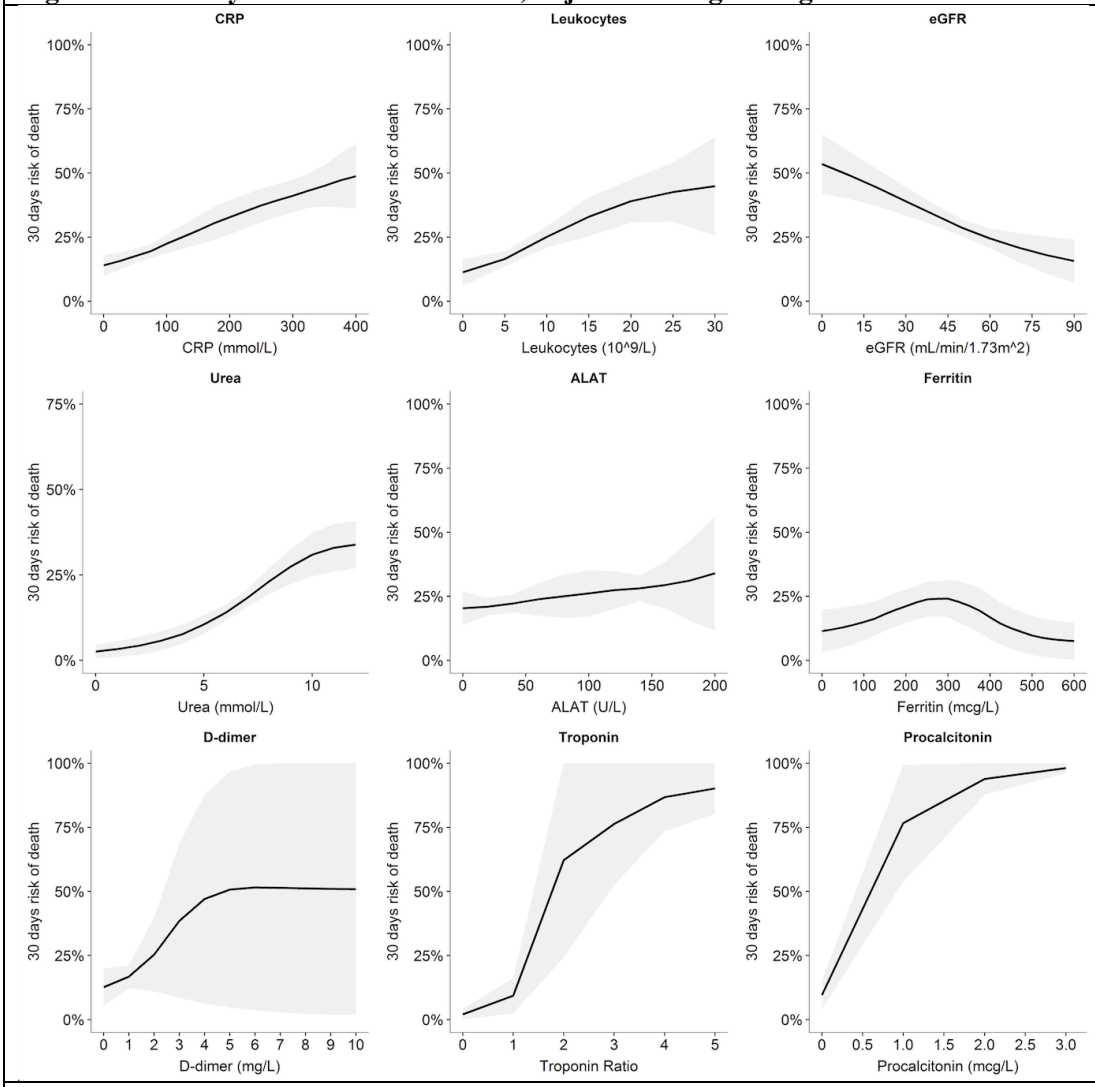
381x381mm (300 x 300 DPI)

Table S1: Characteristics of patients with coronavirus disease 2019 for total cohort, survivors and non-survivors (within 30-days of diagnosis).

	Total	Survivors	Non-survivors	p
Characteristics	1310	1047 (79.9%)	263 (20.1%)	
Age, years (median [IQR])	73.60 [60.50, 81.90]	70.60 [56.55, 79.40]	81.40 [74.10, 86.05]	<0.001
Male sex, n (%)	715 (54.6)	552 (52.7)	163 (62.0)	0.008
Prior ischemic stroke, n (%)	96 (7.3)	66 (6.3)	30 (11.4)	0.008
Prior diabetes, n (%)	221 (16.9)	161 (15.4)	60 (22.8)	0.006
Prior ischemic heart disease, n (%)	165 (12.6)	128 (12.2)	37 (14.1)	0.407
Prior COPD, n (%)	135 (10.3)	93 (8.9)	42 (16.0)	0.001
Prior atrial fibrillation, n (%)	212 (16.2)	139 (13.3)	73 (27.8)	<0.001
Prior chronic kidney disease, n (%)	131 (10.0)	94 (9.0)	37 (14.1)	0.021
Prior hypertension, n (%)	474 (36.2)	358 (34.2)	116 (44.1)	0.003
Prior cancer, n (%)	194 (14.8)	149 (14.2)	45 (17.1)	0.245
Heart failure, n (%)	95 (7.3)	61 (5.8)	34 (12.9)	<0.001
Glimepiride, n (%)	245 (18.7)	184 (17.6)	61 (23.2)	0.042
Aspirin, n (%)	174 (13.3)	122 (11.7)	52 (19.8)	0.001
NSAID, n (%)	140 (10.7)	115 (11.0)	25 (9.5)	0.577
Beta blocker, n (%)	174 (13.3)	122 (11.7)	52 (19.8)	0.001
ACEi, n (%)	203 (15.5)	158 (15.1)	45 (17.1)	0.446
ARB, n (%)	463 (35.3)	361 (34.5)	102 (38.8)	0.195
Loop diuretic, n (%)	217 (16.6)	146 (13.9)	71 (27.0)	<0.001
Thiazide diuretic, n (%)	116 (8.9)	94 (9.0)	22 (8.4)	0.809
CCB, n (%)	241 (18.4)	186 (17.8)	55 (20.9)	0.248
Spironolactones = 1 (%)	69 (5.3)	45 (4.3)	24 (9.1)	0.003
CRP, n (%)	1256 (95.9)	994 (94.9)	262 (99.6)	<0.001
Leucocytes, n (%)	1300 (99.2)	1041 (99.4)	259 (98.5)	0.121
eGFR, n (%)	915 (69.8)	693 (66.2)	222 (84.4)	<0.001
Urea, n (%)	1067 (81.5)	861 (82.2)	206 (78.3)	0.156
ALAT, n (%)	1167 (89.1)	936 (89.4)	231 (87.8)	0.507
Ferritin, n (%)	252 (19.2)	213 (20.3)	39 (14.8)	0.044
D-dimer, n (%)	449 (34.3)	380 (36.3)	69 (26.2)	0.002
Troponin, n (%)	258 (19.7)	223 (21.3)	35 (13.3)	0.003
Procalcitonin, n (%)	249 (19.0)	200 (19.1)	49 (18.6)	0.930
CRP, mg/L (median [IQR])	88.00 [43.00, 160.00]	80.00 [38.00, 143.00]	122.50 [61.25, 210.00]	<0.001
Leucocytes, 10E9/L (median [IQR])	7.40 [5.50, 10.20]	7.20 [5.31, 9.70]	8.50 [6.16, 12.05]	<0.001
eGFR, mL/min/1.73m2 (median [IQR])	73.23 [56.39, 83.55]	74.94 [61.40, 84.27]	62.23 [45.46, 77.93]	<0.001
Urea, mmol/L (median [IQR])	6.50 [4.60, 9.60]	6.00 [4.20, 8.40]	9.80 [7.32, 13.23]	<0.001
ALAT, U/L (median [IQR])	31.00 [21.00, 52.00]	31.50 [21.00, 52.00]	30.00 [20.00, 47.00]	0.177
Ferritin, µg/L (median [IQR])	266.00 [143.00, 446.75]	266.00 [130.00, 449.00]	260.00 [182.50, 388.50]	0.806

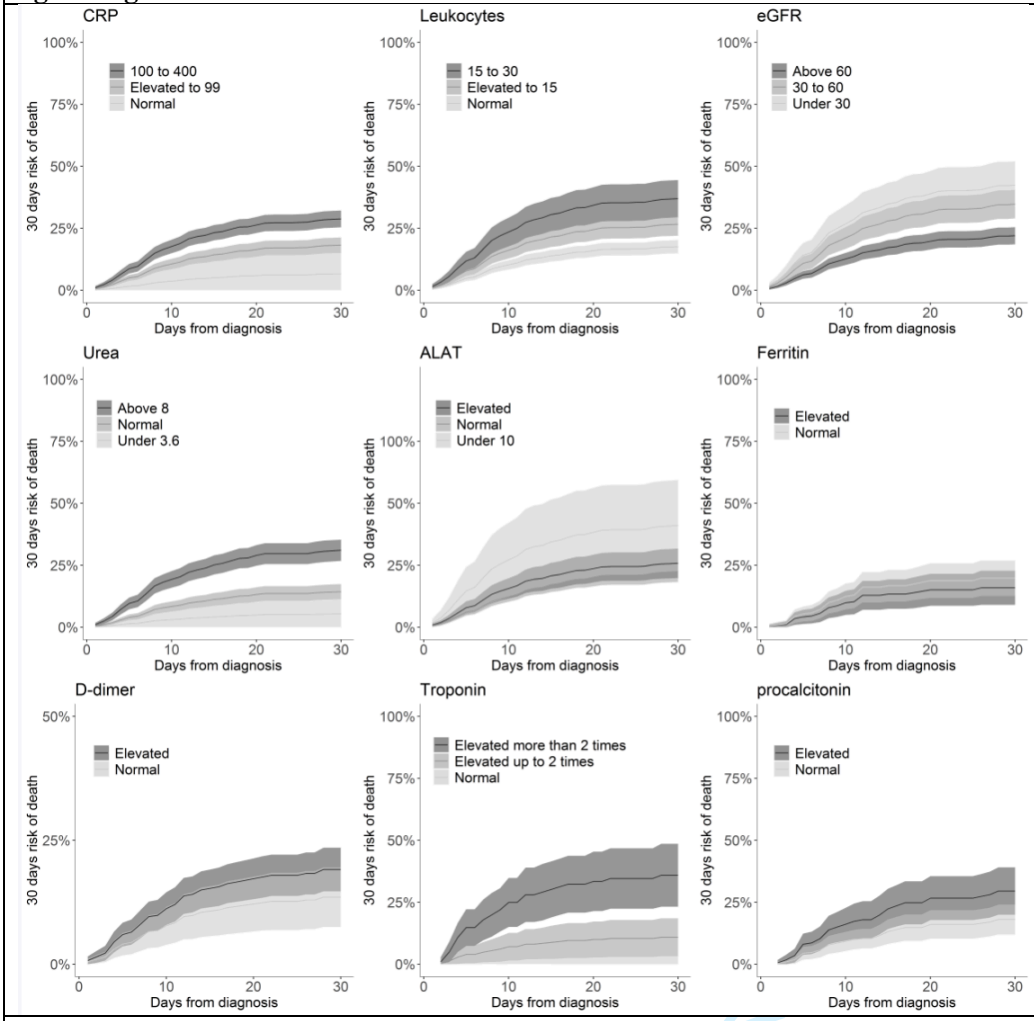
D-dimer, mg/L (median [IQR])	0.94 [0.55, 1.80]	0.90 [0.54, 1.66]	1.40 [0.73, 2.80]	0.001
Troponin ratio (median [IQR])	1.00 [0.57, 1.79]	0.93 [0.45, 1.50]	2.86 [1.82, 4.11]	<0.001
Procalcitonin, µg/L (median [IQR])	0.20 [0.11, 0.49]	0.18 [0.10, 0.40]	0.36 [0.19, 0.92]	<0.001
ACEi, angiotensin-converting enzyme inhibitor; ALAT, alanintransaminase; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, inter-quartile range; NSAID, nonsteroidal anti-inflammatory drug				

Figure S1: 30-day absolute risk of death, adjusted for age and gender.



Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

Figure S2: 30-day absolute risk of death, stratified by normal/elevated ranges, and adjusted for age and gender.



Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-8*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	7-8
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	9-14
		(c) Summarise follow-up time (eg, average and total amount)	9-14
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-14
		(*b*) Report category boundaries when continuous variables were categorized	9-14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-14, supplemental
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	5

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between Biomarkers and COVID-19 Severity and Mortality: A Nationwide Danish Cohort Study

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Association between Biomarkers and COVID-19 Severity and Mortality: A Nationwide Danish Cohort Study

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Abstract

Objective: To evaluate the association between common biomarkers, death and ICU admission in patients with COVID-19.

Design: Retrospective cohort study. From electronic national registry data, we used Cox analysis and bootstrapping to evaluate associations between baseline levels of biomarkers and standardized absolute risks of death/ICU admission, adjusted for age and gender.

Setting: All hospitals in Denmark.

Participants: 1310 patients aged ≥18 years admitted to hospital with COVID-19 from February 27th to May 1st, 2020, with available biochemistry data.

Main outcome measures: A composite of death / ICU admission occurring within 30-days.

Results: Of the 1310 patients admitted to hospital (54.6% male; median age 73.6), 352 (26.9%) experienced the composite endpoint and 263 (20.1%) died. For the composite endpoint, the absolute risks for moderately and severely elevated C-reactive protein (CRP) were significantly higher, 21.5% and 39.2% respectively, compared to 5.0% for those with normal CRP. Moderately and severely elevated leucocytes were significantly higher, 34.5% and 46.6% risk respectively, compared to 23.2% for those with normal leucocytes. Moderately and severely decreased estimated glomerular filtration rate (eGFR) were significantly higher, 41.5% and 45.9% risk respectively, compared to 30.4% for those with normal/mildly decreased eGFR. Normal and elevated urea were significantly higher, 22.3% and 40.6% risk respectively, compared to 7.3% for those with low urea. Elevated D-dimer was significantly higher, 31.8% risk, compared to 17.5% for those with normal D-dimer. Moderately and severely elevated troponins were significantly higher, 27.7% and 57.3% risk respectively, compared to 9.4% for

those with normal troponin. Elevated procalcitonin was significantly higher, 52.1% risk, compared to 28.0% for those with normal procalcitonin.

Conclusion: In this nationwide study of patients admitted with COVID-19, elevated levels of CRP, leucocytes, procalcitonin, urea, troponins and D-dimer, and low levels of eGFR were associated with higher standardized absolute risk of death/ICU admission within 30 days.

Strengths and limitations of this study

- Much of the research concerning COVID-19 describes small case studies from China and Italy, without clearly defined outcomes.
- This study is the first to report the standardized absolute risk of individual laboratory tests on short-term mortality and ICU admission in a relatively large, European cohort of 1310 patients with COVID-19.
- Our study can help the clinician to understand which biomarkers are important in identifying patients with poor prognosis, which may be useful to assess disease severity or to enable early intervention.
- The main limitation of our study is its observational, non-randomized design.
- This study included only patients admitted to the hospital with COVID-19 and measured biochemical data, hence it is likely to represent symptomatic patients at the more severe end of the disease spectrum.

Introduction

Coronavirus disease 2019 (COVID-19) caused by the novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has spread rapidly to become a worldwide pandemic resulting in an enormous strain on healthcare systems globally. As of September 20th, 2020, the number of confirmed cases has surpassed 30 million, affecting 213 countries, although the actual number is likely to be much higher.[1] The clinical course of COVID-19 is variable, but is typically characterized by an initial phase with fever or mild upper respiratory symptoms (though many are asymptomatic). Among hospitalized patients, those with a poor prognosis tend to develop severe viral pneumonia requiring ventilatory support and intensive care unit (ICU) admission.[2] Despite supportive care, a high proportion of patients with COVID-19 suffer rapid deterioration with respiratory failure and death.[3–5] Identifying which patients are at risk of severe disease or death, may be useful in decision making, to determine whether hospitalization or ICU referral is required or to enable early intervention.[6] This is of particular importance given that the fast-pace of the pandemic has led to rationing of scarce resources, most notably mechanical ventilators.[7]

Several studies have demonstrated that older age and chronic diseases are associated with poor outcome in patients with COVID-19.[2,5] Furthermore, in a recently published systematic review, disease severity was associated with more prominent laboratory abnormalities including markers of inflammation and organ damage including elevated troponins, although much of the early research describes small case studies without clearly defined outcomes and the need for further research in more varied cohorts was highlighted.[2,8-11] A recently published study showed that in multivariate analysis, older age, higher Sequential Organ Failure Assessment (SOFA) score, and elevated D-dimer on admission were independently associated with in-hospital death.[5]

The present study aims to expand on these findings and evaluate which biomarkers are associated with death and ICU admission in a large nationwide cohort.

Methods

Data sources

This study is based on four nationwide registers, The Danish National Patient Register, The Civil Registration System, The Danish Registry of Medicinal Product Statistics, and the database on blood samples (LABKA). These four nationwide registers were cross-linked on the individual level using the unique permanent identification number given to all Danish residents at birth or migration.

The Danish National Patient Register holds information on every hospital visit in Denmark, in which each visit is registered with a diagnosis according to the International Classification of Diseases, the 10th revision (ICD-10). The Civil Registration System holds information on the day of birth, sex, and vital status. The Danish Registry of Medicinal Product Statistics contains information on all prescriptions dispensed from Danish pharmacies and is coded according to the Anatomical Therapeutic Chemical (ATC) classification system.[12] The LABKA database holds information on blood samples from all hospital visits, including the emergency departments, outpatient consultations, and admissions to the hospital.

Study design and participants

This study included all laboratory-confirmed COVID-19 patients aged 18 years and older with available biochemistry data, admitted to hospital between February 27th, 2020, and May 1st, 2020 (the first Danish case was recorded on February 27th, 2020). Patients were included on the

first day of diagnosis with COVID-19 and followed for 30 days for the combined endpoint of all-cause mortality and ICU admission. A sub-analysis was also performed for the endpoint of all-cause mortality alone. Blood test results were obtained from electronic registries of laboratory data, with baseline values taken on admission (measured within 24 hours). We focused on readily available laboratory tests associated with inflammation or organ damage, including C-reactive protein (CRP), ferritin, procalcitonin, leucocyte count, estimated glomerular filtration rate (eGFR), urea, alanine aminotransferase (ALAT), D-dimer and troponin (both T and I). The eGFR was calculated using the Modification of Diet in Renal Disease (MDMD) equation, which includes creatinine level, age, race, and sex. In order to compare troponin values with different assays and reference values, a ratio between observed values and highest reference values was performed.

Statistical analysis

Categorical data were presented as counts with percentages, and the statistical difference was tested using Fisher's exact test. Continuous variables were presented as medians with the first and third quartile (Q1 and Q3), and the statistical difference was tested using the Wilcoxon rank-sum test. Cox analysis and bootstrapping with 100 bootstraps were used to derive age and gender adjusted standardized absolute risk and average treatment effects curves with 95% confidence intervals (CI) to evaluate the association between individual biomarkers and the 30-day risk for each endpoint. The above analysis was repeated with stratification by typical normal/elevated ranges used in the clinical setting. For the stratified analysis of troponin the cutoff values were defined as being moderately elevated (>1 to 2 times elevated) and severely elevated (>two times elevated), relative to baseline troponin. As part of a sensitivity analysis, we

performed a Cox multivariate regression analysis to assess CRP in relation to the combined endpoint of all-cause mortality and ICU admission (adjusted for age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease). A two-sided p-value ≤0.05 was considered statistically significant.

Data management and statistical analyses were conducted using R statistics (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.).

Results

A total of 4444 patients with COVID-19 were identified in the study period. We excluded 28 patients aged below 18 years old, 2653 patients who were not admitted to hospital and 453 with no available biochemistry data, leaving 1310 patients for inclusion in the study (54.6% male; median age 73.6). Of these, 352 (26.9%) patients experienced the composite endpoint and 263 (20.1%) died (See Figure 1).

Baseline characteristics for the total cohort and stratified by the composite endpoint, are given in Table 1 (see also Table S1 for baseline characteristics stratified by death and Table S2 stratified according to availability of biochemistry). Patients who experienced the composite endpoint of death/ICU admission within 30-days were more likely to be older, male, with a pre-existing comorbidity (diabetes, chronic obstructive pulmonary disease, atrial fibrillation, hypertension or heart failure); currently receiving treatment with aspirin, beta-blocker, angiotensin II receptor blockers, loop diuretics, calcium channel blockers, or spironolactone; with higher baseline values of leucocytes, urea, D-dimer, troponin or procalcitonin; or lower baseline values of eGFR (p≤0.044 for all). However, the proportion of patients who died or were

admitted to ICU was not significantly different for those with prior ischemic stroke, ischemic heart disease, chronic kidney disease, cancer, or currently receiving treatment with nonsteroidal anti-inflammatory drugs or thiazides.

Biomarkers and Standardized Absolute Risk of Death and Intensive Care Unit Admission

C-reactive protein

Higher baseline CRP was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, moderately elevated (upper reference limit to 99 mmol/L) and severely elevated (100 to 400 mmol/L) baseline CRP were associated with an age and sex adjusted absolute risk of 21.5% (95%CI: 18.1-24.9) and 39.2% (95%CI: 35.6-43.0) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal CRP, absolute risk 5.0% (95%CI: 0.0-12.0) (Figure 3).

Similarly, moderately and severely elevated baseline CRP were associated with an age and sex adjusted absolute risk of 18.1% (95%CI: 15.1-21.3) and 28.8% (95%CI: 25.4-32.1) for 30-day mortality, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal CRP, absolute risk 6.6% (95%CI: 0.0-15.0) (Supplementals, Figure S2).

In a multivariate model, elevated CRP was independently associated with death/ICU admission after adjusting for age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease (Figure 4; Supplementals, Table S3).

Leucocytes

Higher baseline leucocyte count was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, moderately elevated (upper reference limit to 15 x 10^9/L) and severely elevated (15 to 30 x 10^9/L) baseline leucocytes were associated with an age and sex adjusted absolute risk of 34.5% (95%CI: 29.5-39.4) and 46.6% (95%CI: 38.5-54.6) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal leucocytes, absolute risk 23.2% (95%CI: 20.4-22.2) (Figure 3).

In stratified analysis, moderately and severely elevated baseline leucocytes were associated with an age and sex adjusted absolute risk of 26.6% (95%CI: 22.0-31.3) and 37.0% (95%CI: 25.4-32.1) for 30 day-mortality, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal leucocytes, absolute risk 17.6% (95%CI: 15.0-20.2) (Supplementals, Figure S2).

Estimated glomerular filtration rate

Lower baseline eGFR was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, moderately decreased (30 to 60 mmol/L) and severely decreased (0 to 30 mmol/L) baseline eGFR were associated with an age and sex adjusted risk risk of 41.5% (95%CI: 35.1-48.0) and 45.9% (95%CI: 34.9-56.8) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both $p<0.001$) compared to those with normal/mild decreased eGFR (>60 mmol/L), absolute risk 30.4% (95%CI: 26.7-34.1) (Figure 3).

In stratified analysis, moderately elevated and severely elevated baseline leucocytes were associated with an absolute risk of 34.8% (95%CI: 29.0-40.5) and 42.4% (95%CI: 32.9-52.0) for 30-day mortality, respectively, (Figure 3), which was a significantly higher risk (both p<0.001) compared to those with normal/mild decreased eGFR, absolute risk 22.0% (95%CI: 18.5-25.5) (Supplementals, Figure S2).

Urea

Higher baseline urea was associated with higher age and sex adjusted absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, normal (3.6 to 8.0 mmol/L) and elevated (>8 mmol/L) baseline urea were associated with an absolute risk of 22.3% (95%CI: 18.7-25.9) and 40.6% (95%CI: 35.5-45.7) for death/ICU admission within 30 days, respectively, which was a significantly higher risk (both p<0.001) compared to those with low urea, absolute risk 7.3% (95%CI: 1.6-12.9) (Figure 3).

In stratified analysis, normal and elevated baseline urea were associated with an absolute risk of 15.7% (95%CI: 11.2-17.4) and 31.9% (95%CI: 26.8-35.4) for 30-day mortality, respectively, which was a significantly higher risk (both p<0.001) compared to those with low urea, absolute risk 5.4% (95%CI: 0.0-11.0) (Supplementals, Figure S2).

Alanine aminotransferase

Higher baseline ALAT was associated with slightly higher absolute risk of death/ICU admission (Figure 2), and death alone (Supplementals, Figure S1).

In stratified analysis, elevated (>upper reference limit) baseline ALAT was associated with an absolute risk of 36.9% (95%CI: 30.7-43.1) for death/ICU admission within 30 days, which was significantly higher than normal ALAT (10 U/L to upper reference limit), absolute risk 26.9% (95%CI: 23.6-29.3, p=0.002), however not significantly different to low ALAT (<10 U/L), absolute risk 43.8% (95%CI: 21.1-66.4) (Figure 3).

In stratified analysis, low baseline ALAT was associated with an absolute risk of 41.0% (95%CI: 22.7-59.4) for 30-day mortality, which was significantly higher than normal ALAT, absolute risk 20.8% (95%CI: 18.2-23.4, p=0.03), however not significantly different to high ALAT, absolute risk 25.8% (95%CI: 19.9-31.8) (Supplementals, Figure S2).

Ferritin

Higher baseline ferritin was associated with an inverted U shaped risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, elevated (> 300 mmol/L) baseline ferritin was associated with an age and sex adjusted absolute risk of 22.7% (95%CI: 15.1-30.3) for death/ICU admission within 30 days, which was not a significantly higher risk compared to those with normal ferritin (≤300 mmol/L), absolute risk 19.7% (95%CI: 12.5-32.9) (Figure 3).

In stratified analysis, elevated (>300 mmol/L) baseline ferritin was associated with an age and sex adjusted risk of 15.9% (95%CI: 0.09-22.8) for 30-day mortality, which was not a significantly higher risk compared to those with normal ferritin (≤300 mmol/L), absolute risk 19.8% (95%CI: 12.6-26.9) (Supplementals, Figure S2).

D-dimer

Higher baseline D-dimer was associated with an age and sex adjusted higher risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, elevated (>0.5 mg/L) baseline D-dimer was associated with an age and sex adjusted absolute risk of 31.8% (95%CI: 26.7-36.8) for death/ICU admission within 30 days, which was a significantly higher risk ($p<0.001$) compared to those with normal D-dimer, absolute risk 17.5% (95%CI: 10.9-24.1) (Figure 3).

In stratified analysis, elevated baseline D-dimer was associated with an age and sex adjusted risk of 19.1% (95%CI: 14.7-23.5) for 30-day mortality, which was a significantly higher risk ($p<0.001$) compared to those with normal D-dimer, absolute risk 13.5% (95%CI: 8.5-19.5) (Supplementals, Figure S2).

Troponin

Higher baseline troponin ratio was associated with age and sex adjusted higher risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, moderately elevated and severely elevated baseline troponin were associated with an age and sex adjusted absolute risk of 27.7% (95%CI: 16.5-38.9) and 57.3% (95%CI: 43.3-71.3) for death/ICU admission within 30 days, respectively, which was a significantly higher absolute risk ($p=0.003$ and $p<0.001$, respectively) compared to those with normal troponins, absolute risk 9.4% (95%CI: 4.2-14.5) (Figure 3).

In stratified analysis, moderately elevated and severely elevated baseline troponin were associated with an age and sex adjusted absolute risk of 10.8% (95%CI: 3.2-18.5) and 35.9% (95%CI: 23.2-48.6) for 30-day mortality, respectively. However, only the later was a

significantly higher risk (p<0.001) compared to those with normal troponins, absolute risk 3.9% (95%CI: 0.0-8.7) (Supplementals, Figure S2).

Procalcitonin

Higher baseline procalcitonin was associated with age and sex adjusted higher risk of death/ICU admission (Figure 2), and risk of death alone (Supplementals, Figure S1).

In stratified analysis, elevated (>0.5 mcg/L) baseline procalcitonin was associated with an age and sex adjusted risk of 52.1% (95%CI: 41.5-62.6) for death/ICU admission within 30 days, which was a significantly higher risk (p<0.001) compared to those with normal procalcitonin, absolute risk 28.0% (95%CI: 21.1-34.9) (Figure 3).

In stratified analysis, elevated baseline procalcitonin was associated with an age and sex adjusted absolute risk of 29.5% (95%CI: 19.9-39.0) for 30-day mortality, which was a significantly higher absolute risk (p=0.03) compared to those with normal procalcitonin, absolute risk 18.0% (95%CI: 12.0-24.0) (Supplementals, Figure S2).

Discussion

This nationwide study is the first of its kind to examine the association between common biomarkers and risk of early death and ICU admission in adult patients admitted to hospital with laboratory-confirmed COVID-19. In particular, the inflammatory markers CRP, leucocytes and procalcitonin, and markers of organ damage including eGFR, troponins and D-dimer are associated with higher risk of death/ICU admission within 30 days. However, the association between ferritin and ALAT was non-significant.

The novelty of the SARS-CoV-2, coupled with the rapid spread of the COVID-19 pandemic, has led to a tremendous burden on healthcare systems worldwide. To provide optimal care for patients, early diagnosis and identification of vulnerable patients who are at risk of severe disease is needed, as well as recognizing patients who may rapidly deteriorate and require ICU admission and mechanical ventilation. This relies on an accurate knowledge of the critical clinical predictors for disease progression in order to triage patients and allocate scarce resources efficiently. This is especially important when considering which patients should start treatment. For example, in the first trial to offer an effective treatment for COVID-19 disease, results showed that early administration of antiviral treatment with Remdesivir was superior to placebo in reducing time to recovery and in particular, the authors highlighted the need to start antiviral treatment before pulmonary disease progresses to require mechanical ventilation.[13]

In the present study, patients who died or were admitted to ICU were more likely to be older, male, and with a pre-existing comorbidity which is consistent with observed global trends. Our results are aligned with a recent large cohorts of patients hospitalized with COVID-19 in the UK and US, as well as meta-analysis of studies from China.[2,14] The median age of patients who died in our study was 81 years, similar to the values noted in the UK study (median 80 years) but much older compared to patients included in the early studies from the Wuhan region. Similarly, we reported a greater proportion death/ICU admission among males (64%) and patients with pre-existing comorbidity (prior diabetes, COPD, atrial fibrillation and hypertension). Differences are most likely due to a variety of factors including regional differences concerning demographics, preparedness and knowledge of COVID-19 that have drawn on experience from China and other countries as the outbreak has spread.

In terms of clinical laboratory biomarkers, recent systematic reviews of the literature have highlighted many of the challenges inherent in the early studies of COVID-19, as many of the early studies are limited by the small size of the study populations with regional bias (most data is confined to China and Italy), and with limited or poorly defined outcomes.[6,11] Several studies have evaluated level of individual biomarkers in small and selected COVID cohorts. An Italian study including 239 patients, reported that several laboratory parameters including elevated lymphocytes, procalcitonin, interleukin-6, ferritin, CRP, and ALAT was associated with death and ICU admission in unadjusted Cox analysis. However, in adjusted analysis CRP was the only biomarker associated with increased risk of death/ICU admission.[15]

An early meta-analysis identified a range of abnormal biomarkers that was elevated in COVID-19 patients who died, including elevated levels of inflammatory markers and acute phase reactants such as interleukin-6, CRP, ferritin, lymphopenia, as well as reduced CD4 and CD8 counts and coagulation abnormalities including prolonged PT, increased D-dimer and thrombocytopenia.[16] We were able to expand on these findings, reporting age and gender adjusted absolute risk of death/ICU admission of individual biomarkers in a larger, European cohort. In the present study inflammatory markers such as CRP, leucocytes and procalcitonin, and markers of organ damage including eGFR, urea, troponins and D-dimer were associated with higher risk of death/ICU admission within 30 days. However, the association between ferritin and ALAT was non-significant. The association with inflammatory markers, and acute phase reactants likely reflects the cytokine storm associated with severe infection and subsequent end-organ damage from severe sepsis.[17] However, it is also essential to recognize that the acute phase reactants are non-specific markers of inflammation. For example, procalcitonin secretion is not induced by gamma-interferon (produced mainly in response to viral infections), making it

primarily an attractive marker of bacterial infections.[18] Nevertheless, bacterial superinfection is an important consideration in COVID-19; for example, in the study by Wang et al., 81.7% of patients who died with COVID-19 had an associated bacterial infection.[19] Probably, patients with longer and more complex admissions or ICU treatment are more vulnerable to secondary infections e.g. ventilator acquired infection, with an expected rise in procalcitonin levels. Accordingly, Zhou et al. observed that half of the non-survivors experienced a secondary infection, with almost a third due to ventilator-associated pneumonia.[5] However, the association between higher absolute risk of death/ICU admission and elevated baseline procalcitonin in the present study suggests that early concomitant bacterial infection (not only secondary infection) is a significant factor in adverse prognosis for patients with COVID-19 that clinicians should be aware of.

In the study including 191 patients from Wuhan region, leucocytes, older age, higher Sequential Organ Failure Assessment (SOFA) score, and a higher D-dimer (greater than 1 µg/L on admission) were all associated with in-hospital mortality in patients with COVID-19.[5] Although the SOFA score is mainly a diagnostic marker of sepsis and septic shock, it may reflect the state and degree of multi-organ dysfunction due to infection in general.[20] In the present study, abnormal values of biomarkers reflecting organ damage including troponin, eGFR, urea, and D-dimer were all associated with higher absolute risk of 30-day mortality and ICU admission. Myocarditis is a known morbidity amongst patients with COVID-19.[21] Elevated troponin may reflect myocardial injury among COVID-19 patients, including direct damage to the cardiomyocytes, systemic inflammation, myocardial interstitial fibrosis, coronary plaque destabilization and hypoxia.[21] Furthermore, patients with cardiovascular disease are more likely to be admitted with COVID-19, with greater risk of cardiac involvement as their symptoms

develop.[22] With respect to the abnormalities observed in D-dimer, research suggests that respiratory failure in COVID-19 is not only related to development of the acute respiratory distress syndrome (ARDS), but also due to microvascular thrombotic processes, which are associated with elevated D-dimer.[23] Finally, beyond the high mortality observed in those with organ dysfunction and respiratory failure, a generalized coagulopathy has also been noted in those with poor prognosis.[24]

Strengths and limitations:

The main strength of this study is the completeness of data from a nationwide, European cohort and the avoidance of selection bias resulting from race, age, sex, socioeconomic status, affiliation to selected hospitals, or healthcare systems. The Danish National Patient Registry and the Danish Registry of Medicinal Product Statistics are known to be accurate.[25,26] The main limitation of our study is its observational non-randomized design. There is a lack of information about important clinical parameters, including blood pressure, body mass index, and smoking habits.

This study included only patients admitted to the hospital with COVID-19 and with measured baseline biochemical data. Therefore, it is likely to represent symptomatic patients, who are more likely to be elderly or with more comorbidities and at the more severe end of the disease spectrum. Furthermore, patients in the cohort may present to hospital at differing stages of their disease. A large proportion of patients with confirmed COVID-19 in Denmark had missing biochemistry data, which most likely represents patients who attended the emergency room with mild symptoms, which did not warrant admission or blood tests, and were not included in this study, thus leading to selection bias and limiting generalisability. Some

biomarkers (particularly D-dimer, troponin and procalcitonin) are likely to be measured in those with the most severe disease (confounding by indication).

Conclusions:

In this nationwide study of patients admitted with COVID-19, elevated levels of CRP, leucocytes, procalcitonin, urea, troponins and D-dimer, and low levels of eGFR were associated with higher standardized absolute risk of death/ICU admission within 30 days.

Footnotes

Contributors: Authors GH, JP, AMS, PM, MA, MK, KK, LK, GG, CTP, CB made substantial contributions to the conception and design of the work. Author JP was primarily responsible for the acquisition and analysis, and authors CB, GH and JP were primarily responsible for interpretation of data for the work. All authors were involved in the drafting, revision and final approval of the published version and agree to be accountable for all aspects of the work.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no competing interests with regards to the submitted work.

Ethical approval: In Denmark, retrospective register studies do not require approval from the ethics committees or individual informed consent. The data responsible institute (The Capital Region of Denmark) approved this study (ref. no.: P-2019-191), in line with the General Data Protection Regulation (GDPR) and The Data Protection Act. Data were made available in a pseudonymized format such that specific individuals could not be identified. Dissemination to study patients is not possible/applicable.

Patient and Public Involvement: This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Transparency declaration: The lead authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data availability statement: No additional data are available.

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Tables

Table 1: Characteristics of patients with coronavirus disease 2019 for total cohort, and stratified by death/ICU admission (within 30-days of diagnosis).

	Total	No Death/ICU admission	Death/ICU admission	p
Characteristics	1310	958 (73.1)	352 (26.9)	
Age, years (median [IQR])	73.60 [60.50, 81.90]	71.15 [56.52, 79.80]	77.50 [70.18, 84.53]	<0.001
Male sex, n (%)	715 (54.6)	489 (51.0)	226 (64.2)	<0.001
Prior comorbidities:				
Ischemic stroke, n (%)	96 (7.3)	63 (6.6)	33 (9.4)	0.094
Diabetes, n (%)	221 (16.9)	145 (15.1)	76 (21.6)	0.008
Ischemic heart disease, n (%)	165 (12.6)	116 (12.1)	49 (13.9)	0.398
COPD, n (%)	135 (10.3)	86 (9.0)	49 (13.9)	0.010
Atrial fibrillation, n (%)	212 (16.2)	132 (13.8)	80 (22.7)	<0.001
Chronic kidney disease, n (%)	131 (10.0)	88 (9.2)	43 (12.2)	0.119
Hypertension, n (%)	474 (36.2)	323 (33.7)	151 (42.9)	0.002
Cancer, n (%)	194 (14.8)	140 (14.6)	54 (15.3)	0.727
Heart failure, n (%)	95 (7.3)	60 (6.3)	35 (9.9)	0.030
Prior medication				
Glimepiride, n (%)	245 (18.7)	164 (17.1)	81 (23.0)	0.017
Aspirin, n (%)	174 (13.3)	111 (11.6)	63 (17.9)	0.004
NSAID, n (%)	140 (10.7)	103 (10.8)	37 (10.5)	1.000
Beta blocker, n (%)	174 (13.3)	111 (11.6)	63 (17.9)	0.004
ACEi, n (%)	203 (15.5)	144 (15.0)	59 (16.8)	0.439
ARB, n (%)	463 (35.3)	323 (33.7)	140 (39.8)	0.044
Loop diuretic, n (%)	217 (16.6)	138 (14.4)	79 (22.4)	0.001
Thiazide diuretic, n (%)	116 (8.9)	87 (9.1)	29 (8.2)	0.742
CCB, n (%)	241 (18.4)	157 (16.4)	84 (23.9)	0.003
Spironolactones, n (%)	69 (5.3)	41 (4.3)	28 (8.0)	0.012
Baseline laboratory values				
CRP, n (%)	1256 (95.9)	906 (94.6)	350 (99.4)	<0.001
Leucocytes, n (%)	1300 (99.2)	952 (99.4)	348 (98.9)	0.472
eGFR, n (%)	915 (69.8)	619 (64.6)	296 (84.1)	<0.001
Urea, n (%)	1067 (81.5)	781 (81.5)	286 (81.2)	0.936
ALAT, n (%)	1167 (89.1)	850 (88.7)	317 (90.1)	0.549
Ferritin, n (%)	252 (19.2)	205 (21.4)	47 (13.4)	0.001
D-dimer, n (%)	449 (34.3)	332 (34.7)	117 (33.2)	0.646
Troponin, n (%)	258 (19.7)	197 (20.6)	61 (17.3)	0.210
Procalcitonin, n (%)	249 (19.0)	164 (17.1)	85 (24.1)	0.005
CRP, mg/L (median [IQR])	88.00 [43.00, 160.00]	74.50 [35.25, 130.00]	131.00 [68.25, 218.00]	<0.001

Leucocytes, 10E9/L (median [IQR])	7.40 [5.50, 10.20]	7.10 [5.30, 9.60]	8.40 [6.12, 11.90]	<0.001
eGFR, mL/min/1.73m2 (median [IQR])	73.23 [56.39, 83.55]	74.80 [60.97, 84.17]	66.45 [48.43, 81.33]	<0.001
Urea, mmol/L (median [IQR])	6.50 [4.60, 9.60]	5.90 [4.20, 8.30]	9.05 [6.60, 12.47]	<0.001
ALAT, U/L (median [IQR])	31.00 [21.00, 52.00]	31.00 [21.00, 51.00]	32.00 [22.00, 53.00]	0.169
Ferritin, µg/L (median [IQR])	266.00 [143.00, 446.75]	264.00 [130.00, 439.00]	311.00 [189.50, 464.00]	0.110
D-dimer, mg/L (median [IQR])	0.94 [0.55, 1.80]	0.86 [0.51, 1.63]	1.40 [0.77, 2.40]	<0.001
Troponin ratio (median [IQR])	1.00 [0.57, 1.79]	0.92 [0.39, 1.34]	2.14 [1.29, 3.43]	<0.001
Procalcitonin, µg/L (median [IQR])	0.20 [0.11, 0.49]	0.15 [0.08, 0.28]	0.40 [0.20, 0.92]	<0.001

ACEi, angiotensin-converting enzyme inhibitor; ALAT, alanintransaminase; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, inter-quartile range; NSAID, nonsteroidal anti-inflammatory drug.

Figure caption:

Figure 1: Flowchart of study cohort.

Figure 2: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for age and gender.

Figure 3: 30-day absolute risk for the composite outcome of death or ICU admission, stratified by normal/elevated ranges, and adjusted for age and gender.

Figure 4: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for CRP level, age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease.

Figure S1: 30-day absolute risk of death, adjusted for age and gender.

Figure S2: 30-day absolute risk of death, stratified by normal/elevated ranges, and adjusted for age and gender.

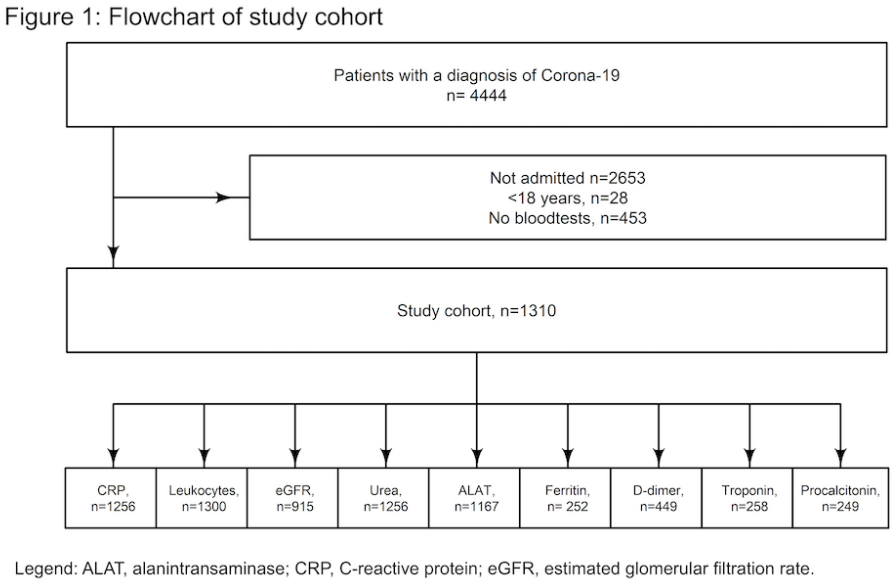


Figure 1: Flowchart of study cohortLegend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

86x51mm (300 x 300 DPI)

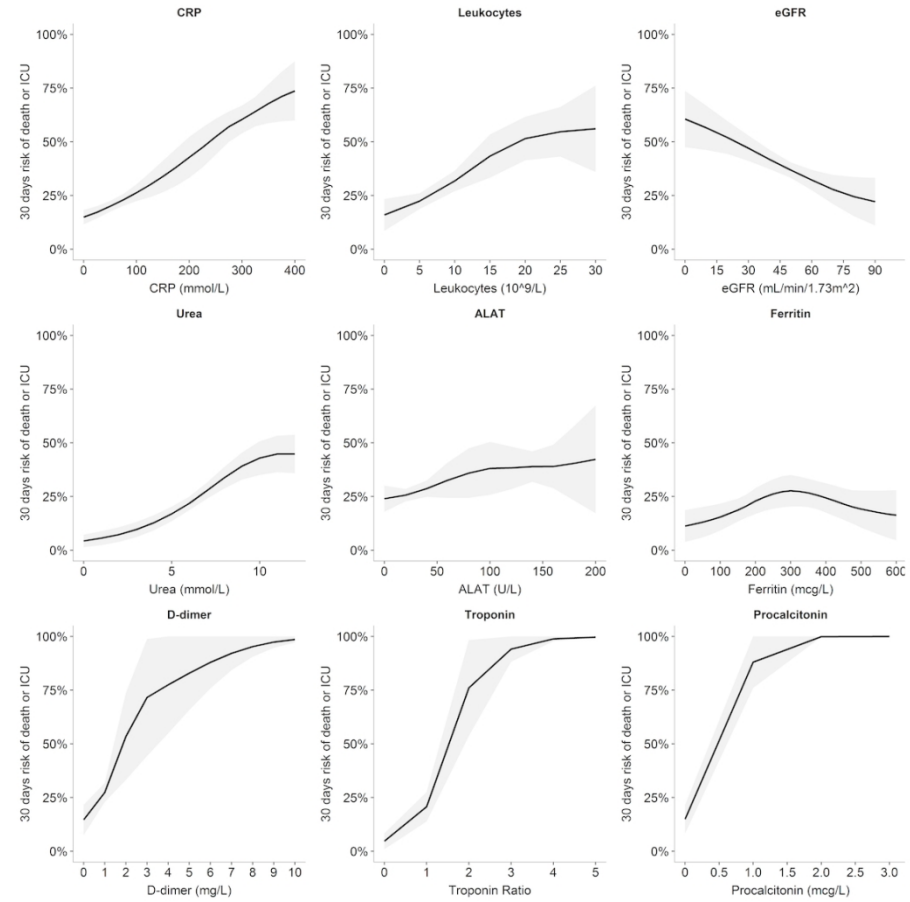


Figure 2: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for age and gender. Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit.

108x108mm (300 x 300 DPI)

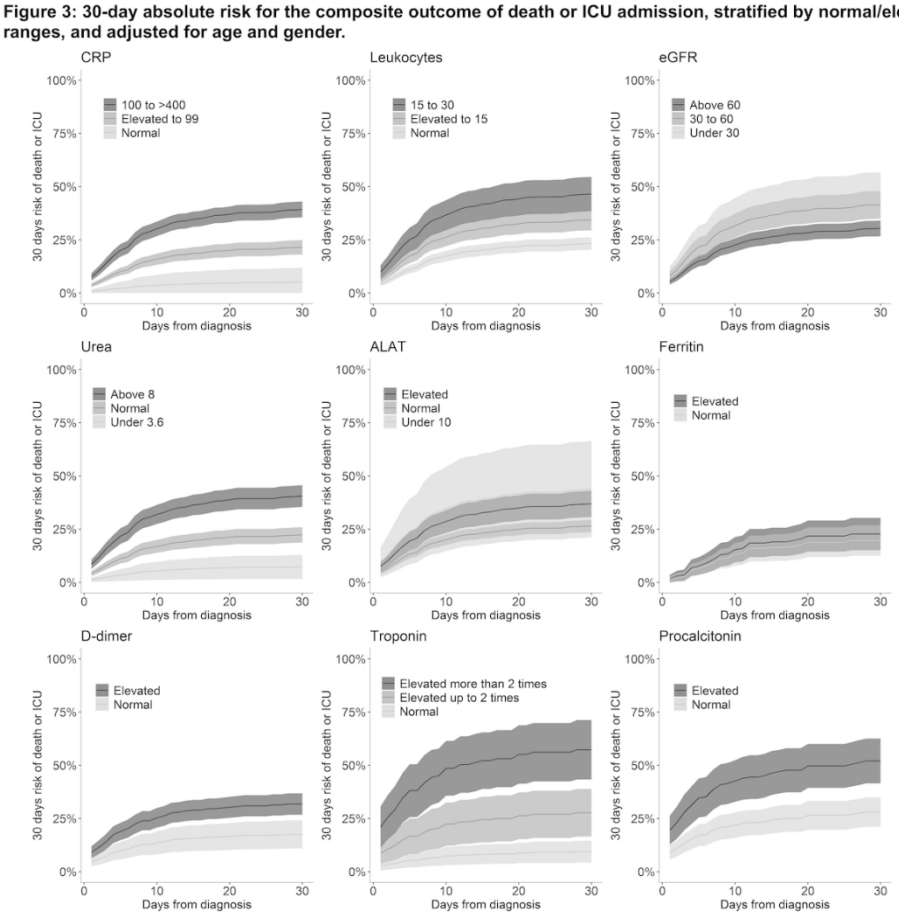
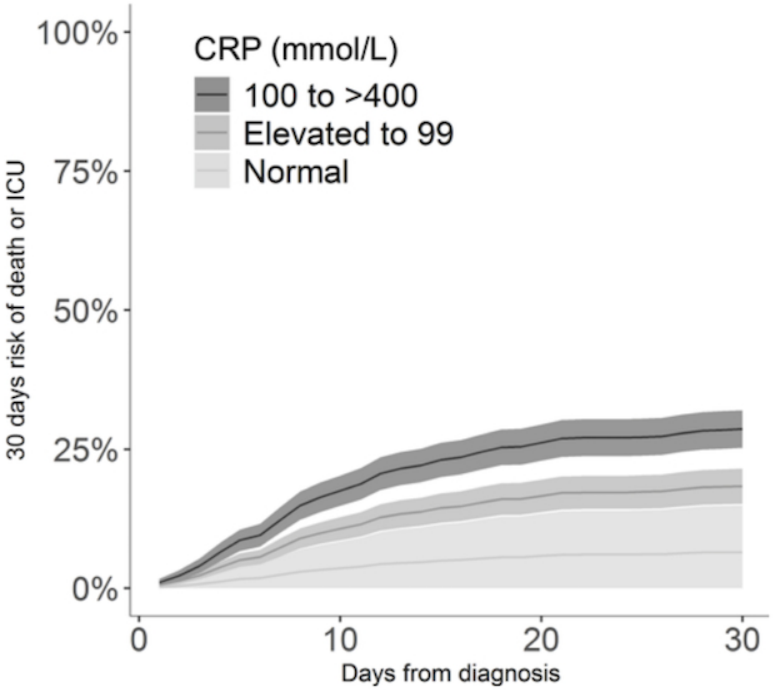


Figure 3: 30-day absolute risk for the composite outcome of death or ICU admission, stratified by normal/elevated ranges, and adjusted for age and gender. Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit.

108x108mm (300 x 300 DPI)

Figure 4: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for CRP level, age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease.

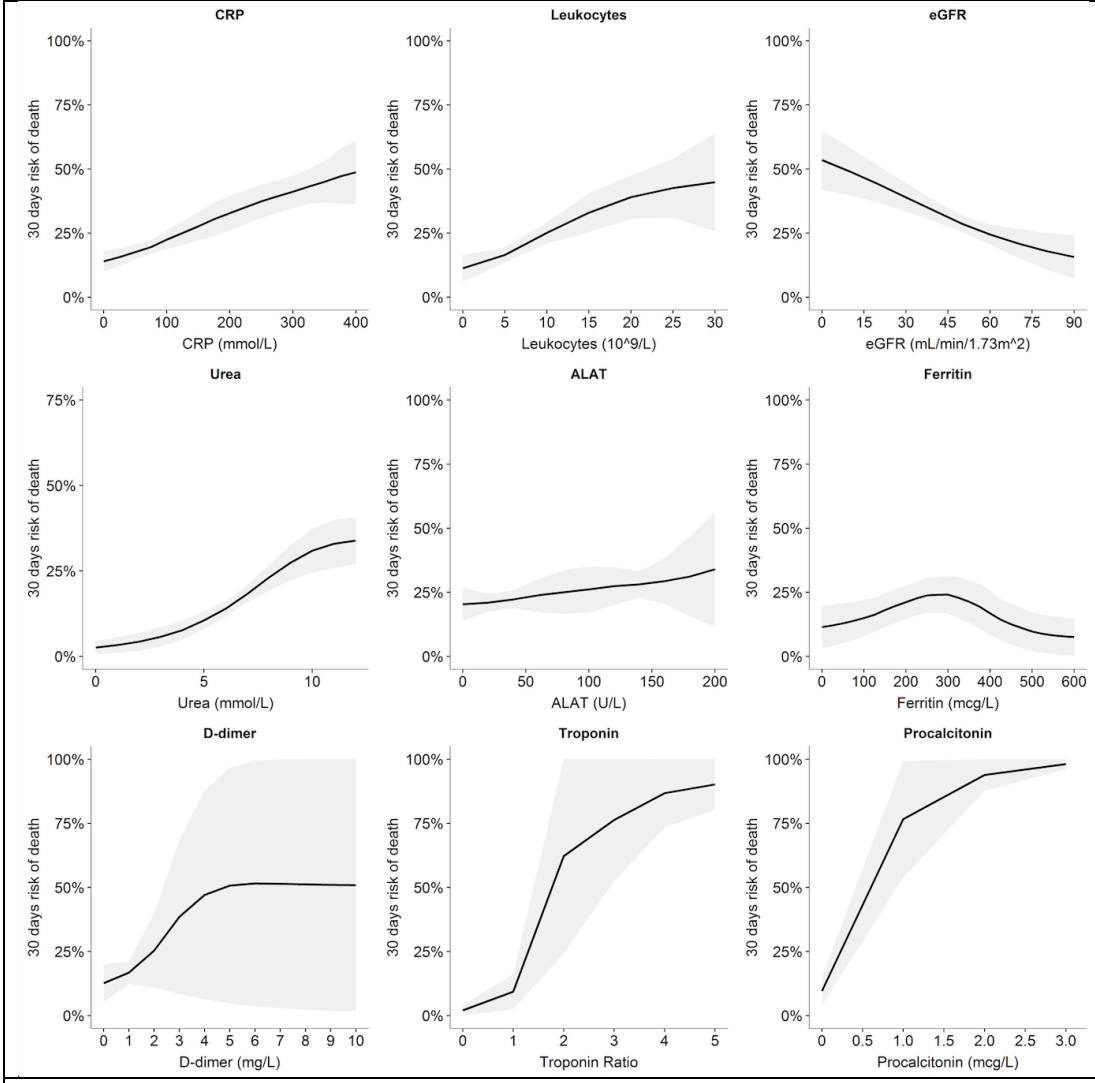


Legend: CRP, C-reactive protein; ICU, intensive care unit.

Figure 4: 30-day absolute risk for the composite outcome of death or ICU admission, adjusted for CRP level, age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease.
Legend: CRP, C-reactive protein; ICU, intensive care unit.

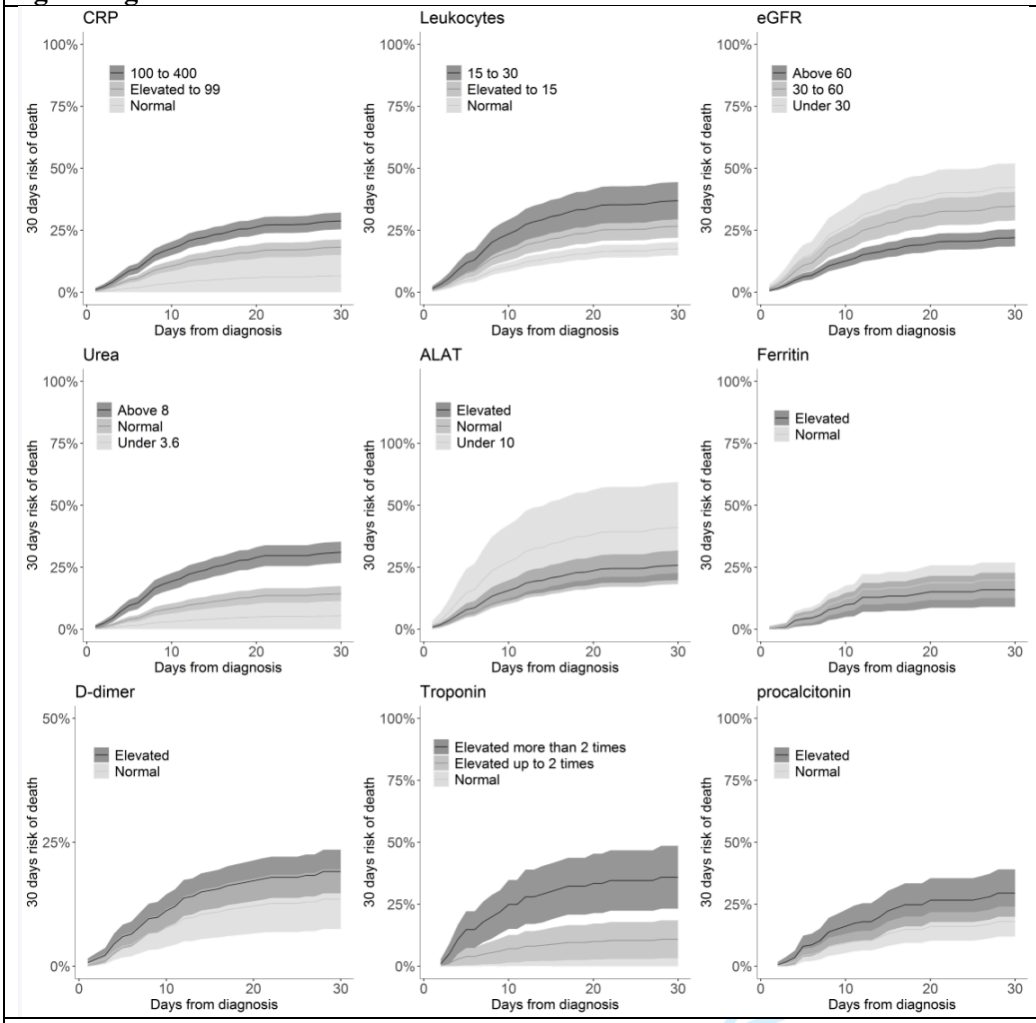
67x60mm (300 x 300 DPI)

Figure S1: 30-day absolute risk of death, adjusted for age and gender.



Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

Figure S2: 30-day absolute risk of death, stratified by normal/elevated ranges, and adjusted for age and gender.



Legend: ALAT, alanintransaminase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

Table S1: Characteristics of patients with coronavirus disease 2019 for total cohort, survivors and non-survivors (within 30-days of diagnosis).

	Total	Survivors	Non-survivors	p
Characteristics	1310	1047 (79.9%)	263 (20.1%)	
Age, years (median [IQR])	73.60 [60.50, 81.90]	70.60 [56.55, 79.40]	81.40 [74.10, 86.05]	<0.001
Male sex, n (%)	715 (54.6)	552 (52.7)	163 (62.0)	0.008
Prior ischemic stroke, n (%)	96 (7.3)	66 (6.3)	30 (11.4)	0.008
Prior diabetes, n (%)	221 (16.9)	161 (15.4)	60 (22.8)	0.006
Prior ischemic heart disease, n (%)	165 (12.6)	128 (12.2)	37 (14.1)	0.407
Prior COPD, n (%)	135 (10.3)	93 (8.9)	42 (16.0)	0.001
Prior atrial fibrillation, n (%)	212 (16.2)	139 (13.3)	73 (27.8)	<0.001
Prior chronic kidney disease, n (%)	131 (10.0)	94 (9.0)	37 (14.1)	0.021
Prior hypertension, n (%)	474 (36.2)	358 (34.2)	116 (44.1)	0.003
Prior cancer, n (%)	194 (14.8)	149 (14.2)	45 (17.1)	0.245
Heart failure, n (%)	95 (7.3)	61 (5.8)	34 (12.9)	<0.001
Glimepiride, n (%)	245 (18.7)	184 (17.6)	61 (23.2)	0.042
Aspirin, n (%)	174 (13.3)	122 (11.7)	52 (19.8)	0.001
NSAID, n (%)	140 (10.7)	115 (11.0)	25 (9.5)	0.577
Beta blocker, n (%)	174 (13.3)	122 (11.7)	52 (19.8)	0.001
ACEi, n (%)	203 (15.5)	158 (15.1)	45 (17.1)	0.446
ARB, n (%)	463 (35.3)	361 (34.5)	102 (38.8)	0.195
Loop diuretic, n (%)	217 (16.6)	146 (13.9)	71 (27.0)	<0.001
Thiazide diuretic, n (%)	116 (8.9)	94 (9.0)	22 (8.4)	0.809
CCB, n (%)	241 (18.4)	186 (17.8)	55 (20.9)	0.248
Spironolactones = 1 (%)	69 (5.3)	45 (4.3)	24 (9.1)	0.003
CRP, n (%)	1256 (95.9)	994 (94.9)	262 (99.6)	<0.001
Leucocytes, n (%)	1300 (99.2)	1041 (99.4)	259 (98.5)	0.121
eGFR, n (%)	915 (69.8)	693 (66.2)	222 (84.4)	<0.001
Urea, n (%)	1067 (81.5)	861 (82.2)	206 (78.3)	0.156
ALAT, n (%)	1167 (89.1)	936 (89.4)	231 (87.8)	0.507
Ferritin, n (%)	252 (19.2)	213 (20.3)	39 (14.8)	0.044
D-dimer, n (%)	449 (34.3)	380 (36.3)	69 (26.2)	0.002
Troponin, n (%)	258 (19.7)	223 (21.3)	35 (13.3)	0.003
Procalcitonin, n (%)	249 (19.0)	200 (19.1)	49 (18.6)	0.930
CRP, mg/L (median [IQR])	88.00 [43.00, 160.00]	80.00 [38.00, 143.00]	122.50 [61.25, 210.00]	<0.001
Leucocytes, 10E9/L (median [IQR])	7.40 [5.50, 10.20]	7.20 [5.31, 9.70]	8.50 [6.16, 12.05]	<0.001
eGFR, mL/min/1.73m2 (median [IQR])	73.23 [56.39, 83.55]	74.94 [61.40, 84.27]	62.23 [45.46, 77.93]	<0.001
Urea, mmol/L (median [IQR])	6.50 [4.60, 9.60]	6.00 [4.20, 8.40]	9.80 [7.32, 13.23]	<0.001
ALAT, U/L (median [IQR])	31.00 [21.00, 52.00]	31.50 [21.00, 52.00]	30.00 [20.00, 47.00]	0.177
Ferritin, µg/L (median [IQR])	266.00 [143.00, 446.75]	266.00 [130.00, 449.00]	260.00 [182.50, 388.50]	0.806

D-dimer, mg/L (median [IQR])	0.94 [0.55, 1.80]	0.90 [0.54, 1.66]	1.40 [0.73, 2.80]	0.001
Troponin ratio (median [IQR])	1.00 [0.57, 1.79]	0.93 [0.45, 1.50]	2.86 [1.82, 4.11]	<0.001
Procalcitonin, µg/L (median [IQR])	0.20 [0.11, 0.49]	0.18 [0.10, 0.40]	0.36 [0.19, 0.92]	<0.001
ACEi, angiotensin-converting enzyme inhibitor; ALAT, alanintransaminase; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IQR, inter-quartile range; NSAID, nonsteroidal anti-inflammatory drug				

Table S2: Characteristics of all patients with coronavirus disease 2019 (admitted and not-admitted) stratified by those with or without recorded biochemistry data.

	No biochemistry data	Biochemistry data	p
Characteristics	**2683 (62.0%)**	**1647 (38.0%)**	
Age, years (median [IQR])	**47.50 [36.10, 58.50]**	**72.20 [58.15, 80.95]**	**<0.001**
Male sex, n (%)	**1181 (44.0)**	**898 (54.5)**	**<0.001**
Prior comorbidities:			
Ischemic stroke, n (%)	**41 (1.5)**	**115 (7.0)**	**<0.001**
Diabetes, n (%)	**97 (3.6)**	**261 (15.8)**	**<0.001**
Ischemic heart disease, n (%)	**91 (3.4)**	**189 (11.5)**	**<0.001**
COPD, n (%)	**57 (2.1)**	**164 (10.0)**	**<0.001**
Atrial fibrillation, n (%)	**95 (3.5)**	**253 (15.4)**	**<0.001**
Chronic kidney disease, n (%)	**89 (3.3)**	**168 (10.2)**	**<0.001**
Hypertension, n (%)	**274 (10.2)**	**575 (34.9)**	**<0.001**
Cancer, n (%)	**123 (4.6)**	**243 (14.8)**	**<0.001**
Heart failure, n (%)	**42 (1.6)**	**118 (7.2)**	**<0.001**
COPD, chronic obstructive pulmonary disease; IQR, inter-quartile range.			

Table S3: Multivariable Cox regression analysis for the composite outcome of death or ICU admission, adjusted for CRP level, age, gender, diabetes, chronic obstructive pulmonary disease, hypertension and ischemic heart disease. Legend: CRP, C-reactive protein; ICU, intensive care unit.

Variable	Hazard ratio (95% CI)	p
CRP 100-400 mmol/L	**10.32 (2.56 – 41.61)**	**0.001**
CRP elevated to 99 mmol/L	**4.89 (1.21-19.81)**	**0.026**
Age, years	**1.04 (1.03-1.05)**	**<0.001**
Male sex	**1.54 (1.23-1.93)**	**<0.001**
Hypertension	**1.01 (0.81-1.26)**	**0.924**
Ischemic heart disease	**0.91 (0.66-1.24)**	**0.533**
COPD	**1.26 (0.93-1.71)**	**0.142**
Diabetes	**1.25 (0.97-1.62)**	**0.087**
CI, confidence interval; COPD, chronic obstructive pulmonary disease; IQR, inter-quartile range.		

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6-8*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	7-8
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	9-14
		(c) Summarise follow-up time (eg, average and total amount)	9-14
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-14
		(*b*) Report category boundaries when continuous variables were categorized	9-14
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-14, supplemental
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	5

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.