Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Lipoprotein(a) during COVID-19 hospitalization: Thrombosis, inflammation, and mortality

Morten Kaltoft [a,b,1], Kathrine Sofia Glavind [a,b,1], Sune Fallgaard Nielsen [a], Anne Langsted [a,b], Kasper Karmark Iversen [b,c], Børge Grønne Nordestgaard [a,b], Pia Rørbæk Kamstrup [a,*]

[a] Department of Clinical Biochemistry, Copenhagen University Hospital – Herlev and Gentofte, Denmark
[b] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[c] Department of Cardiology and Department of Emergency Medicine, Copenhagen University Hospital – Herlev and Gentofte, Denmark

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ABSTRACT

Background and aims: High levels of lipoprotein(a) could worsen the outcome of COVID-19 due to prothrombotic and proinflammatory properties of lipoprotein(a). We tested the hypotheses that during COVID-19 hospitalization i) increased thrombotic activity and inflammation are associated with lipoprotein(a) levels, and ii) lipoprotein(a) levels are associated with rate of hospital death and discharge.

Methods: We studied 211 patients admitted to Copenhagen University Hospital in 2020 with COVID-19, that is, prior to any vaccination. Thrombotic activity was marked by elevated D-dimer while inflammation was marked by elevated interleukin-6, C-reactive protein, and procalcitonin. Patients were followed until death (N = 36) or discharge (N = 175).

Results: A 2-fold higher D-dimer was associated with 14% (95%CI: 8.1–20%) higher lipoprotein(a). Conversely, 2-fold higher interleukin-6, C-reactive protein, and procalcitonin were associated with respectively 4.3% (0.62–7.8%), 5.7% (0.15–5.2%), and 8.7% (5.2–12%) lower lipoprotein(a).

For hospital death, the multivariable adjusted hazard ratio per 2-fold higher lipoprotein(a) was 1.26 (95% CI:0.91–1.73). Corresponding hazard ratios per 2-fold higher biomarker were 0.93 (0.75–1.16) for D-dimer, 1.42 (1.17–1.73) for interleukin-6, 1.44 (0.95–2.17) for C-reactive protein, and 1.44 (1.20–1.73) for procalcitonin. For hospital discharge, the multivariable adjusted hazard ratio per 2-fold higher lipoprotein(a) was 0.91 (95% CI:0.79–1.06). Corresponding hazard ratios per 2-fold higher biomarker were 0.86 (0.75–0.98) for D-dimer, 0.84 (0.76–0.92) for interleukin-6, 0.80 (0.71–0.90) for C-reactive protein, and 0.76 (0.67–0.88) for procalcitonin.

Conclusions: In COVID-19 patients, thrombotic activity marked by elevated D-dimer was associated with higher lipoprotein(a) while elevated inflammatory biomarkers of interleukin-6, C-reactive protein, and procalcitonin were associated with lower lipoprotein(a); however, elevated lipoprotein(a) was not associated with rate of hospital death or discharge.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic including >200 million cases worldwide [1] with a clinical profile similar to that seen for previous betacoronavirus epidemics, i.e. severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), but also with clinical features different from anything seen before [2,3]. Thus, while the majority of individuals infected remain asymptomatic or mildly symptomatic, some patients develop acute respiratory distress syndrome and thrombi in both the arterial and venous system to a far greater extent than patients with similar viral infections [4,5]. While high age, male sex, comorbidities, and severe overweight has been associated with poor outcome [6,7], it is presently unclear which other patient specific factors may predispose to serious complications.

It was recently hypothesized that high levels of lipoprotein(a), found in 1 in 5 individuals of European descent [8,9], could worsen the outcome of COVID-19 due to prothrombotic and proinflammatory

* Corresponding author. Department of Clinical Biochemistry, Copenhagen University Hospital - Herlev & Gentofte, Borgmester Ib Juuls Vej 73, 2730, Herlev, Denmark.
E-mail address: pia.roerbaek.kamstrup@regionh.dk (P.R. Kamstrup).
[1] These authors contributed equally to this work.

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properties of lipoprotein(a) [10]. Lipoprotein(a) consists of a cholesterol-laden LDL-like particle covalently bound to a large plasminogen-like glycoprotein, apolipoprotein(a), which may interact with fibrinolysis during disease pathogenesis [8,9,11–14]. The lipoprotein(a) particle has additionally been identified as a primary carrier of proinflammatory oxidized phospholipids [15]. Further, while lipoprotein(a) levels are primarily genetically determined, high lipoprotein (a) levels have been linked to high interleukin-6; importantly, interleukin-6 greatly increases during the "cytokine storm" in severely ill COVID-19 patients [10,16,17], which in combination with elevated lipoprotein(a) could worsen the outcome. Finally, lipoprotein(a) may be consumed during the acute phase, possibly during thrombosis in wound healing [18–21], and thus the role of lipoprotein(a) in COVID-19 is presently unclear.

We tested the hypothesis that during COVID-19 hospitalization i) increased thrombotic activity and inflammation are associated with plasma lipoprotein(a) levels, and ii) lipoprotein(a) levels are associated with rate of death during hospitalization and hospital discharge. For this purpose, we recruited 211 patients admitted to Copenhagen University Hospital – Herlev and Gentofte in 2020 (prior to vaccinations) with a positive Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) PCR test. Increased thrombotic activity was marked by elevated D-dimer while inflammation was marked by elevated interleukin-6, C-reactive protein, and procalcitonin.

2. Patients and methods

This study of hospitalized Danish COVID-19 patients was approved by the Ethical Committee in the Capital Region of Denmark (H-20040649). Due to the urgency of the SARS-CoV-2 pandemic, it was approved without the need for patients to provide written informed consent.

2.1. Study population

From March 2020 through August 2020, we examined serial samples collected from 211 consecutive Danish COVID-19 patients aged 24–97 years admitted to Herlev and Gentofte Hospital, Copenhagen University Hospital. Patients were included if they had a positive SARS-CoV-2 PCR test and at least one sample with measurements of either lipoprotein(a) (N = 195), interleukin-6 (N = 198), C-reactive protein (N = 206), procalcitonin (N = 199), or D-dimer (N = 196) (Supplementary Fig. 1) Follow-up was from admission date until death during hospitalization or hospital discharge, both of which was verified in the medical records for each patient. Investigators were blinded to death, discharge status and other hospital records, when performing laboratory analyses.

2.2. Laboratory analyses

Serial plasma samples were drawn during patient admission and subsequently frozen at −40 °C until measurement in December 2020. The samples were thawed at room temperature and analyzed at the same/next day. Lipoprotein(a) was measured on a Siemens Atellica (Siemens, Munich, Germany) platform and using the immunoturbidimetric Denka Seiken developed Roche 2nd generation assay (Roche Professional Diagnostics, Rotkreuz, Switzerland) with minimal apolipoprotein(a) isoform size bias [22]. Measurements were performed in both mg/dL and nmol/L using separate sets of calibrators with traceability to the WHO-IFCC approved lipoprotein(a) calibrator SRM-2B. Procalcitonin and interleukin-6 were measured from the same thawed samples, while C-reactive protein and D-dimer were ordered as part of the routine patient care and were thus measured on fresh samples. Thus, these two latter biomarkers were not necessarily blinded to the physicians treating the patients. All biomarkers used in the study were measured using standard assays from Siemens and on Siemens Atellica equipment in one laboratory. If a sample was below the range of the assay, the lowest value within the range was assigned to the sample, that is, e.g. lipoprotein(a) < 6 mg/dL was set to 6 mg/dL. 33% of all lipoprotein(a) samples were thus set to 6 mg/dL and included in the analyses. Of note, for lipoprotein(a) and other biomarkers there were no maximum values, as samples above the analytical measurement range were diluted and remeasured. The highest measured lipoprotein(a) was 164 mg/dL.

2.3. Covariates

Age and sex were obtained from the patients' Danish civil registration number. Date of admission, date of hospital discharge, death during hospitalization, comorbidities at admission, admission to intensive care unit, and medical treatment during hospitalization were obtained through reviews of the medical records by a medical doctor for each patient. Comorbidities were categorized into cardiovascular, cancer, neurological, pulmonary, endocrine including diabetes, and other diseases. Medical treatment was categorized into antibiotics, anticoagulants, intravenous steroids, remdesivir, and inhalation treatment. It was further noted if intensive care was deemed futile or inappropriate in which case the patient would be unsuitable for admission to an intensive care unit.

2.4. Statistical analyses

We used Stata 13 and RStudio 1.2.5001 with R 3.6.1. Differences across groups of discharged patients and patients who died were tested with Welch T-test for continuous variables and Fisher's exact test for categorical variables. As no baseline information on levels of lipoprotein (a) or biomarkers of thrombosis or inflammation was available and levels of thrombotic and inflammatory biomarkers are assumed elevated at admission, we did not stratify according to normal and elevated levels but rather according to quartiles or medians in the dataset and used the same approach for lipoprotein(a) for consistency.

Mean lipoprotein(a) for quartiles of D-dimer, interleukin-6, C-reactive protein, and procalcitonin was plotted using the geometrical mean (95% confidence interval of the mean) of lipoprotein(a) for all measured samples, that is, both samples taken at admission and samples taken during hospitalization. Thus, some samples are from the same individual taken at different timepoints to ensure that as much available information as possible was used. The biomarkers were divided into quartiles to investigate the whole distribution including both low, intermediary, high, and very high levels, to maintain groups of sufficient sample size, and to capture possible non-linear relationships. Consequently, D-dimer was categorized into <0.7, 0.7–1.6, 1.7–3.5, and >3.5 mg/L (Fibrinogen Equivalent Units, FEU), interleukin-6 into <9, 9–20, 21–52, and >52 ng/L, C-reactive protein into <38, 38–71, 72–141, and >141 mg/L, and procalcitonin into <0.09, 0.09–0.18, 0.19–0.44, and >0.44 μg/L. All subgroups used in analyses were prespecified.

Associations between lipoprotein(a) and the four other biomarkers, D-dimer, interleukin-6, C-reactive protein, and procalcitonin were evaluated with general linear mixed random effects models taking into account that multiple measurements were performed at different days for each patient. Lipoprotein(a) was log-transformed and the other biomarkers were log2-transformed, thus, reporting the results in percent difference of lipoprotein(a) per 2-fold higher biomarker level. Only samples with information on both lipoprotein(a) and the other biomarker in question were used, that is, e.g. for testing the association between lipoprotein(a) and D-dimer, the sample should contain valid measurements of both with identical sample collection times. Thus, lipoprotein(a) was only compared to another biomarker if they were taken at the same time. An example of how biomarkers were compared to each other is illustrated in Supplementary Fig. 2.

For cumulative incidence curves, patients were categorized into two groups by the median of the first available value of the biomarker for each patient, that is the sample closest to admission in order to avoid

immortal time bias [23] (Supplementary Fig. 2). For lipoprotein(a) the median was 11.6 mg/dL, for D-dimer 0.99 mg/L (FEU), for Interleukin-6 22 ng/L, for C-reactive protein 59 mg/L, and for procalcitonin the median was 0.11 μg/L. Cumulative incidence curves for death during hospitalization were plotted using the Aalen-Johansen-estimator with 95% Wald confidence intervals (CI) taking competing risk of hospital discharge into account [24], and *vice versa*. The R package prodlim was used for this purpose. Overall differences between cumulative incidences were evaluated with Gray's test.

Hazard ratios of death during hospitalization and hospital discharge per 2-fold higher lipoprotein(a), D-dimer, interleukin-6, C-reactive protein, and procalcitonin were evaluated using Cox proportional hazards models with admission time as underlying timescale. For these analyses, with death during hospitalization or hospital discharge as outcomes, we used values for the first available sample as the exposure, that is, the sample value as close to admission as possible as above (Supplementary Fig. 2). The number of exposure samples, and thus individuals, available for each outcome is shown in Fig. 4. Analyses were carried out i) unadjusted, ii) with adjustment for age and sex, and iii) with multivariable adjustment. With lipoprotein(a) as exposure variable, multivariable adjustment was for age, sex, D-dimer, interleukin-6, C-reactive protein, procalcitonin, and steroid treatment during admission. For D-dimer, multivariable adjustment was for age, sex, lipoprotein (a), interleukin-6, C-reactive protein, procalcitonin, and steroid treatment. For interleukin-6, C-reactive protein, and procalcitonin, multivariable adjustment was for age, sex, lipoprotein(a), D-dimer, and steroid treatment, but not for each other, as all three are markers of inflammation.

3. Results

Of the 211 included patients, 36 died during hospitalization while 175 were discharged from hospital. Median age was 69 years (interquartile range:55–80 years) among the discharged and 79 years (71–83 years) for patients who died ($p = 4*10^{-5}$) (Table 1). Fifty nine percent of discharged patients suffered from at least one comorbidity compared to 92% of those who died during hospitalization ($p = 9*10^{-5}$).

Twenty-four patients were discharged at the same day of their admission and were not included for longitudinal analyses. For the remaining 187 patients, median follow-up was 6 days ranging from 1 to 74 days. A total of 539 plasma samples of lipoprotein(a), 942 of D-dimer, 560 of interleukin-6, 976 of C-reactive protein, and 560 of procalcitonin were collected from the 211 patients. Means and medians of these biomarkers for the first samples taken at admission are shown in Table 1, while values for serial samples taken during follow-up hospitalization are shown in Supplementary Tables 1 and 2. Distribution plots of all biomarkers including both samples taken at admission and during hospitalization are shown in Supplementary Fig. 3. Distribution of lipoprotein(a) samples taken at admission was similar to distribution of lipoprotein(a) in the Copenhagen General Population Study (N = 70,639, Supplementary Fig. 4).

3.1. Lipoprotein(a) levels by quartiles of D-dimer and inflammatory biomarkers

In combined analyses of samples taken at admission and during hospitalization, there were 462 samples with measurements of both lipoprotein(a) and D-dimer. For samples with D-dimer <0.7 mg/dL (FEU) mean lipoprotein(a) was 17.6 mg/dL (N = 118, 95%CI:13.4–21.7 mg/dL) while for D-dimer >3.5 mg/L (FEU) mean lipoprotein(a) was 32.7 mg/dL (N = 114, 95%CI:28.5–37.0 mg/dL) (Fig. 1A; p for trend across all quartiles <0.001).

Likewise, for interleukin-6 and lipoprotein(a), 524 samples were available. For samples with interleukin-6 <9 ng/L, mean lipoprotein(a) was 27.5 mg/dL (N = 131, 95%CI: 23.3–31.7 mg/dL) while for interleukin-6 >52 ng/L mean lipoprotein(a) was 18.8 mg/dL (N = 131,

Table 1
Characteristics of COVID-19 patients.

		Discharged	Died	*p*-value
Individuals		175	36	
Age, years		69 (55–80)	79 (71–83)	$4{\cdot}10^{-5}$
Women		87 (50)	14 (39)	0.27
Admitted to intensive care unit		23 (13)	8 (22)	0.19
Unsuitable for intensive care		12 (6.9)	24 (67)	$9{\cdot}10^{-8}$
Comorbidities				
Any		103 (59)	33 (92)	$9{\cdot}10^{-5}$
Cardiovascular		54 (31)	16 (44)	0.12
Cancer		18 (10)	5 (14)	0.56
Neurological		18 (10)	9 (25)	0.026
Lung		32 (18)	6 (17)	1.00
Endocrine system including diabetes		30 (17)	10 (28)	0.16
Endocrine system including Other		18 (10)	8 (22)	0.056
Medication initiated at admission or during hospitalization				
Antibiotics		113 (65)	36 (100)	$1{\cdot}10^{-6}$
Anticoagulants		61 (35)	16 (44)	0.34
Systemic steroid		17 (9.7)	3 (8.3)	1.00
Inhalation treatment		13 (7.4)	3 (8.3)	0.74
Remdesivir		4 (2.3)	1 (2.8)	1.00
At admission sample values				
Lipoprotein(a), mg/dL	Mean	20.2 (16.5–24.0)	23.0 (12.9–33.1)	
	Median	11.3 (6.0–20.5)	13.0 (6.0–31.7)	
D-dimer, mg/L (FEU)	Mean	2.0 (1.4–2.6)	5.5 (2.1–9.0)	
	Median	0.8 (0.5–1.9)	1.9 (0.8–4.3)	
Interleukin-6, ng/L	Mean	39 (30–49)	406 (0–850)	
	Median	18 (8.1–46)	43 (28–154)	
C-reactive protein, mg/L	Mean	73 (63–84))	136 (104–168)	
	Median	53 (25–93)	113 (64–196)	
Procalcitonin, μg/L	Mean	0.35 (0.21–0.50)	4.5 (0.34–8.62)	
	Median	0.09 (0.06–0.21)	0.39 (0.12–1.67)	

Values are shown in numbers (percent), mean (95% confidence interval of the mean), or median (interquartile range).
Means and medians for biomarkers are based on varying numbers of individuals, as not all individuals had values for all biomarkers.
Mean number of days from collection of positive SARS-CoV-2 test to first samples were 0.6 days for lipoprotein(a), 0.4 days for D-dimer, 0.6 days for interleukin-6, 0.1 days for C-reactive protein, and 0.5 days for procalcitonin.

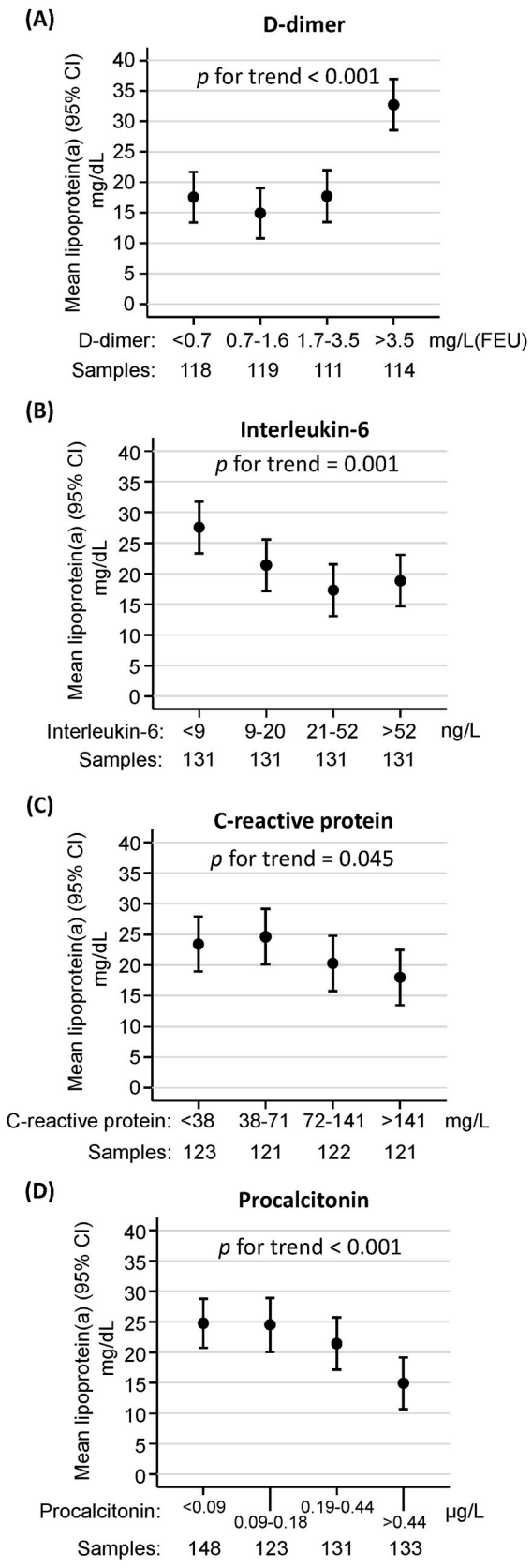

Fig. 1. Mean lipoprotein(a) across quartiles of D-dimer, interleukin-6, C-reactive protein, and procalcitonin.
Results are from all available samples containing information on both lipoprotein(a) and the quartiled biomarker. Mean is geometric mean with 95% CI. *p* for trend is from Cuzick's test. CI: Confidence interval.

95%CI:14.6–23.1 mg/dL) (Fig. 1B; p for trend across all quartiles = 0.001).

Likewise, for C-reactive protein and lipoprotein(a), 487 samples were available. For samples with C-reactive protein <38 mg/L mean lipoprotein(a) was 23.4 mg/dL (N = 123, 95%CI:18.9–27.9 mg/dL) while for C-reactive protein >141 mg/L mean lipoprotein(a) was 18.0 mg/dL (N = 121, 95%CI:13.5–22.5 mg/dL) (Fig. 1C; p for trend across all quartiles = 0.045).

Finally, there were 535 samples with measurement of both procalcitonin and lipoprotein(a) when combining samples taken at admission and during hospitalization. For samples with procalcitonin <0.09 µg/L mean lipoprotein(a) was 24.8 mg/dL (N = 148, 95%CI:20.7–28.8 mg/dL) while for procalcitonin >0.45 µg/L mean lipoprotein(a) was 15.0 mg/dL (N = 133, 95%CI:10.7–19.2 mg/dL) (Fig. 1, panel D; p for trend across all quartiles <0.001). Results showing medians and interquartile ranges of lipoprotein(a) are shown in Supplementary Fig. 5, and results for samples taken only at admission or samples only during follow-up hospitalization are shown in Supplementary Fig. 6.

3.2. Lipoprotein(a) levels by D-dimer and inflammatory biomarkers on continuous scales

In an unadjusted generalized linear mixed effects model allowing for multiple serial measurements on the same individual over the course of admittance to the hospital, a 2-fold higher D-dimer was associated with 14% (95% CI:8.1–20%, $p < 0.001$) higher lipoprotein(a). Conversely, 2-fold higher inflammatory biomarkers were associated with 4.3% (0.62–7.8%, $p = 0.02$) lower lipoprotein(a) for interleukin-6, 5.2% (0.15–5.2%, $p = 0.04$) lower lipoprotein(a) for C-reactive protein, and 8.7% (5.2–12%, $p < 0.001$) lower lipoprotein(a) for procalcitonin (Fig. 2). When adjusting for age and sex, corresponding values were 13% (7.2–19%, $p < 0.001$) higher for D-dimer, 5.7% (2.0–9.3%, $p = 0.003$) lower for interleukin-6, 7.1% (2.0–12%, $p = 0.007$) lower for C-reactive protein, and 10% (6.5–14%, $p < 0.001$) lower for procalcitonin. Results for samples taken only at admission or samples taken only during follow-up hospitalization are shown in Supplementary Fig. 7.

3.3. Cumulative incidence of death during hospitalization and hospital discharge

For patients with first lipoprotein(a) measurement above the median (=high) versus below or equal to the median (=low), cumulative incidences of death during hospitalization and hospital discharge were similar (Gray's test $p = 0.60$ for death and $p = 0.97$ for discharge) (Fig. 3A). After 30 days of admission, the cumulative incidence of death during hospitalization was 17% (95%CI:9.0–24%) for low lipoprotein(a) and 19% (11–28%) for high lipoprotein(a), while the cumulative incidence of hospital discharge was 71% (62–81%) for low lipoprotein(a) and 75% (65–84%) for high lipoprotein(a).

For patients with high versus low D-dimer, the cumulative incidence of death during hospitalization was higher ($p = 0.026$) while the cumulative incidence of hospital discharge was lower ($p = 0.013$) (Fig. 3B). After 30 days, the cumulative incidences of death during hospitalization were 10% (95%CI: 3.5–17%) for low and 25% (16–34%) for high D-dimer. For hospital discharge, corresponding values were 80% (71–88%) and 67% (57–76%), respectively.

For patients with high versus low interleukin-6, the cumulative incidence of death during hospitalization was higher ($p = 2*10^{-5}$) and of hospital discharge lower ($p = 2*10^{-7}$) (Fig. 3 C), with similar patterns for C-reactive protein and procalcitonin (Fig. 3D and E). After 30 days the cumulative incidences of death during hospitalization were 4.9% (0.22–9.7%) and 28% (19–37%) for low and high interleukin-6, 8.4% (2.5–14%) and 26% (17–34%) for low and high C-reactive protein, and 8.9% (2.6–15%) and 25% (16–33%) for low and high procalcitonin. For hospital discharge, corresponding values were 90% (84–97%) and 60% (50–70%) for interleukin-6, 88% (81–95%) and 61% (52–71%) for C-

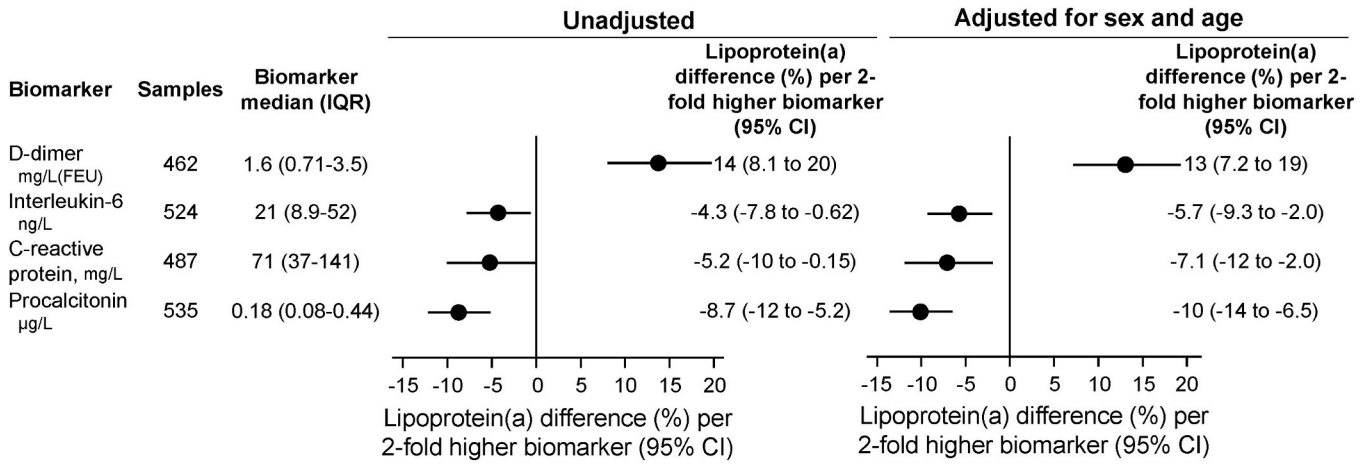

Fig. 2. Association of lipoprotein(a) with D-dimer, interleukin-6, C-reactive protein, and procalcitonin on continuous scales.
Results are from a general linear mixed random effects model taking into account that multiple measurements were performed at different days for each patient. CI: Confidence interval. IQR: Interquartile range.

reactive protein, and 87% (80–95%) and 62% (52–73%) for procalcitonin, respectively.

3.4. Hazard ratios of death during hospitalization and hospital discharge

Per 2-fold higher lipoprotein(a), the multivariable adjusted hazard ratio for death during hospitalization was 1.26 (95%CI:0.91–1.73) (Fig. 4). The corresponding hazard ratios were 0.93 (0.75–1.16) per 2-fold higher D-dimer, 1.42 (1.17–1.73) per 2-fold higher interleukin-6, 1.44 (0.95–2.17) per 2-fold higher C-reactive protein, and 1.44 (1.20–1.73) per 2-fold higher procalcitonin. When additionally adjusting the multivariable adjusted model for preexisting cardiovascular disease, the hazard ratio for death during hospitalization increased for lipoprotein(a); however, results were still not statistically significant (Supplementary Fig. 8). There was no interaction between lipoprotein (a) and preexisting cardiovascular disease (p for interaction = 0.4) on hazard ratio for death.

For hospital discharge, the multivariable adjusted hazard ratio per 2-fold higher lipoprotein(a) was 0.91 (0.79–1.06) (Fig. 4). Corresponding hazard ratios were 0.86 (0.75–0.98) per 2-fold higher D-dimer, 0.84 (0.76–0.92) per 2-fold higher interleukin-6, 0.80 (0.71–0.90) per 2-fold higher C-reactive protein, and 0.76 (0.67–0.88) per 2-fold higher procalcitonin.

4. Discussion

In this study of 211 hospitalized COVID-19 patients, increased thrombotic activity marked by elevated D-dimer was associated with higher plasma lipoprotein(a) while inflammatory biomarkers of elevated interleukin-6, C-reactive protein, and procalcitonin were associated with lower plasma lipoprotein(a); however, elevated lipoprotein(a) was not associated with risk of death during hospitalization or hospital discharge. These findings are novel.

Mechanistically, our finding that elevated lipoprotein(a) during COVID-19 hospitalization was associated with elevated D-dimer, a marker of thrombotic activity, supports the theory that apolipoprotein (a) may compete with plasminogen to decrease fibrinolysis, which could increase the tendency for thromboembolic activity, including alteration of fibrin clotting which ultimately results in a higher level of D-dimer [9,13,25,26]. Likewise, apolipoprotein(a) has also been implicated as a procoagulant directly inhibiting tissue factor pathway inhibitor (TFPI), promoting blood coagulation which may ultimately also increase D-dimer, D-dimer being a degradation product of crosslinked fibrin in clots [27].

It is, however, somewhat surprising that high levels the inflammatory biomarkers interleukin-6, C-reactive protein, and procalcitonin were associated with low levels of lipoprotein(a), as previous studies have indicated that lipoprotein(a) has properties of an acute phase reactant possibly due to decreased clearance in the liver [16,28–30]. A potential mechanism behind this association could be increased aggregation of lipoprotein(a) in damaged or inflammatory tissue during thrombosis formation in wound healing and tissue repair [18–21], or alternatively, decreased synthesis of lipoprotein(a) during acute inflammatory responses by liver cells represent an alternative theoretical explanation.

Using a very different design, a recent study from the UK Biobank did not find that lipoprotein(a) measured in healthy individuals many years prior to COVID-19 infection was a risk modulator for thromboembolic events in 6937 SARS-CoV-2 positive individuals compared to 435,104 population controls [31]. Importantly, in that study baseline lipoprotein (a) levels did not differ between cases and controls indicating that lipoprotein(a) does not increase or decrease susceptibility to become SARS-CoV-2 positive, which may otherwise have influenced our findings. Although the UK Biobank study is on a different population answering a different question, that study indirectly supports our finding that levels of lipoprotein(a) are not related to COVID-19-associated death or hospital discharge. If high lipoprotein(a) increases the risk of thromboembolic events, this would be expected to lead to higher risk of death and/or lower discharge rate as more patients would subsequently die or have prolonged admission times. While the UK Biobank study reported a higher frequency of thromboembolic events in COVID-19 subjects, the authors did not find an association between lipoprotein(a) levels and such events. Thus, it could be speculated that the patients in our study represent a clinically more severe group with a more pronounced association between lipoprotein(a) levels and thrombotic activity.

In line with our findings, a recent study including 219 hospitalized COVID-19 patients in the Netherlands [32], did not find lipoprotein(a) levels or inflammatory markers at admission to be associated with risk of venous thromboembolism; however, increases in lipoprotein(a) – but not changes in inflammatory markers – during hospitalization were associated with higher risk of venous thromboembolism. Additionally, baseline interleukin-6 was associated with risk of being admitted to an intensive care unit, which is in line with our finding that inflammatory markers were associated with risk of death. In contrast to our findings, the Dutch study found high interleukin-6 to be associated with higher levels of lipoprotein(a). This difference could be explained by the fact that the study also showed that in the later phases of admission,

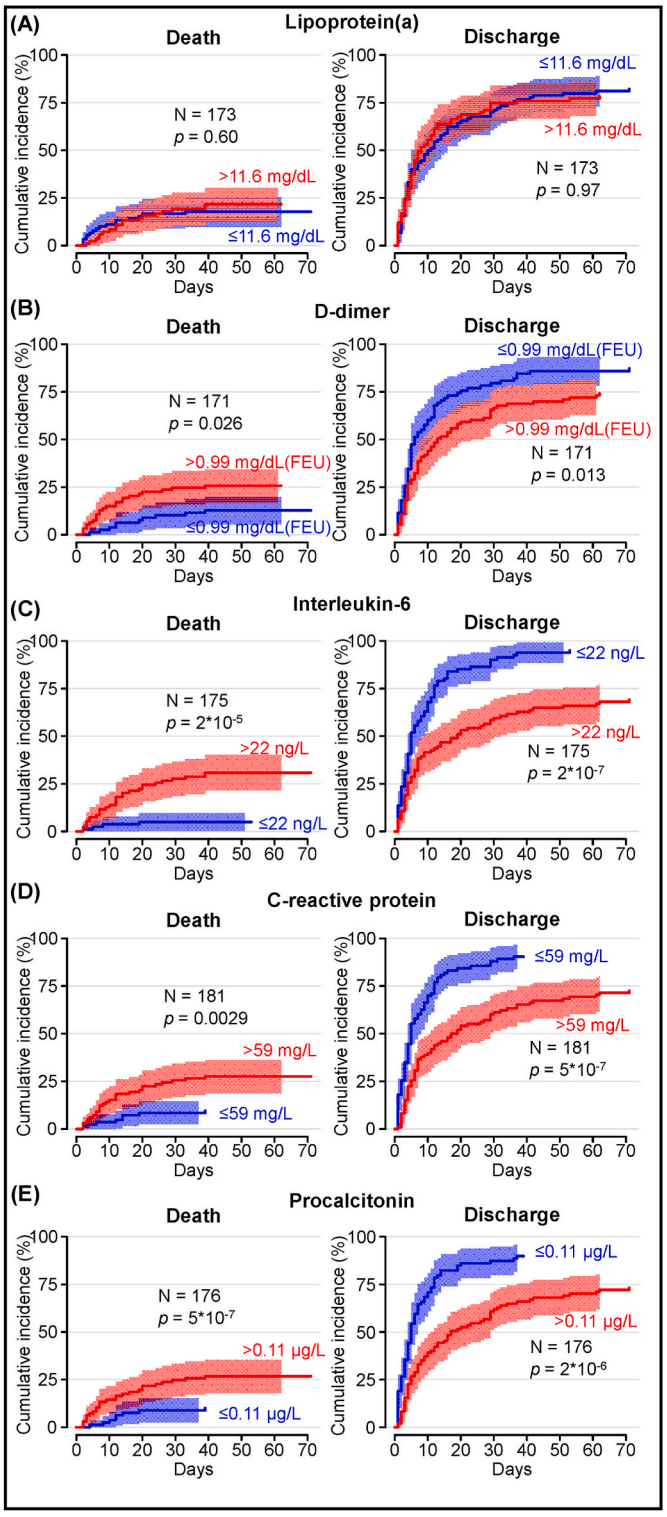

Fig. 3. Cumulative incidence of death during hospitalization and hospital discharge for top 50% and bottom 50% patients according to levels of lipoprotein(a), D-dimer, interleukin-6, C-reactive protein, and procalcitonin.
Cumulative incidence of death and discharge are from the Aalen-Johansen product limit estimator considering that death during hospitalization and hospital discharge are competing events. Confidence intervals are 95% Wald confidence intervals. Red indicates patients with the top 50% of the biomarker and blue indicates patients with the bottom 50% of the depicted biomarker. P-values are from Gray's test. N: Number of individuals.

lipoprotein(a) rose while interleukin-6 decreased.

In the present study, particularly interleukin-6 and procalcitonin were robustly associated with increased risk of death during hospitalization and reduced hospital discharge. Our study also indicates that a measurement of lipoprotein(a) does not offer any additional information in a clinical setting neither as a biomarker to indicate risk of death or hospital discharge in COVID-19 patients, nor as a useful marker of disease progression as other markers of inflammation and thrombotic activity including interleukin-6, C-reactive protein, procalcitonin, and D-dimer are simply better, as demonstrated in the present study and in support of previous findings [33,34].

An important strength of our study is the use of serial blood samples on verified COVID-19 patients, making our study representative of typical hospitalized COVID-19 patients not vaccinated against COVID-19. Associations between levels of lipoprotein(a) with levels of D-dimer, interleukin-6, C-reactive protein, and procalcitonin during COVID-19 hospitalization were thoroughly investigated based on these samples. Further, follow-up was 100% complete, as all patients were accounted for during their hospital admission. Finally, full access to medical records of all patients provided high validity of endpoints and covariates.

A limitation of our study is the relatively few patients who died during hospitalization. For that reason, we cannot exclude that a study with more statistical power could potentially find an association between lipoprotein(a) levels and risk of death or hospital discharge during COVID-19 hospitalization; however, it is unlikely that the risk estimate would be large enough to have clinical impact, and more importantly our study indicates that other biomarkers are already performing better for the purpose of disease and prognosis monitoring [33,34]. Also, as our results are only observational, we cannot imply causal relationships, and possible mechanistic explanations are only speculative. Further, the physicians treating the included patients were not masked to levels of D-dimer, C-reactive protein, and procalcitonin if they ordered these analyses as part of their assessments of the patient. Thus, the association between, for example, C-reactive protein and rate of hospital discharge could be influenced by the fact that C-reactive protein is used to assess patients with COVID-19 and physicians may base their decision to discharge patients partly on this biomarker. However, treating physicians were blinded to lipoprotein(a) and interleukin-6 measurements indicating the robustness of these findings. Additionally, even though information on treatments was available, we cannot exclude that these treatments could have altered our results.

Also, in analyses involving endpoints of death during hospitalization or hospital discharge, only the first sample value of the biomarker and not the change over time was used. Notably, using this study design, the values at admission of D-dimer, C-reactive protein, and procalcitonin were able to show associations with death and hospital discharge, whereas lipoprotein(a) was not. Thus, lipoprotein(a) measured during infection did not correlate with death or hospital discharge at any level. Further, while a biomarker value at admission may be clinically relevant if able to predict the course of disease in advance of disease worsening, the value only reflects a specific time point, and biomarker changes during hospitalization, not examined in this study due to lack of statistical power, may provide additional information. Of note, the lipoprotein(a) values used to investigate risk of death or discharge were from frozen samples taken on or close to the day of admission of symptomatic patients with active disease, and hence, these values do not necessarily reflect disease-free baseline values. Furthermore, the present study is unable to exclude if there is any matrix effect disturbing the measurements during the acute setting of COVID-19 hospitalization; however, we are unaware of any data that suggests such an effect. Lipoprotein(a) levels obtained at timepoints far away from the COVID-19 hospitalization were unfortunately not available. As we do not have these usual, highly genetically determined lipoprotein(a) levels, this study cannot be used to assess the relationship between low and high levels of lipoprotein(a) and inflammatory or thrombotic markers in the general

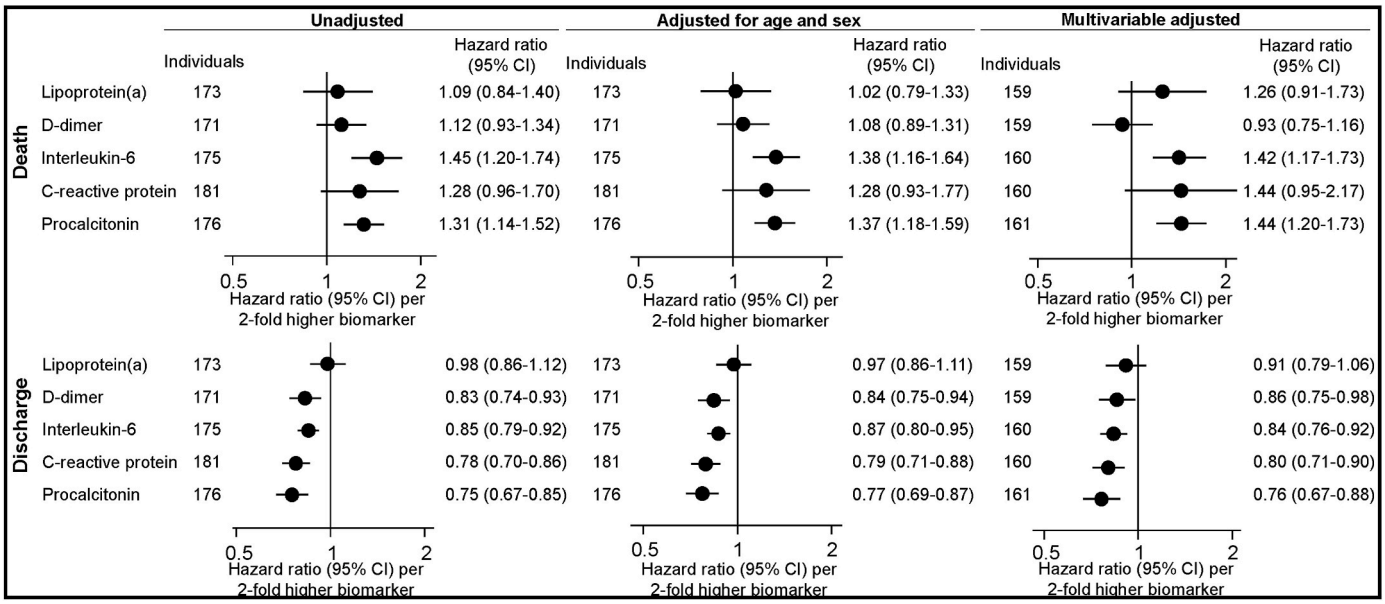

Fig. 4. Hazard ratios of death during hospitalization and hospital discharge per 2-fold higher lipoprotein(a), D-dimer, interleukin-6, C-reactive protein, and procalcitonin.

Hazard ratios are from Cox regression with time admitted to hospital as the underlying timescale. For lipoprotein(a) as exposure variable, multivariable adjustment was for age, sex, D-dimer, interleukin-6, C-reactive protein, procalcitonin, and steroid treatment during admission. For D-dimer, multivariable adjustment was for age, sex, lipoprotein(a), interleukin-6, C-reactive protein, procalcitonin, and steroid treatment. For interleukin-6, C-reactive protein, and procalcitonin, multivariable adjustment was for age, sex, lipoprotein(a), D-dimer, and steroid treatment, but not for each other, as all three are markers of inflammation. Medians (interquartile range) were 10 (6.0–23) mg/dL for lipoprotein(a), 1.1 (0.56–2.5) mg/L (FEU) for D-dimer, 25 (11–58) ng/L for interleukin-6, 64 (35–118) mg/L for C-reactive protein, and 0.13 (0.07–0.31) μg/L for procalcitonin. CI: Confidence interval.

population, that is, in individuals who do not suffer from COVID-19. Indeed, in a previous study of the Danish general population, high lipoprotein(a) levels associated with increased c-reactive protein levels in observational but not in genetic analyses [35].

Further, we exclusively used D-dimer to track thrombotic activity and did not measure apolipoprotein(a)-like plasminogen usually found in large molar excess of lipoprotein(a) in plasma.

Finally, for some multivariable adjustments, biomarkers were not always taken at the same timepoint as the exposure-biomarker tested. However, as adjustment did not alter results significantly, this potential limitation is unlikely to change the conclusions of our results. Ideally, all samples would have been from the same time points and all would have been measured for the same parameters including covariates; however, this setup was not possible, as clinicians would also request parameters only used for clinical assessment. Despite this limitation, we were able to robustly demonstrate the associations found.

In conclusion, increased thrombotic activity marked by elevated D-dimer was associated with higher plasma lipoprotein(a) while elevated inflammatory biomarkers of interleukin-6, C-reactive protein, and procalcitonin were associated with lower plasma lipoprotein(a); however, elevated lipoprotein(a) was not associated with rate of death during hospitalization or hospital discharge.

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CRediT authorship contribution statement

Morten Kaltoft: Formal analysis. **Sune Fallgaard Nielsen:** Formal analysis. **Pia Rørbæk Kamstrup:** Formal analysis.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

BGN reports consultancies and talks sponsored by AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka, Amarin, Novartis, Novo Nordisk, Silence Therapeutics, and Esperion. PRK reports talks and consultancies sponsored by Physicians Academy of Cardiovascular Education (PACE), Silence Therapeutics, and Novartis. The other authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2022.07.015.

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