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Daily monitoring of viral load measured as SARS-CoV-2 antigen and RNA in blood, IL-6, CRP and complement C3d predicts outcome in patients hospitalized with COVID-19

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Abstract

Objectives: We hypothesized that the amount of antigen produced in the body during a COVID-19 infection might differ between patients, and that maximum concentrations would predict the degree of both inflammation and outcome for patients.

Methods: Eighty-four hospitalized and SARS-CoV-2 PCR swab-positive patients, were followed with blood sampling every day until discharge or death. A total of 444 serial EDTA plasma samples were analyzed for a range of biomarkers: SARS-CoV-2 nuclear antigen and RNA concentration, complement activation as well as several inflammatory markers, and KL-6 as a lung marker. The patients were divided into outcome groups depending on need of respiratory support and death/survival.

Results: Circulating SARS-CoV-2 nuclear antigen levels were above the detection limit in blood in 65 out of 84 COVID-19 PCR swab-positive patients on day one of hospitalization, as was viral RNA in plasma in 30 out of 84. In all patients, complete antigen clearance was observed within 24 days. There were definite statistically significant differences between the groups depending on their biomarkers, showing that the concentrations of virus RNA and antigen were correlated to the inflammatory biomarker levels, respiratory treatment and death.

Conclusions: Viral antigen is cleared in parallel with the virus RNA levels. The levels of antigens and SARS-CoV-2 RNA in the blood correlates with the level of IL-6, inflammation, respiratory failure and death. We propose that the antigens levels together with RNA in blood can be used to predict the severity of disease, outcome, and the clearance of the virus from the body.

Keywords: biomarkers; correlation; COVID-19; prognosis; severity.

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Introduction

There have been several hypotheses regarding the disease mechanism causing tissue damage in SARS-CoV-2 infection, primarily targeting the interstitial cells of the lung and in the circulatory system [1, 2]. From a theoretical point of view this can occur by

- 1) A direct effect of the invasion of virus through the Angiotensin-converting enzyme 2 receptors of the cell.
- 2) A T-cell initiated SARS-CoV-2 antigen specific cell lysis as indicated by the remarkable increase in interleukin-6 (IL-6) known as cytokine storm.
- 3) A complement mediated direct activation of antibody dependent cytolysis after the appearance of specific IgM and IgG antibodies.
- 4) A combination of these and other inflammatory mechanisms such as e.g. activation of the coagulation system.

Whatever the mechanism, the result is tissue damage targeting the infected cells primarily in the respiratory tract, the lungs and the kidneys but also the endothelial cells of the circulation, resulting in dyspnea, reduced oxygenation, myalgia and/or symptoms from micro thrombosis [2].

Deposition of complement products in the lung, suggesting a local complement activation as part of an elimination process of the viral antigens by an immune protective response through the production of IgM and IgG antibodies has previously been reported [3]. Such a process, however, also leads to cell destruction by an innocent by-stander process unless stopped. However, no papers have reported measuring and monitored complement activation by quantitating complement split products from C3 at admittance and by daily monitoring. September 2nd, 2020 WHO started recommending treatment using dexamethasone to reduce inflammation [4], which in large doses would reduce the complement reaction making it impossible to quantify the complement reaction. Approximately 90% of the patients in this study were included before these recommendations.

Our primary questions of importance for treatment of Covid-19 patients admitted to hospital were:

- 1) Is the level of antigen and/or virus RNA levels in the blood, i.e. viral load a determining factor for T-cell and complement activation?
- 2) Is the degree of complement activation, as measured by circulating complement biomarkers, determining of outcome?
- 3) Are both the T-cell mediated tissue damage, as reflected by IL-6 levels, and the humoral (Antigen/Antibody) complement mediated cell lysis activated?
- 4) Which inflammation markers are useful in following and predicting disease course and outcome?

To answer these questions, we monitored routine and newly described biomarkers together with the nuclear antigen levels, the RNA levels in the blood and complement biomarkers, daily during hospitalization.

Materials and methods

Patients

All patients admitted to Kolding Hospital, Denmark with a PCR-positive pharyngeal swab-test for SARS-CoV-2 infection within the period of March 30th to September 15th 2020 were included in the study. This resulted in a total of 84 patients of which 69 had blood drawn from the day of hospital admittance and 15 were included although they were already admitted when inclusion started. Blood

samples were drawn at admittance and for routine monitoring and treatment purposes on remaining days of hospitalization. Blood was centrifuged at 2,650 g at 21 °C for 6 min and residual EDTA-plasma was stored at minus 80 °C and thawed prior to analysis.

Clinical data and laboratory data already produced were retrieved from the electronic health records

We grouped patients according to intensity of respiratory treatment as follows: Group 1 was not treated with supplemental oxygen support, group 2 received nasal oxygen support, group 3 received nasal high-flow oxygen therapy or non-invasive ventilation, while group 4 was treated with mechanical ventilation. Some patients were not candidates for intensive care due to comorbidity and age, and therefore treated with high-flow oxygen therapy as highest level of ventilation. We therefore also divided the patients into two binary categories in terms of respiratory treatment, the abovementioned groups 1 + 2 vs. 3 + 4, to detect whether any biomarker could predict the need of intensive oxygen therapy and the outcome (alive or dead at discharge). We investigated levels for each biomarker at the first day of admittance and the day of the maximum concentration for their predictive value and correlation to each other.

SARS-CoV-2 nucleocapsid antigen in plasma

The SARS-CoV-2 nucleocapsid antigen assay was purchased from Quanterix (Billerica, MA, USA), and measured on the automated Simoa HD-X Analyzer platform (Quanterix). The assay procedure was followed according to the instructions of the manufacturer. Plasma EDTA samples were diluted four-fold in diluent provided with the kit and run as single measurements. Four internal quality controls were applied in each run, two were provided in the kit and two were plasma samples taken from SARS-CoV-2 PCR positive individuals. The cutoff for a positive result was 0.1 pg/mL. The analytical variations were calculated from runs across multiple days resulting in 19, 14, 10 and 20% at level 1.7, 6.2, 12.5 and 134 pg/mL, respectively.

RNA extraction and RT-droplet digital PCR

Viral RNA was extracted from 1.0 mL EDTA plasma using QIAasympyony DSP Virus/Pathogen Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A total of 1.2 mL sample were loaded to the QiaSymphony Robot (Qiagen) and in the case sample volume was less than 1.2 mL, Nuclease-free water (Sigma-Aldrich) was added for adjustment. Extracted RNA was eluted in 60 µL. One-step RT-ddPCR was initiated immediately after RNA extraction using the Bio-Rad SARS-CoV-2 ddPCR kit (Bio-Rad, Hercules, CA, USA) according to manufacturer's specifications. All samples were spiked with non-human CPP1 as control of extraction [5]. For each sample extraction batch, a positive template control (COV019, Bio-Rad), and two negative controls (COV000, Bio-Rad and nuclease-free water, Sigma-Aldrich) were included. ddPCR results were analyzed using the QX Manager standard edition (1.1.341, Bio-Rad) and the concentration of SARS CoV-2 RNA was calculated per mL plasma. A sample is considered positive for SARS-CoV-2 if the SARS-CoV-2 markers N1 and/or N2 exhibit ≥2 positive droplets, as described by Bio-Rad.

Complement activation

Complement C3d (C3d) was measured by double-decker rocket immunoelectrophoresis [6] using an intermediate gel containing anti-C3c (Dako (Agilent), Copenhagen), and anti-C3d (Dako (Agilent), Copenhagen) in the top gel.

Complement factor 3 (C3) and Complement factor 4 (C4) were analyzed on a Roche Cobas c702 module. All assays are CE-label Roche assays and run according to the manufacturer's protocols. The reference intervals for the analyses were C3: 0.9–1.8 g/L, C4: 0.02–1.0 g/L and C3d: 20–52 U/L.

Other biomarkers

C-reactive protein (CRP, reference interval <6.0 mg/L) was analyzed on a Roche Cobas c702 module, whereas interleukin-6 (IL-6, reference interval <7 ng/L) and Growth/differentiation factor 15 (GDF-15) (upper reference limit 831–2,199 ng/L depending on age) analyzed on a Roche cobas e801 module (Roche, Basel, Switzerland). All assays are CE-labeled Roche assays and were run according to the manufacturer's protocols. Krebs von den lungen-6 (KL-6, reference interval 98–313 U/mL) was analyzed by the Nanopai®-KL-6 reagent (Sekisui diagnostic, Burlington, MA, USA), on a Roche cobas c502 module. Similarly, soluble urokinase plasminogen activator receptor (suPAR) (reference interval <3 µg/L) (Virogates, Denmark) and Neutrophil gelatinase-associated lipocalin (NGAL) (reference interval 25–113 µg/L for women and 26–97 µg/L for men) (Bioporto, Denmark) were analyzed according to the manufacturer's protocol on a Roche cobas c502 module.

Procalcitonin (reference interval <0.10 µg/L) was measured on a Kryptor Compact platform (BRAHMS GmbH, Hennigsdorf, Germany).

Quantitative levels of SARS-CoV-2 IgM and SARS-CoV-2 IgG in plasma samples were measured using the iFlash 1800 platform (Shenzhen Yhlo Biotech Co.LTD, Shenzhen, China), a chemiluminescent immunoassay (CLIA) method, which uses paramagnetic particles coated with antigens from both the spike protein (S) and the nucleocapsid protein (N). According to the recommendations of the manufacturer, both IgM and IgG results ≥12 AU/mL were considered positive. Three pools of human plasma were prepared in-house and used as internal quality controls for evaluating assay performance. Intra-assay as well as total coefficient of variations were <10%.

Data analysis

Data were analyzed using R-3.6.2 statistical software. Wilcoxon rank sum test was used to compare patient groups. Correlations were calculated using Spearman's rank correlation rho. p values less than 0.05 were considered statistically significant. Data are presented as median and 2.5–97.5 percentiles.

Ethics

The project was approved by The Regional Committees on Health Research Ethics for Southern Denmark (registration number 20/22417). A clinical biobank was created by storing residual blood from the patient samples. The study was performed according to the Danish data protection laws and EU GDPR.

Results

A total of 84 patients with SARS-CoV-2 PCR-positive swabs were included and followed with daily blood samples. Some of the patients were included in the study after admission as noted in the Table 1 showing patient characteristics.

Examples of the changes of the biomarkers during the course of two patient's hospital stay are illustrated in Figure 1.

Figure 2 shows serial measurement of SARS CoV-2 N antigen and RNA concentration in the blood during hospitalization in absolute values within 24 days in all patients. Notably is the large difference in concentration from 0.1–100,000 pg/mL.

There is a strong correlation between antigen and RNA concentrations ($R=0.59$, $p<5 \times 10^{-5}$, Supplemental Table 1). Figure 2 shows that the antigen and the virus RNA are eliminated at the same time.

Strong associations exists between the need for supplemental oxygen supply and artificial ventilation and virus levels, complement activation, IL-6, CRP and the other inflammation markers as listed in Table 2.

This is further supported by interrelated correlation between causative determinants and tissue damaging mediators as seen in Figure 3. Specifically a strong correlation exist between virus maximum levels and IL-6, C3d and CRP, illustrating both T-cell activation and complement mediated cell damage. A full statistical correlation is shown for all biomarkers in Supplemental Table 1.

In all patients, the viral RNA and antigen were cleared at the same time as IgM and IgG antibodies concentrations begin to increase.

Other biomarkers correlated to treatment grouping, such as CRP, C3d, IL-6 and procalcitonin as expected given the known cause and effect, reflecting general inflammation and hence cell damage.

Table 1: Patient characteristics.

	Patients including first day sample	All patients
Number of patients	69	84
Age, years	61.4 [20.4–90.3]	65.6 [22.7–90.9]
Sex, % male	53.6%	57.1%
BMI, kg/m ²	27.7 [19.0–40.1]	27.7 [19.3–52.1]

First column shows characteristics of the patients who were admitted before participating in this study. The second column shows characteristics of the patients who participated in this study from their first day at the hospital. BMI, body mass index.

Figure 1: Patient timeline. Examples of the timeline for two patients, all biomarkers are listed from hospital admittance to discharge. X-axis shows days from admittance. Y-axis shows the concentration of each biomarker. Both patients were included on the first day of admittance.

Figure 2: Show the change in concentration of SARS-CoV-2 antigen (A) and RNA (B) from hospital admittance in absolute values.

Comparing group 1 and 2 against groups 3 and 4 (high-flow and ventilation support) and survived against dead, a clear correlation was observed to antigen levels and other markers, as seen in Figure 4.

Based on increased levels of several predictive markers, a worse outcome could be predicted based on risk-ratios. Figure 4 shows ROC-curves for four biomarkers.

Discussion

Our study shows a strong association between the level of SARS CoV-2 N protein and disease severity as well as between SARS CoV-2-RNA level and disease severity in terms of both morbidity and mortality (Figure 4). This is in line with the association between viral load and critical illness as showed by Bermejo-Martin et al. [7] We also show a strong association between levels of SARS CoV-2 N protein and SARS CoV-2-RNA against C3d and IL-6, which means that both the complement system as well as the T-cells are activated as decisive components of the clearance and inflammatory cell damage mechanisms.

In all patients, the viral RNA and antigen were cleared at the same time as IgM and IgG antibodies concentrations

began to increase, indicating a preponderance of cell damage through the T-cell system but a clearance through antibodies and complement system.

We propose that measuring and monitoring levels of antigen by the Simoa method could determine both who should be treated early with anti-T-cell therapies (Abatacept e.g.), but also tell when it is safe to stop the specific treatment when the virus is cleared from the body. This could guide to a more personalized treatment with Remdesivir to reduce side effects in patients without antigen or RNA in the body. Monitoring levels of antigen could also possibly guide the clinicians to determine when it is safe to terminate isolation of the patient.

Usually, when detecting complement activation, C3 and C4 quantification is used. As we have previously shown limited value of C3 and C4 for detection of complement activation [6], we propose instead the use of C3d for monitoring complement activation and effect of steroid treatment.

Others have shown a deposition of complement factors around the lung cells [3]. However, this is the first time that a blood circulating complement split product C3d, with strong statistical significance, is shown to be produced during the disease process. Furthermore it is shown that even though steroids are given, a complement activation

Table 2: Biomarker concentrations measured.

Biomarker	Group 1	Group 2	Group 3	Group 4	Group 1 + 2	Group 3 + 4	Survived	Dead
SARS-CoV-2 N protein	42.1 (0.005–22,227.247)	18,933 (3,944–33,921) 0.038	288 (44.2–52,972.6) 0.011	12,005 (0.199–422,446.187) 0.002	47 (0.005–23,681.816)	10,497 (0.392–319,497.768) 4e-04	53.6 (0.005–24,812.052)	17,328 (61.7–97,109.0) 4.45e-05
SARS-CoV-2 RNA	0.005 (0.005–269.178)	1,720 (145–3,295) 0.014	136 (0.005–2,091.076) 0.000708	620 (0.005–4,680.921) 0.000134	0.005 (0.005–1,579.557)	287 (0.005–4,371.007) 8.12e-06	0.005 (0.005–797.669)	420 (4.26–4,618.94) 1.21e-05
SARS-CoV-2 IgM	7.99 (0.47–90.52)	9.54 (1.29–17.79) n.s.	39 (0.496–445.023) n.s.	23.1 (2.73–142.47) 0.035	7.99 (0.475–89.606)	36.4 (0.515–432.205) 0.013	12.4 (0.495–420.552)	8.05 (0.501–63.222) n.s.
SARS-CoV-2 IgG	57.6 (0.715–139.665)	4.55 (1.36–7.74) n.s.	74.6 (4.41–102.88) n.s.	111 (13.4–164.8) 0.013	50 (0.744–139.110)	88.4 (2.29–160.69) 0.032	63.8 (0.863–150.015)	42.4 (0.959–136.694) n.s.
C3	1.46 (0.91–2.51)	1.71 (1.69–1.73) n.s.	1.27 (0.821–3.758) n.s.	2.37 (0.895–3.842) 0.015	1.48 (0.91–2.50)	1.6 (0.8–4.2) n.s.	1.52 (0.895–2.937)	1.3 (0.791–3.377) n.s.
C4	0.33 (0.170–0.527)	0.475 (0.347–0.603) n.s.	0.3 (0.201–0.737) n.s.	0.32 (0.0975–0.6125) n.s.	0.34 (0.17–0.58)	0.3 (0.125–0.710) n.s.	0.33 (0.164–0.668)	0.3 (0.196–0.488) n.s.
C3d	53.4 (26.3–88.3)	64.4 (59.9–68.9) n.s.	58.2 (36.9–92.3) n.s.	81.9 (60–171) 2.07e-05	55.1 (26.4–87.5)	72.7 (40.4–162.6) 0.000163	57.9 (26.6–118.6)	67.1 (37.2–118.6) n.s.
CRP	31.5 (0.246–221.040)	98.9 (58.3–139.5) n.s.	157 (60.8–293.6) 0.000557	271 (76.9–389.8) 6.23e-06	34.2 (0.25–219.28)	199 (59.1–382.9) 1.22e-07	41 (0.266–341.255)	176 (64.8–268.8) 0.000921
IL-6	22.1 (1.5–261.0)	40.5 (37.3–43.6) n.s.	158 (44.4–795.2) 5.47e-05	344 (66.6–1,411.2) 3.3e-06	22.5 (1.5–260.5)	191 (47.8–1,270.0) 6.93e-09	26.4 (1.5–480.9)	266 (42.3–869.9) 4.08e-05
Procalcitonin	0.103 (0.0311–1.7465)	0.22 (0.135–0.306) n.s.	0.862 (0.261–39.112) 5.2e-06	2.56 (0.383–15.365) 1.03e-06	0.109 (0.0312–1.6632)	1.08 (0.277–31.272)	0.12 (0.0317–9.9634)	2.91 (0.262–36.612) 5.57e-06
suPAR	6.88 (2.27–25.99)	12.2 (12.0–12.5) n.s.	23.4 (12.0–81.6) 1.23e-05	22.1 (10.2–42.5) 9.79e-06	7.04 (2.27–25.99)	22.1 (9.41–74.41) 5.82e-09	7.42 (2.29–57.21)	20 (11.4–38.1) 9.97e-05
NGAL	60.7 (38.4–373.2)	98.5 (90–107) n.s.	256 (77.3–1,059.7) 5.73e-05	202 (124–555) 1.41e-05	61.4 (38.5–373.0)	236 (90.3–1,036.7) 2.5e-08	69.8 (38.5–427.0)	236 (73.5–877.5) 4e-05
GDF-15	1,708 (400–10,834)	6,379 (1,983–10,775) n.s.	8,955 (4,758–27,958) 3.56e-05	19,928 (7,200–69,036) 7.87e-07	1,752 (400–11,058)	16,398 (5,141–57,727) 2.36e-09	2,117 (400–28,993)	11,006 (6,194–66,774) 1.68e-05
KL-6	396 (131–2,476)	397 (284–510) n.s.	499 (373–1,854) 0.04	801 (327–1,773) 0.004	396 (135–2,408)	615 (323–2,002) 0.000953	443 (149–2,335)	468 (230–1,245) n.s.

Patient grouping: group 1 = no oxygen treatment, group 2 = nasal/mask oxygen treatment, group 3 = high-flow and NIV oxygen treatment, group 4 = respirator treatment. The median (2.5–97.5 percentiles) maximum concentration of each biomarker is noted together with the p-value for comparison with group 1 (for group 2, 3 and 4), group 1 + 2 (for group 3 + 4) and survived (for dead), respectively. n.s., not significant; C3, complement C3; C4, complement C4; C3d, complement C3d; CRP, C-reactive protein; IL-6, interleukin-6; suPAR, soluble urokinase plasminogen activator receptor; NGAL, neutrophil gelatinase-associated lipocalin; GDF-15, growth/differentiation factor 15; KL-6, Krebs von den lungen-6.

Figure 3: Correlation plots between SARS CoV-2 N protein on the X-axis and the analyses of SARS CoV-2 RNA, CRP and IL-6. Spearman's rank correlation coefficient and p-value are noted on each sub-Figure.

process continues with 51 out of 84 exceeding upper reference limit of 52 mU/L (>60%). A total of 20 patients received steroid treatment at any time during hospitalization, doses ranging from 5–100 mg prednisolone-equivalent doses daily, but only three patients were treated with a high dose of 100 mg/day.

From these observations, it can be hypothesized whether the doses of steroids given to our patients have been too low to terminate the complement activation cascade [8]. Indeed, the Recovery trial has showed that 40 mg prednisolone daily is advantageous [9].

Inflammatory diseases, which are primarily complement driven, such as systemic Lupus Erythematosus, severe disease activity with high immune complex levels and C3d levels around 100 mU/L is treated with prednisolone doses at 1 mg/kg body weight. Hence, it could be considered to treat severe COVID-19 patients displaying high antigen, RNA, IL-6 and CRP levels with the similar doses to stop the complement activation. This has been tried in a small Iranian study [10], which found no significant clinical difference between doses, although the study might be too small. A larger Scandinavian study is underway [11].

In Table 2 illustrating correlations to disease, the markers SARS CoV-2 antigen and RNA, C3d, CRP, IL-6, procalcitonin, suPAR, NGAL, GDF-15 and KL-6 all exhibit low p-values for the need for oxygen supply. In contrast, the same biomarkers except C3d and KL-6 are associated to a fatal outcome and hence all reflect disease severity.

To illustrate predictive values for need of intensive ventilation support or death, IL-6 as first and maximum value during hospitalization is shown in Figure 4. However, other markers as SARS CoV-2 antigen and RNA, CRP, procalcitonin, suPAR, NGAL, GDF-15 and KL-6 also show high predictive value. Figure 4 shows AUC and ROC curves for sars cov2 antigen, crp, IL-6 and c3d.

KL-6 is a marker of lung-tissue damage [12] and as expected, the levels of KL-6 are strongly correlated with the antibody markers, complement marker (C3 and C3d) as well as the inflammation markers CRP, IL-6, procalcitonin, suPAR, NGAL and GDF-15 and also with the need of ventilation support. This indicates that the inflammatory process causes lung damage and hence KL-6 release (Supplemental Table 1). KL-6 has been suggested as a biomarker for lung fibrosis and a marker for need of

Figure 4: Figure (A) shows a box-plot depiction of the IL-6-concentrations in each group with p-values between each compared dichotomous group set. Figure (B) shows ROC-curves for SARS CoV2 N protein, CRP, IL-6, C3d in terms of how well each biomarker detects death and intensive oxygen treatment (group 3 + 4), respectively.

mechanical ventilation [12]. Interestingly, we found KL-6 concentrations typically rising throughout the duration of the hospital stay for this study cohort (see Figure 1) suggesting that KL-6 may be more a biomarker for long term fibrosis than for mechanical ventilation. This would indicate that KL-6 should not be measured before the end of the hospital stay or perhaps even after the patient has been discharged.

NGAL is usually considered an early biomarker for kidney damage although NGAL is also found expressed in lung tissue [13]. In this study, NGAL shows a strong increase in many patients correlated with both inflammatory markers and KL-6 indicating NGAL might be used as a biomarker of lung cell damage.

As an overall conclusion, it is reasonable to believe that the primary noxious event leading to a sequence of clinical consequences is two-fold. First, the higher the virus and antigen levels are in the blood, the more severe disease is seen. Second, the slower the elimination of the virus is (up to 24 days and some do not eliminate the virus before death), the larger the total inflammatory damage.

Concerning the time for elimination, the exact “normal” clearance time cannot be established, as these patients were not followed from the time of Covid-19 diagnosis with a PCR-positive swab sample, but from the time of hospitalization.

We suggest that the most important drug in need of development would be an inhibitor of virus replication, as used for HIV, to reduce the production of new virus particles in the body, at an early stage with low virus load, as measured in the blood with the proposed viral antigen and ddPCR RNA quantification methods.

The strengths of this study is that we followed 84 patients longitudinally in the early phase of the COVID-19 pandemic with daily blood samples before international recommendations with respect to anti-IL-6 and steroid hormone therapy were established. We can therefore 1) describe the disease mechanisms not being highly modified by drugs; 2) measure the viral load using both a sensitive quantitative assay developed for measuring SARS-CoV-2 antigens on the Simoa HDX platform and compared it with parallel measurements of viral RNA with ddPCR; 3) quantify a panel of inflammation markers. Furthermore, the statistics done points beyond a reasonable doubt to causative effects of antigen and RNA-levels, and that the moderate steroid doses given in our study seem not enough to stop complement mediated cell damage.

The limitations of our study is that it is a descriptive study monitoring daily developments in biomarkers pointing to statistical significant predictive correlations with severity of disease and outcome, but the study cannot establish firm causative mechanisms. On the other hand,

the proposed biomarkers can be used in the future to do that. Another limitation is the number of patients was not sufficient to subgroup the cohort.

Finally, it seems reasonable to include measured levels of these biomarkers, especially antigen level, IL-6, C3d and CRP, in the decision to hospitalize Covid-19 patients or not based on the high predictive value of these biomarkers together with the clinical evaluation.

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Ethical approval: The project was approved by The Regional Committees on Health Research Ethics for Southern Denmark (registration number 20/22417). A clinical biobank was created by storing residual blood from the patient samples. The study was performed according to the Danish data protection laws and EU GDPR.

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