SARS-CoV-2 RNA in serum as predictor of severe outcome in COVID-19: a retrospective cohort study

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Summary

SARS-CoV-2 RNA in serum at admission was associated with a seven-fold increased risk of critical disease and an eight-fold increased risk of death in a cohort of 167 patients hospitalised for COVID-19.

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Abstract

Background

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causes Coronavirus disease 2019 (COVID-19). This study aimed to determine if SARS-CoV-2 RNA in serum at admission correlated with clinical outcome in COVID-19.

Methods

COVID-19 patients admitted to the Infectious Diseases department of a tertiary level Swedish hospital, and sampled for SARS-CoV-2 RNA in serum at admission, April 10 to June 30 2020 were included in a cohort. Primary outcomes were day 28 all-cause mortality and progress to critical disease.

Results

The cohort (N=167) consisted of 106 SARS-CoV-2 RNA serum negative and 61 positive patients. Median sampling time for initial SARS-CoV-2 in serum was 1 (IQR 1-2) day after admission corresponding to day 10 (IQR 8-12) after symptom onset. Median ages were 53 (IQR 44-67) and 63 (IQR 52-74) years for the PCR-negative and positive patients, respectively. In the serum PCR negative and positive groups 3/106 and 15/61 patients died, respectively.

The hazard ratios for critical disease and all-cause mortality were 7.2 (95% CI 3.0-17) and 8.6 (95% CI 2.4-30), respectively for patients that were serum PCR positive compared to serum PCR negative.

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Conclusion

SARS-CoV-2 RNA in serum at hospital admission indicates a high-risk of progression to critical disease and death.

Keywords

SARS-CoV-2; COVID-19; Viremia; Viral load; Mortality

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Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the cause of the current Coronavirus disease 2019 (COVID-19) pandemic [1]. COVID-19 is characterised by fever, respiratory tract, and gastrointestinal symptoms with multiple organ involvement in severe disease [2]. A proportion of patients develop critical disease with Acute Respiratory Distress Syndrome and high mortality rates [2, 3].

A "cytokine storm" has been proposed as the cause of severe COVID-19, based on the detection of high levels of cytokines [4]. Similar findings were described in critically ill patients with *Severe Acute Respiratory Syndrome* (SARS) [5]. In SARS, high serum viral load and detection of virus at multiple sites were predictive of adverse clinical outcome. These findings indicated that adverse outcome was associated with continued, uncontrolled viral replication [6]. Similarly, a high viral load in respiratory samples was associated with more severe disease in *Middle East Respiratory Syndrome* (MERS) and has also been described in COVID-19 [7-10]. Oropharyngeal viral load also appears to increase with age in COVID-19 [11]. In line with these findings, SARS-CoV-2 RNA in serum was detected in five of 17 critically ill patients of whom two died and in none of 31 patients with severe or moderate COVID-19 [12]. Moreover, in a very recent study, SARS-CoV-2 RNA was detected in serum in 43/58 (74%) of hospitalised COVID-19 patients with an ultrasensitive Polymerase Chain Reaction (PCR) method [13]. The presence and levels of SARS-CoV-2 RNA in serum were correlated to disease severity, and there was a tendency towards a higher risk of poor outcome in the serum positive group. An inability to control viral replication, and a disseminated hematogenous spread to multiple organs, is thus a possible cause of severe disease or possibly the driving force behind the inflammatory reaction that characterises severe COVID-19. Detection of viral-RNA in serum may thus be an early prognostic marker that is simpler to use compared to more complex risk scoring systems [14, 15]. However, the prognostic value of detecting serum SARS-CoV-2 RNA with a commonly available method has not been evaluated. The primary aim of this study was to determine if SARS-

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CoV-2 RNA in serum, detected using standard Reverse Transcriptase (RT) PCR methods, correlated with clinical outcome in COVID-19.

Methods

Study design and setting

This was a retrospective cohort study of patients with confirmed COVID-19, consecutively admitted to the 38 bed Department of Infectious Diseases at Danderyd Hospital, between the 10th of April and the 30th of June 2020. Danderyd Hospital is a 500-bed tertiary care hospital in Stockholm, Sweden that serves a population of approximately 500.000 people.

The study was approved by the Swedish Ethical Review Authority (Registration number 2020-01770).

Study population

The study population consisted of patients with confirmed COVID-19 that were admitted to the Department of Infectious Diseases and had a SARS-CoV-2 RNA serum sample taken within three days of admission. All patients admitted to the hospital during the study period with symptoms suggestive of COVID-19 were screened for SARS-CoV-2 in airway (nasopharyngeal, throat, or sputum) samples by PCR assays. Patients with positive results were considered to have a confirmed diagnosis.

At the beginning of the study period the influx of patients was considerable and patients with confirmed COVID-19 were admitted to medical and surgical wards as well as the Department of Infectious Diseases. During this period there was no particular selection of any patient category to any particular ward.

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A phase I/II safety and dose-finding study on convalescent plasma was conducted at the Department of Infectious Diseases during the study period. Febrile patients requiring supplemental oxygen therapy were initially eligible for entry into the phase I study. Subsequently, patients with SARS-CoV-2 RNA detected in serum were eligible for entry to the phase II study. No other interventional studies were ongoing in this population. No antivirals or chloroquine derivatives were in use for the treatment of COVID-19 during the study period. Corticosteroid treatment was introduced for patients with critical disease from the 18th of June.

Data collection

Serum sampling was part of routine and evolving clinical practice. Between the 10th of April and the 10th of May, serum samples from patients perceived to be more ill were collected for SARS-CoV-2 PCR analysis. Serum samples for SARS-CoV-2 PCR analysis were systematically collected from patients with confirmed COVID-19 admitted to the Department of Infectious Diseases between 11th of May and 30th of June.

Department records were accessed to identify all patients with a PCR-confirmed diagnosis of COVID-19 between 10th of April and 30th of June 2020. The research team accessed the electronic medical records of the patients in the cohort and extracted epidemiological, clinical, laboratory, treatment, and outcome data retrospectively. Patient characteristics, including routine blood samples and clinical parameters, were noted the same day as the first SARS-CoV-2 PCR-analysis from serum was taken. If data was missing, we used the closest known value from $\pm$ 1 day. No imputation of missing data was done.

All patients were followed for 28 days after SARS-CoV-2 RNA serum sampling. The electronic medical records are linked and synchronized to the Swedish Population Register, which enables follow-up of mortality regardless of cause and place of death.

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PCR analyses

PCR analyses were performed at the Karolinska University Laboratory according to normal routine procedures for SARS-CoV-2 RNA detection. Airway and serum samples were analysed on the cobas® 6800 instrument, a fully automated test including RNA extraction (Roche Molecular Diagnostics, Pleasanton, CA, USA) or an in-house method with RNA-extraction (MagNA Pure 96, Roche Diagnostics) followed by a modified version (unpublished) of the Drosten protocols for PCR analysis [16, 17]. The two PCR methods had similar performance in in-house assessments (Supplementary data). The cobas® target the *envelope* (E) and *Open Reading Frame* (ORF) 1 genes. The in-house assay targets the E-gene and the *RNA-dependent RNA polymerase* (RdRp) gene. Amplification of at least one gene was considered a positive test. Airway samples were also analysed using the GeneXpert® system (Cepheid, Sunnyvale, USA) from April 27 [18]. CT values were recorded from serum PCRs. CT values were not recorded for airway PCRs as nasopharyngeal, throat swabs and sputum samples were analysed and assessed on three different systems preventing meaningful analyses.

Outcome

The primary outcome was all-cause mortality at 28 days after first SARS-CoV-2 serum sampling.

The secondary outcome was progression to critical disease within 28 days of the initial SARS-CoV-2 serum sampling. Critical disease was defined as a composite of ICU-care and mortality. The hospital's ICU consisted of one ward for SARS-CoV-2 patients requiring mechanical ventilation, and two wards for patients requiring Non-Invasive Ventilation (NIV), High Flow Nasal Oxygen (HFNO) Therapy, or inotropic drugs. During the study period, the ICUs had an admission criterion of peripheral capillary

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oxygen saturation <90% despite 12 litres/min oxygen on a mask (PaO2/FiO2-ratio approximately 75). At the Department of Infectious Diseases, oxygen was given using a mask but not using NIV or HFNO.

Statistics

Patients that were SARS-CoV-2 serum PCR negative at study entry, in effect on admission, were classed as *PCR negative*. Patients that were SARS-CoV-2 serum PCR positive at entry were classed as *PCR positive*. Continuous variables are presented as medians (interquartile range - IQR) and were calculated using quantile regression with bootstrap (100 reps). Hazard Ratios (HR) were calculated with 95% confidence intervals (95% CI) using univariate and multivariate Cox regression. Variables that have been previously described as predictors for severe outcome (age, gender, body mass index (BMI), hypertension, diabetes, C-reactive protein, lymphocyte count, cardiovascular and pulmonary disease), were included in the univariate analyses [19]. Due to missing data and difficulties with standardized interpretations, lactate dehydrogenase and chest x-rays were not included in the statistical analyses. Variables with a p-value < 0.05 in the univariate analysis were included in the multivariate analysis. The proportional hazards assumption was tested with a log-log plot of survival. We constructed Kaplan-Meier survival curves for all-cause mortality and progression to critical disease. The log-rank test was used to test for equality of survivor functions. All tests were two-sided and a p-value <0.05 was considered significant. Statistical analyses were done with STATA version 13.1 (StataCorp, TX, USA).

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Results

A total of 258 patients with confirmed COVID-19 were admitted to the Department of Infectious Diseases during the study period. Of these, 167 were tested for SARS-CoV-2 RNA in serum within three days of admission. SARS-CoV-2 RNA in serum was detected in 61/167 (37%) patients and not detected in 106/167 (63%) patients. A total of 91 patients were excluded from the study. Of these,

77 were not tested, 10 tested more than three days after admission, and four tested during/after ICU-care. The flow of patients through the study are shown in Figure 1. Baseline characteristics are shown in Table 1. Routine serum sampling of patients admitted to the Department of Infectious Diseases was implemented from the 10th of May until the 30th of June. In this subgroup, 69/106 (65%) patients were PCR negative and 37/106 (35%) were PCR positive.

The median time from admission to initial PCR testing in serum was 1 (IQR 1-2) day in both the PCR negative and positive group. The median durations of illness before testing were 10 days (IQR 8-12) and 9 days (IQR 8-11) in the PCR negative and positive groups, respectively. Median time to first outcome was 2 (IQR 1-2) days after sampling in the PCR positive group.

Progression to critical disease and mortality

In total, 18/167 (11%) patients died during follow-up, of which 3/106 (3%) were in the PCR-negative group and 15/61 (25%) in the PCR-positive group. The number of patients reaching the composite outcome (ICU-care or death) were 34/167 (20%) in the whole cohort; 7/106 (7%) in the PCR-negative and 27/61 (44%) in the PCR positive group. Age, PCR positivity, hypertension, cardiovascular disease, and C-reactive protein were correlated to death or critical diseases in the univariate regression and included in the multivariate Cox regression (Table 2 and 3). Only PCR positivity and age were significantly associated with mortality and progression to critical disease in the multivariate analysis.

Hazard ratios for serum PCR positivity were 8.6 (95% CI 2.4-30) for all-cause mortality and 7.2 (95% CI 3.0-17) for critical disease. HR for age were 1.2 (95% CI 1.1-1.2) per year for all-cause mortality and 1.1 (95% CI 1.0-1.1) for critical disease. There was no significant interaction between age and serum PCR positivity. Kaplan-Meier survival graphs of all-cause mortality and critical disease, comparing serum PCR positive and negative patients are presented in Figures 2 and 3. Performing

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the analyses after dividing the cohort into the period before and after systematic testing of admitted patients (see Supplementary Material) did not significantly alter the findings.

Correlation between age and SARS-CoV-2 detection in serum

The correlation was assessed in patients admitted between the 11th of May and 30th of June 2020, when serum PCR samples were collected systematically. PCR negative patients were significantly younger (median 53 years [IQR 42-67]) compared to PCR positive patients (median 62 years [IQR 50-74]) years ($p<0.001$). The proportion of serum PCR positive patients increased with age with a distinct increase in patients older than 60 years, as shown in Figure 4.

RT-PCR results

The in-house PCR method was used in 44/61 (72%) of the positive serum samples and 17/61 (28%) were analysed with the cobas® assay. Median Cycle Threshold (CT) values were 34 (IQR 32-35) for the E-gene and 33 (IQR 30-35) for the RdRp-gene with the in-house-assay, and 37 (IQR 35-38) for the E-gene with the cobas® assay. Median CT value for the ORF1-gene from the cobas® assay was not analysable due to insufficient number of positive samples.

Correlation between Convalescent plasma therapy and mortality

Convalescent plasma was given to 28/61 serum PCR positive patients. Plasma from several donors at 7-fold varying doses were used. The proportion of patients that died amongst serum PCR positive patients that did and did not receive convalescent plasma were 6/28 and 9/33, respectively.

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Discussion

The principal finding was that detection of SARS-CoV-2 RNA in serum, within three days of admission, identified a population with a seven-fold increased risk of progress to critical disease and an eight-fold increased risk of death, compared to patients in whom SARS-CoV-2 RNA was not detected in serum. Equally valuable, not detecting SARS-CoV-2 RNA in serum indicated a high chance of uncomplicated recovery. The data are consistent with a study which reported SARS-CoV-2 RNA in the serum of critically ill patients only (N=5) and a recent study that identified higher serum viral loads in more severely ill patients [12, 13]. Veyer et. al. also showed a trend towards worse outcome in the PCR positive group. The results are also in line with SARS, MERS, and COVID-19 data indicating that detection of virus in serum and viral load in respiratory samples correlated with outcome [6, 7, 9, 10]. However, detection of SARS-CoV-2 in serum was not significantly associated with disease severity in a cohort of 96 patients [8]. A possible explanation is that disease severity was based on clinical characteristics in that cohort, in which there was zero mortality. In the current analysis, the presence of SARS-CoV-2 RNA in serum was assessed as an early predictor of clinical outcome, essentially asking the question the other way around. Thus, these data do not necessarily oppose each other and detection of SARS-CoV-2 in the serum of hospitalized patients seems to be an early indicator of a population at a significantly increased risk of critical disease and death. However, the results need to be verified in other populations.

As shown previously, age was also an independent predictor of critical disease and death [11]. In addition, the proportion of serum PCR positive patients increased with age, especially in those over the age of 60 years. An age correlated increased nasopharyngeal viral load has been reported previously supporting this result [9]. These data suggest that there is an age correlated, decreased ability to control viral replication that may in turn be causally linked to severe disease. Furthermore, SARS-CoV-2 was found in serum of 78% (28/36) of patients admitted to Danderyd hospital ICU due to an anticipated need of invasive ventilation during a similar time period (unpublished data), which

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matches the 88% (23/26) PCR positive patients with critical disease in a study by Veyer et. al. [13]. Taken together this suggests that most patients who developed critical disease had a continued, age correlated, uncontrolled viral replication as found in SARS [6].

Dysregulation of the inflammatory response resulting in a "cytokine storm" has been proposed as an important factor in the pathogenesis of severe COVID-19 [4]. In line with this, high IL-6 levels commonly occur in critically ill patients [3, 12, 20, 21]. Moreover, detection of SARS-CoV-2 in serum correlated with extremely high levels of IL-6 [12]. Detection of SARS-CoV-2 in serum could thus be a result of leakage from tissues damaged by the inflammatory response. However, the presence of SARS-CoV-2 in serum prior to the development of severe disease suggests that the virus is, at least initially, driving the inflammatory response. Detecting SARS-CoV-2 in serum is in line with the multi-organ involvement seen in patients with critical disease and autopsy data reporting viral infection in several organs, indicating hematogenic spread of the virus [2, 22, 23].

The serum PCR CT values in this study were high with low variance as reported previously, and unlike CT values from respiratory samples where virus is known to replicate [8]. The low variance and the dichotomous nature of PCR positivity suggests a total viral load reaching a detectable level, predictive of a severe course. In a study using an ultrasensitive PCR method, the viral load in serum differed significantly between patients of different disease severity and 74% of tested patients were PCR positive [13]. This difference is most likely due to a greater method sensitivity enabling detection of even lower RNA levels in serum, of potential clinical utility in the future.

Early identification of groups with a high or low risk of critical disease, using a commonly available PCR method, helps us make informed treatment decisions. Recent preliminary data indicate that remdesivir given early to moderately ill patients may be an effective COVID-19 treatment [24]. Serum PCR positive patients are a group in which to primarily assess the efficacy of remdesivir while the supply is limited. Other potential antiviral therapies such as convalescent plasma may also be most effective if given to serum PCR positive patients before the development of critical disease. The

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PCR positive group may also be a target for immunomodulating therapies such as corticosteroids, IL-6 and IL-1 inhibitors.

Limitations

This study has several limitations. Firstly, at the start of the study, only more severely ill patients were tested. This affected the total distribution of negative versus positive samples. However, it did not appear to affect the predictive value of the results as the HR were not significantly affected by analysing the data before and after the period of systematic inclusion.

Secondly, the use of convalescent plasma may have affected the outcome. The use of convalescent plasma from several donors, with differing antibody titres, at 7-fold varying doses prevented a meaningful analysis. However, receiving convalescent plasma did not obviously increase the risk of serum PCR positive patients dying, at least indicating that convalescent plasma did not cause the deaths seen in the PCR positive group. Moreover, in a large safety study (n = 20.000) of COVID-19 patients receiving convalescent plasma, the incidence of serious adverse events was low [25].

Thirdly, lactate dehydrogenase and radiological results have been described as predictors for severe outcome. Unfortunately, we were not able to include these variables in our statistical models due to missing data and difficulties with standardized interpretations.

Conclusion

SARS-CoV-2 RNA in serum at admission to hospital is associated with a seven-fold increased risk of progression to critical disease and an eight-fold increased risk of death. PCR positivity in serum at admission is thus a predictor for risk of progression to critical illness, and identifies a group of patients to whom treatment should be prioritized in a setting with limited drug availability.

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Notes

Acknowledgements

Contributions

KH, MH, PG-J, JD, and JU designed the study.

KH, MH, and JU organised and entered the data.

KH, MH, PG-J, JD, and JU contributed to the data analysis.

BH, MG, and JD contributed to the microbiological analyses.

KH and JU drafted the manuscript.

All writers contributed to data interpretation, critically revised the drafted manuscript, and approved of the submitted manuscript.

KH and JU accepts full responsibility for the conduct of the study, had full access to all the data, and controlled the decision to publish.

KH attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Conflicts of Interest

PG-J reports personal fees from Pfizer co., outside the submitted work. The other authors report no conflict.

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Tables

Table 1. Demographic and Clinical Characteristics at Time for First SARS-CoV-2 PCR in Serum According to SARS-CoV-2 Serum PCR Status.			
Demographics	***PCR negative (N = 106)***	***PCR positive (N = 61)***	***Complete data (%)***
Age, years	53 (45-67)	63 (52-74)	167 (100)
Sex Female:Male	36:70 (34:66)	23:38 (38:62)	167 (100)
Any comorbidity	74 (70)	48 (79)	167 (100)
Pulmonary disease	19 (18)	10 (16)	167 (100)
Any Cardiovascular disease	15 (14)	18 (30)	167 (100)
Hypertension	35 (33)	30 (49)	167 (100)
Diabetes mellitus	21 (20)	13 (21)	167 (100)
Liver Disease	3 (3)	0 (0)	167 (100)
Chronic Renal Disease	4 (4)	8 (13)	167 (100)
Malignancy	3 (3)	1 (2)	167 (100)
Immunosuppression	1 (1)	5 (8)	167 (100)
Body Mass Index, kg/m^2	29 (25-32)	27 (25-31)	157 (94)
Days after symptom onset for sampling	10 (8-12)	9 (8-11)	165 (99)
Days after admission for sampling	1 (1-2)	1 (1-2)	167 (100)
Peak temperature, °C	38.1 (37.4-38.9)	39 (38.4-39.5)	167 (100)
Peak oxygen demand, L/min	0 (0-2)	3 (1-6)	167 (100)
C-reactive protein, mg/L	93 (49-168)	115 (74-240)	165 (99)
Procalcitonin, µg/L	0.17 (0.1-0.4)	0.32 (0.2-1.2)	107 (64)
Neutrophil, $10^9/L$	4.7 (3.4-6.6)	5.6 (3.6-8.0)	142 (85)
Lymphocyte, $10^9/L$	1.1 (0.8-1.4)	0.8 (0.6-1.3)	142 (85)

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Neutrophil/Lymphocyte ratio	4.6 (2.8-7.6)	6.4 (4.1-10.9)	142 (85)
Troponin T, ng/L	8 (5-10)	13 (8-24)	110 (66)
D-dimer, mg/L	0.8 (0.5-1.3)	1.0 (0.7-1.8)	105 (63)
Part of phase 1 or 2 convalescent plasma therapy study	4 (4)	28 (46)	167 (100)
Corticosteroid treatment	1 (1%)	3 (5%)	167 (100)

Data are median (IQR) or number (%).

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Table 2. Hazard Ratios for All-cause Mortality at 28 Days.

	Univariate			Multivariate		
Variable	**HR**	**95% CI**	***P* Value**	**HR**	**95% CI**	***P* Value**
PCR positive	9.9	2.8-34	< 0.001	8.6	2.4-30	0.001
Age (year)	1.1	1.1-1.2	< 0.001	1.2	1.1-1.2	< 0.001
Male gender	0.8	0.3-2.1	0.7			
BMI (kg/m^2)	0.9	0.8-1.0	0.07			
Hypertension	2.6	1.0-6.8	0.04	1.1	0.4-2.9	0.9
Diabetes	2.0	0.8-5.0	0.17			
Cardiovascular Disease	3.6	1.4-9.1	0.007	0.7	0.2-2	0.5
Pulmonary Disease	1.9	0.3-3.3	0.9			
C-reactive protein (mg/L)	1.0	1.0-1.0	1.0			
Lymphocyte Count (10^9/L)	0.4	0.1-1.3	0.11			

Hazard Ratios (HR) with 95 % Confidence intervals (95% CI) for all-cause mortality calculated by Cox regression. Data on age, gender, PCR status and co-morbidities were available for all patients. CRP, BMI and Lymphocyte Count were available for 165, 157 and 142 subjects.

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Table 3. Hazard Ratios for Critical Disease and Death at 28 Days.

	Univariate			Multivariate		
Variable	**HR**	**95% CI**	***P* Value**	**HR**	**95% CI**	***P* Value**
PCR positive	8.4	3.6-19	< 0.001	7.2	3.0-17	< 0.001
Age (year)	1.1	1.0-1.1	< 0.001	1.1	1.0-1.1	0.001
Male gender	1.3	0.6-2.8	0.4			
BMI (kg/m^2)	1.0	0.9-1.0	0.2			
Hypertension	2.1	1.1-4.2	0.03	0.9	0.4-2.0	0.8
Diabetes	1.0	0.4-2.3	1.0			
Cardiovascular Disease	2.3	1.2-4.7	0.02	0.8	0.3-1.8	0.6
Pulmonary Disease	0.8	0.3-2.0	0.6			
CRP (mg/L)	1.0	1.0-1.0	0.04	1.0	1.0-1.0	0.3
Lymphocyte count (10^9/L)	0.5	0.2-1.1	0.10			

Hazard Ratios (HR) with 95% Confidence intervals (95% CI) for critical disease calculated by Cox regression. Data on age, gender, PCR status and co-morbidities were available for all patients. CRP, BMI and Lymphocyte Count were available for 165, 157 and 142 subjects.

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Figure legends

Figure 1. Flowchart for Study Population.

Figure 2. Kaplan-Meier Estimates of All-cause Mortality.

Survival of patients without (dotted) and with (full) SARS-CoV-2 RNA in serum at admission.

Figure 3. Kaplan-Meier Estimates of Progression to Critical Disease.

Estimates of progression to critical disease, defined as a composite of ICU-care and all-cause mortality. Patients without (dotted) and with (full) SARS-CoV-2 RNA in serum at admission.

Figure 4. Proportion of serum PCR positive patients in different age groups.

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Figure 1

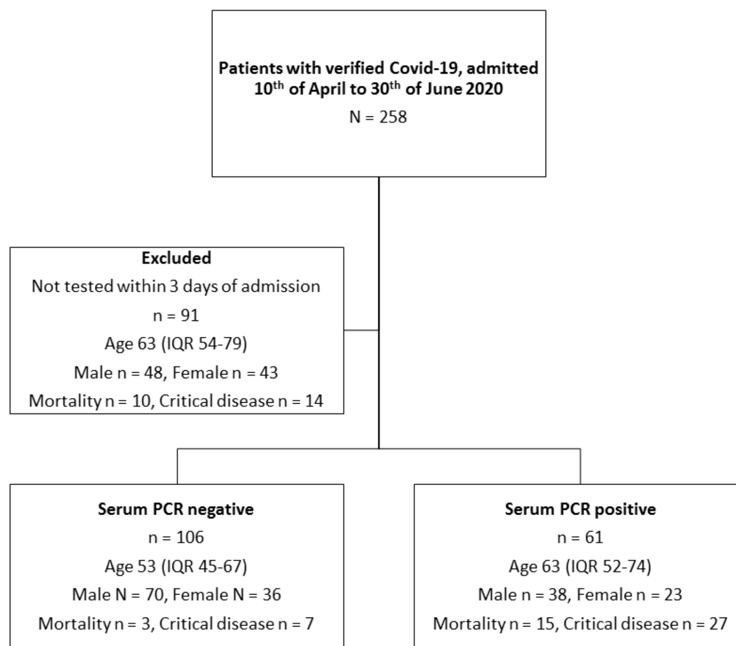

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Figure 2

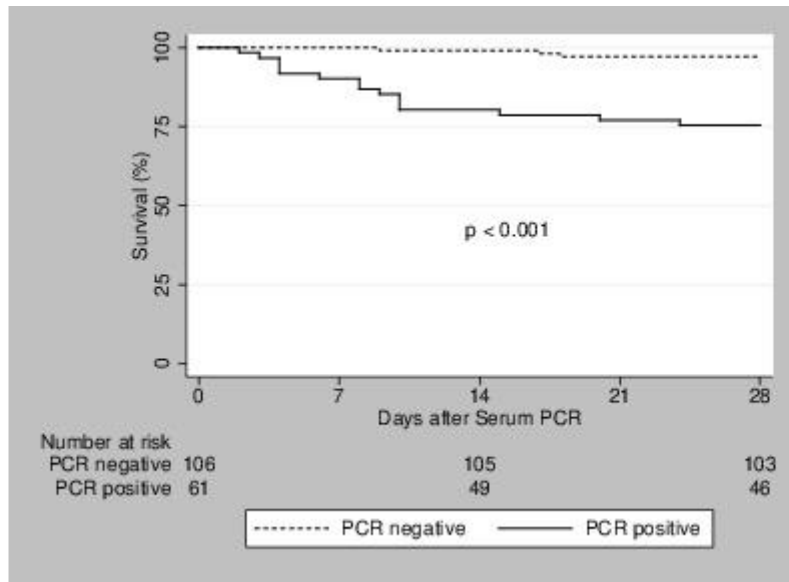

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Figure 3

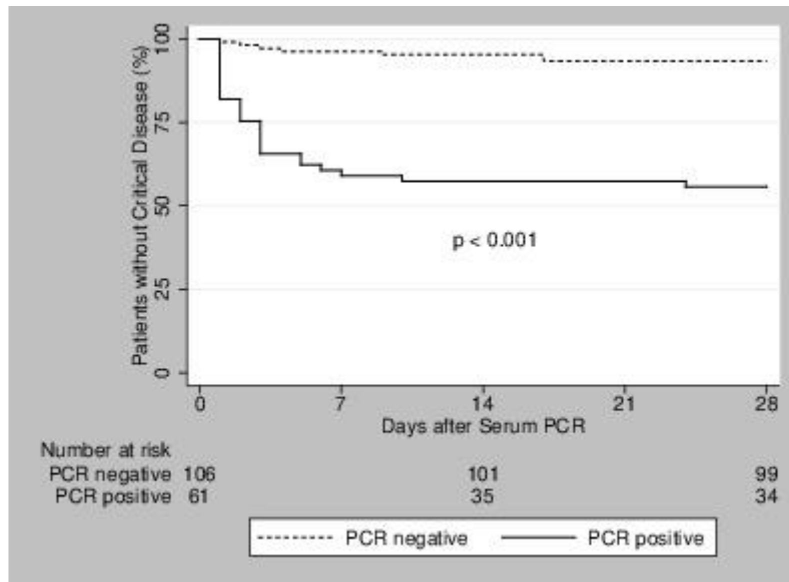

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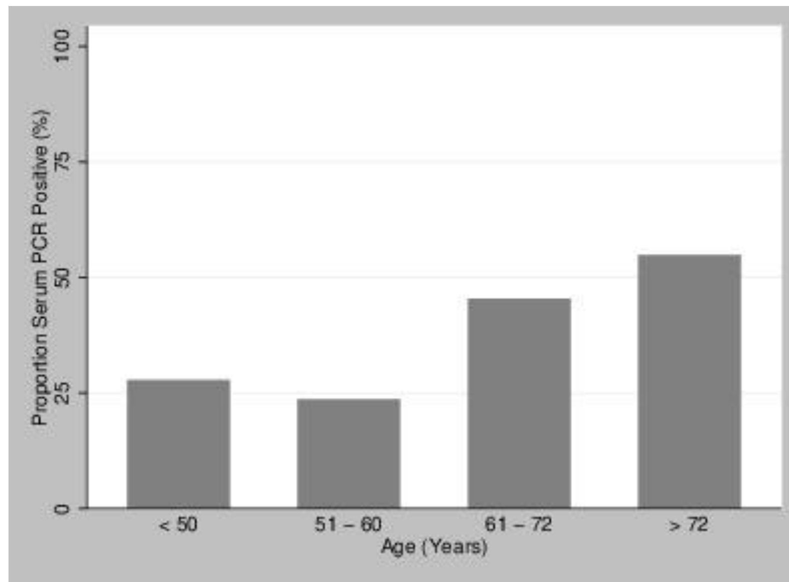
Figure 4



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