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Prof. Dr. med. B. Ondruschka

Predictive value of standard diagnostic procedures for SARS-CoV-2 infections before and after death

Dissertation

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Fabian Heinrich
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95% CI	95% Confidence Interval
AIDS	Acquired Immunodeficiency Syndrome
ARDS	Acute Respiratory Distress Syndrome
AgRDT	Antigen Rapid Diagnostic Test
AWMF	Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften (Working Group of the Scientific Medical Societies)
BMI	Body Mass Index
COVID-19	Coronavirus Disease-2019
DNA	Desoxyribonucleic Acid
DFG	Deutsche Forschungsgemeinschaft (German Research Fundation)
E2	Envelope SARS-CoV-2
ELISA	Enzyme-Linked Immunosorbent Assay
EDTA	Ethylenediaminetetraacetic acid
ECMO	Extracorporeal Membrane Oxygenation
GLMM	Generalised Linear Mixed Model
H1N1	H1N1 Swine Flu
HIV	Human Immunodeficiency Virus
IF	Impact Factor
ILM	Institute of Legal Medicine
IMMVH	Institute of Medical Microbiology, Virology, and Hygiene
ICU	Intensive Care Unit
IQR	Interquartile Range
LDT	Laboratory Developed Test
LOD	Lower Limit of Detection
LOQ	Lower Limit of Quantification
LRT	Lower Respiratory Tract
MV	Mechanical Ventilation
NATON	Nationales Obduktionsnetzwerk (National Autopsy Network)
NAAT	Nucleic Acid Amplification Tests
N1	Nucleocapsid SARS-CoV-2
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PMI	Postmortem Interval
RT-qPCR	Reverse Transcription-Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
SOFA	Sequential Organ Failure Assessment Score
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
SAPS II	Simplified Acute Physiology Score II
SNP	Single Nucleotide Polymorphism
SD	Standard Deviation
USA	United States of America
UTM	Universal Transport Medium
UTR	Untranslated Region
URT	Upper Respiratory Tract
UCT	Utility Channel

VOC	Variants of Concern
WHO	World Health Organisation

Open Forum Infectious Diseases

MAJOR ARTICLE

SARS-CoV-2 Blood RNA Load Predicts Outcome in Critically Ill COVID-19 Patients

Fabian Heinrich,[1,a] Michael F. Nentwich,[2,a] Eric Bibiza-Freiwald,[1] Dominik Nörz,[1] Kevin Roedl,[2] Martin Christner,[1] Armin Hoffmann,[1] Flaminia Olearo,[1] Stefan Kluge,[2] Martin Aepfelbacher,[1] Dominic Wichmann,[2,a] Marc Lütgehetmann,[1,a] and Susanne Pfefferle[1,a]

[1]Center for Diagnostics, Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, and [2]Department of Intensive Care Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA loads in patient specimens may act as a clinical outcome predictor in critically ill patients with coronavirus disease 2019 (COVID-19).

Methods. We evaluated the predictive value of viral RNA loads and courses in the blood compared with the upper and lower respiratory tract loads of critically ill COVID-19 patients. Daily specimen collection and viral RNA quantification by reverse transcription quantitative polymerase chain reaction were performed in all consecutive 170 COVID-19 patients between March 2020 and February 2021 during the entire intensive care unit (ICU) stay (4145 samples analyzed). Patients were grouped according to their 90-day outcome as survivors (n = 100) or nonsurvivors (n = 70).

Results. In nonsurvivors, blood SARS-CoV-2 RNA loads were significantly higher at the time of admission to the ICU ($P = .0009$). Failure of blood RNA clearance was observed in 33/50 (66%) of the nonsurvivors compared with 12/64 (19%) survivors ($P < .0001$). As determined by multivariate analysis, taking sociodemographic and clinical parameters into account, blood SARS-CoV-2 RNA load represents a valid and independent predictor of outcome in critically ill COVID-19 patients (odds ratio [OR; $\log_{10}$], 0.23; 95% CI, 0.12–0.42; $P < .0001$), with a significantly higher effect for survival compared with respiratory tract SARS-CoV-2 RNA loads (OR [$\log_{10}$], 0.75; 95% CI, 0.66–0.85; $P < .0001$). Blood RNA loads exceeding 2.51 × 103 SARS-CoV-2 RNA copies/mL were found to indicate a 50% probability of death. Consistently, 29/33 (88%) nonsurvivors with failure of virus clearance exceeded this cutoff value constantly.

Conclusions. Blood SARS-CoV-2 load is an important independent outcome predictor and should be further evaluated for treatment allocation and patient monitoring.

Keywords. kinetics; SARS-CoV-2; SARS-CoV-2 RNA load; viremia.

Risk assessment and stratification of coronavirus disease 2019 (COVID-19) patients are challenging, notably in intensive care units (ICUs), as it is still unclear which factors correlate with severe courses or fatal outcomes. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA load in blood and respiratory tract specimens as detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR) has been suggested to correlate with disease severity and mortality. However, previous studies on the impact of viremia on patient outcomes were mostly limited to single-point measurements and did not consider the level and course of viral loads [1–7]. By analyzing the course of viremia in a small cohort of critically ill patients with hemato-oncologic disorders, we recently reported that failure to clear SARS-CoV-2 RNA from the bloodstream is associated with a high risk of death [8, 9], as confirmed in small patient cohorts [10, 11].

To investigate the prognostic value of viral load in a mixed patient population for the present study, we evaluated the level and course of viral RNA load in the upper respiratory tract (URT), lower respiratory tract (LRT), and bloodstream of 170 critically ill patients. Multivariate analysis, considering primary sociodemographic data and relevant clinical parameters, was performed.

METHODS

Patients and Ethics

All patients (n = 170) were hospitalized at the Department of Intensive Care Medicine (ICU), University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany, between March 2020 and March 2021. Patients were hospitalized for COVID-19 and/or COVID-19-associated conditions. Patients

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[a]Equal contribution.

Correspondence: Susanne Pfefferle, MD, Center for Diagnostics, Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany (s.pfefferle@uke.de).

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were grouped according to their 90-day outcome status as survivors (n = 100) or nonsurvivors (n = 70). Readmissions of patients were counted as 1 intensive care unit stay. Relevant covariables evaluated were age, sex, body mass index, preexisting medical conditions (ie, prior myocardial infarction, congestive heart failure, peripheral vascular disease, rheumatologic disease, peptic ulcer disease, mild, moderate, or severe liver disease, diabetes mellitus, cerebrovascular [hemiplegia] event, moderate to severe renal disease, diabetes with chronic complications, cancer without metastases, leukemia, lymphoma, metastatic solid tumor, acquired immune deficiency as part of the Charlson comorbidity index, chronic lung diseases, and arterial hypertension), immunosuppression due to preexisting medical conditions, time from COVID-19 diagnosis to ICU admission, presence and degree of acute respiratory distress syndrome (ARDS) according to the Berlin definition, disease severity according to Simplified Acute Physiology Score II (SAPS II) and Sepsis-related Organ Failure Assessment Score (SOFA), need for mechanical ventilation (MV), need for extracorporeal membrane oxidation (ECMO), and need for COVID-19-related treatments (ie, dexamethasone, remdesivir, monoclonal antibodies, therapeutic plasma exchange [TPE]).

The Ethics Committee of the Hamburg Chamber of Physicians was informed about the study. Due to the retrospective nature of the study, the need for informed consent was waived (WF-094/21). Partial data of a subset of the cohort (30 out of 170) have been previously analyzed and published elsewhere [8, 9].

Sampling, Molecular Diagnostics, and Epidemiology

For the upper respiratory tract (URT), nasopharyngeal swabs in UTM (MANTACC, Shenzhen, China) or Amies Medium (E-swab, Copan, Brescia, Italy) were collected. Sputum, bronchial fluid samples, or bronchial lavage samples (all native) were assessed for the lower respiratory tract (LRT). EDTA plasma samples (Sarstedt, Nümbrecht, Germany) were obtained to analyze the blood RNA load. Samples were collected regularly during the ICU stay. All samples were obtained as part of routine clinical practice. In total, 4145 samples were analyzed by qPCR (621 upper respiratory tract, 1455 lower respiratory tract, and 2069 EDTA-plasma samples).

SARS-CoV-2 RNA in respiratory specimens (URT and LRT) was quantified and detected as described previously using the commercially available assays Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA), cobas SARS-CoV-2 (Roche, Mannheim, Germany), and laboratory-developed assays (LDTs) run on the cobas6800 system (Roche), the NeuMoDx system (Qiagen, Germantown, MD, USA), or the Light Cycler 480 II (Roche) [12–15]. Standard RNA reference material (obtained from INSTAND, Düsseldorf, Germany) was used for quantification. To calculate $\log_{10}$ RNA copies/mL (y) based on ct values (x), the following targets and conversion formulae were used for respiratory samples: y = −0.29x + 12.83 (Xpert Xpress SARS-CoV-2, target E2) y = −0.308x + 13.81 (cobas SARS-CoV-2, target T2), y = −0.291x + 12.97 (SARS-CoV-2_UCT (LDT), E-gene), y = −0.425x + 14.8 (NeuMoDx (LDT), E-gene), y = −0.318x + 13.32 (LightCycler 480 II, E-gene). For plasma EDTA samples, the cobas SARS-CoV assay was used with the conversion formula y = −0.247x + 12.27 (cobas SARS-CoV-2, target T2). A threshold of 1×10^3 copies/mL was set for quantification; RNA loads below this cutoff were excluded from the quantitative analysis. For all patients, initial respiratory samples were analyzed in a multiplex typing PCR, identifying and distinguishing SARS-CoV-2 spike variants [16].

Statistical Analysis

Estimation of Virus RNA Clearance

Successful RNA clearance was defined as the absence of SARS CoV-2 RNA (in RT-qPCRs) from the respective compartment for at least 3 days.

Multivariate Analysis

Assuming nonparametric data distribution, the Wilcoxon-Mann-Whitney *U* test was used to compare viral loads between 2 groups. Categorical variables were compared using the 2-sided Fisher exact test or 2-sided chi-square test. The survival distribution of 2 groups was compared using the log-rank test. A generalized mixed model with logistic regression and Firth approximation was used to identify predictors of adverse outcomes in the URT, LRT, and blood. The patients' 90-day outcome status served as the dependent variable. Fixed effects were age [years; metric variable], sex [male = 0; female = 1], body mass index [kg/m^2; metric variable], Charlson comorbidity index [1–13; pseudo-metric variable], the need for mechanical ventilation [no = 0; yes = 1], the presence of ARDS [no = 0; yes = 1], the need for extracorporeal membrane oxygenation [no = 0; yes = 1], and the viral RNA load in the particular compartment [1 $\log_{10}$ level; copies/mL; metric variable]. The patient (correlation structure: compound symmetry) and time (correlation structure: first order autoregression) were set as random factors. The initial selection of statistically independent variables was performed on a clinical and scientific basis. Top-down variable selection was made in the form of a stepwise Akaike Information Criterion (AIC)–guided elimination of predictors. Model optimization was done for the LRT, and the model was subsequently transferred without further adjustments to the other compartments to ensure comparability. Results < limit of detection (<LOD; eg, negative) and < the threshold of 1×10^3 copies/mL were excluded from the generalized mixed model analyses. *P* values <.05 were considered significant.

Statistics were performed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). GraphPad Prism software, version 9.0.0 (GraphPad Software, San Diego, CA, USA), was used for data illustration.

RESULTS

Patients Characteristics

The median patient age (IQR) was 63 (55–73) years, with 35% being female. The overall case fatality rate in our single-center cohort was 41% (70/170), with a median observation time (IQR) of 22 (11–34) days. Baseline characteristics of survivors and nonsurvivors are illustrated in Table 1. The presence of ARDS and, accompanying this, the need for mechanical ventilation (MV) and ECMO were predominantly observed in the nonsurvivor group (90% vs 50%, 93% vs 57%, and 46% vs 17%, respectively).

In the survivor group, 23% of patients were considered immunosuppressed, while in the nonsurvivor group this proportion was significantly higher at 40%. The proportions of patients receiving COVID-19-related antiviral therapy were comparable, meaning 39/100 (39%) survivors received dexamethasone, compared with 35/70 (50%) nonsurvivors. Remdesivir was administered to 22/100 (22%) survivors compared with 11/70 (16%) nonsurvivors. None of the survivors received monoclonal antibody therapy, and only 3/70 (4%) nonsurvivors were treated with monoclonal antibodies. Therapeutic plasma exchange was performed in 3/100 (3%) survivors and 3/70 (4%) nonsurvivors.

The presence of mutant spike variants of concern (VOC) was ruled out in all patients by multiplex typing PCR [16]. Of note, this is in line with the GSAID database, which documented the first entries of SARS-CoV-2 VOCs (such as B.1.1.7) in Northern Germany only by the end of the entire observation period.

Viral Loads at ICU Admission

Median blood viral loads at the time of ICU admission (IQR) were significantly different between the 2 groups, with 3.56×10^3 (<limit of detection [LoD]—1.93×10^4) SARS-CoV-2 RNA copies/mL in nonsurvivors compared with $<1.00 \times 10^3$ (<LoD—2.79×10^3) SARS-CoV-2 RNA copies/mL in survivors ($P = .0009$) (Figure 1A).

Median LRT RNA loads at the time of admission showed no significant differences, with median LRT RNA loads (IQR) of 4.77×10^5 (8.38×10^3–1.11×10^7) SARS-CoV-2 RNA copies/mL in nonsurvivors compared with 1.70×10^5 (<LoD—5.39×10^6) SARS-CoV-2 RNA copies/mL ($P = .14$) in survivors (Figure 1A).

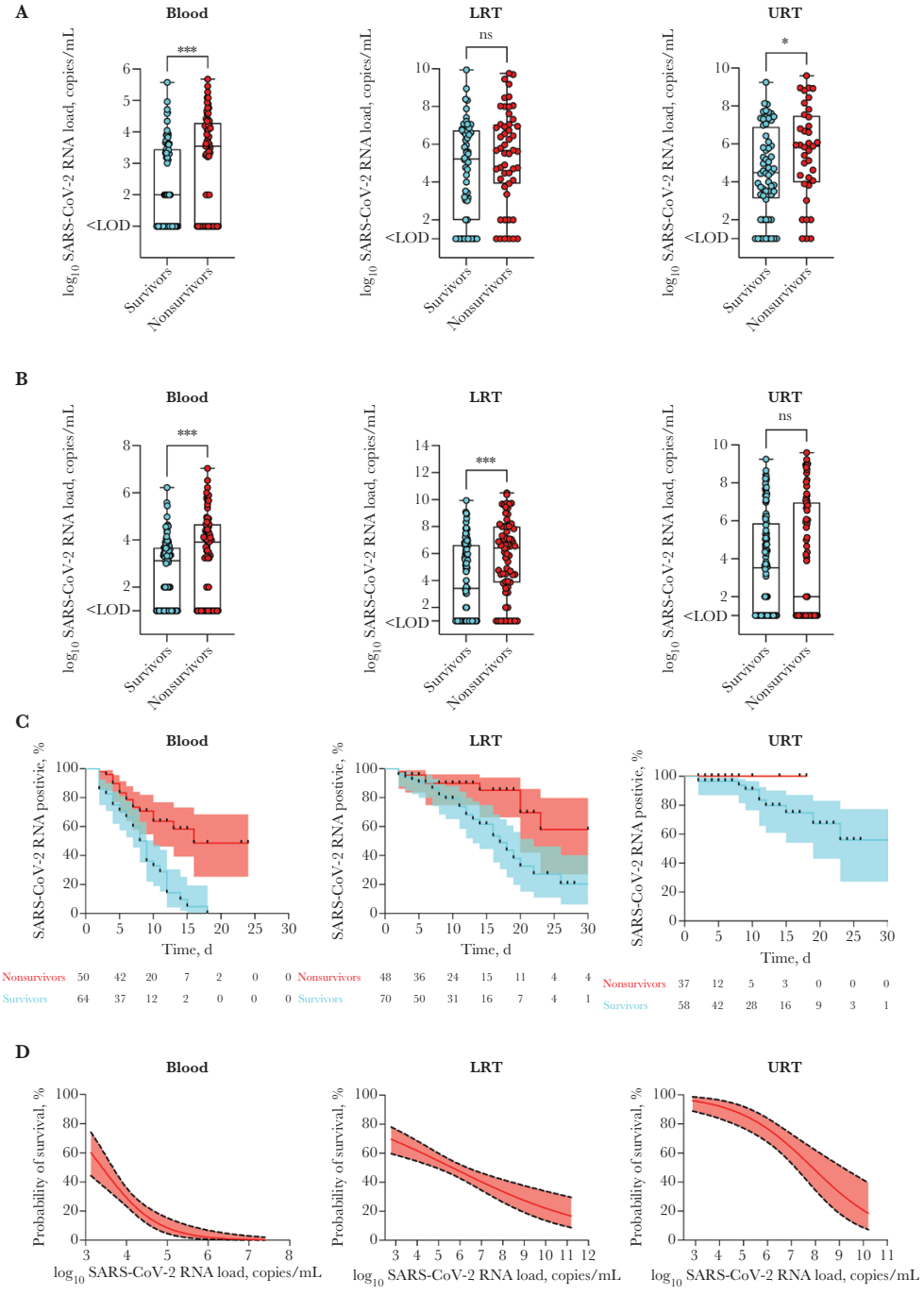
Median URT RNA loads at the time of admission were found to be different, with median URT viral RNA loads (IQR) of

Table 1. Patient Characteristics of ICU Patients of the University Medical Center Hamburg-Eppendorf, Hamburg, Germany

	Survivors, No. (%) or Median (IQR) (n = 100)	Nonsurvivors, No. (%) or Median (IQR) (n = 70)	Comparative Statistics, *P* Value	Total, No. (%) or Median (IQR) (n = 170)
Age, y	60 (51–72)	67 (59–76)	.01	63 (55–73)
Sex	Male: 63 (63)	Male: 48 (69)	.45	Male: 111 (65)
	Female: 37 (37)	Female: 22 (31)		Female: 59 (35)
Body mass index, kg/m^2	28 (25–32)	26 (24–32)	.22	27 (25–32)
Charlson comorbidity index at ICU admission	1 (1–3)	2 (1–4)	.05	2 (1–3)
Comorbidities				
Chronic lung disease	13 (13)	11 (16)	.62	24 (14)
Type II diabetes mellitus	35 (35)	23 (33)	.77	58 (34)
Arterial hypertension	55 (55)	43 (61)	.40	98 (58)
Immunosupression	23 (23)	28 (40)	.02	51 (30)
Duration of illness/time from COVID-19 diagnosis until ICU admission, d	3 (1–8)	7 (1–13)	.14	4 (1–11)
COVID-19 disease severity				
Clinically diagnosed ARDS	50 (50)	63 (90)	<.0001	113 (67)
Sepsis-related Organ Failure Assessment Score	5 (3–11)	10 (5–13)	.001	7 (3–12)
Simplified Acute Physiology Score II	37 (30–43)	42 (37–52)	<.0001	40 (32–48)
ICU-specific treatment				
Mechanical ventilation	57 (57)	65 (93)	<.0001	122 (72)
ECMO	17 (17)	32 (46)	<.0001	49 (29)
COVID-19-related treatment				
Dexamethasone	39 (39)	35 (50)	.16	74 (44)
Remdesivir	22 (22)	11 (16)	.31	33 (19)
Monoclonal antibodies	0 (0)	3 (4)	.07	3 (2)
Therapeutic plasma exchange	3 (3)	3 (4)	.69	6 (4)

The groups are divided according to survival.

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.



7.56×10^5 (9.66×10^3–3.04×10^7) SARS-CoV-2 RNA copies/mL compared with 3.03×10^4 (1.37×10^3–7.90×10^6) SARS-CoV-2 RNA copies/mL ($P = .04$) in nonsurvivors vs survivors (Figure 1A).

Maximum Viral Loads

The maximum blood RNA loads during the course of the disease were significantly higher in nonsurvivors compared with survivors (median [IQR], 8.11×10^3 [<LoD—4.60×10^4] SARS-CoV-2 RNA copies/mL; vs median [IQR], 1.32×10^3 [<LoD—4.67×10^3] SARS-CoV-2 RNA copies/mL; $P = .0009$) (Figure 1B). Maximum LRT RNA loads during the course of the disease were significantly higher among nonsurvivors compared with survivors (median [IQR], 2.63×10^6 [7.26×10^3–9.81×10^7] SARS-CoV-2 RNA copies/mL; vs median [IQR], 2.61×10^3 [<LoD—4.12×10^6] SARS-CoV-2 RNA copies/mL; $P < .0001$) (Figure 1B).

During the course of the disease, maximum URT levels showed no significant differences between the 2 groups (median RNA load [IQR], $<1.00 \times 10^3$ [<LoD—9.27×10^6] SARS-CoV-2 RNA copies/mL in nonsurvivors; vs median RNA load [IQR], 3.30×10^3 [<LoD– 7.36×10^5] SARS-CoV-2 RNA copies/mL in survivors; $P = .73$) (Figure 1B).

Viral Load Kinetics and Clearance

Mean SARS-CoV-2 RNA loads in the 3 compartments normalized to COVID-19 diagnosis are illustrated in Figure 2 (for individual patient kinetics, refer to Supplementary Figure 1). Analyses of viral load kinetics revealed significant differences in viral clearance rates between nonsurvivors and survivors in the blood (median time to clearance, 16 days vs 9 days; $P < .0001$) and LRT samples ($P = .01$) (Figure 1C), but not for URT samples ($P = .13$) (Figure 1C). The Kaplan-Meier curves show the proportion of patients with continuous SARS-CoV-2 detection throughout the observation period. Accordingly, failure of viral RNA clearance from the bloodstream was observed in the majority of nonsurvivors (33/50 [66.0%]) compared with survivors (12/64 [18.8%]; $P < .0001$).

Multivariate Analysis

The generalized linear logistic mixed models incorporating blood or respiratory tract (URT and LTR) viral loads in addition to relevant clinical covariables (age, sex, body mass index, Charlson comorbidity index, ARDS, the need for mechanical ventilation [MV], and ECMO) confirmed blood viral RNA load as a strong independent predictor of adverse outcomes (odds ratio [OR; unit: 1 $\log_{10}$], 0.23; 95% CI, 0.12–0.42; $P < .0001$), with a significantly higher effect for survival if compared with SARS-CoV-2 RNA loads in the LRT and URT (Figure 1D; details of the analysis are given in Table 2). For the URT model, refer to Figure 1D and Table 2.

In our model, patients with blood SARS-CoV-2 RNA loads exceeding 2.51×10^3 SARS-CoV-2 RNA copies/mL had a probability of death exceeding 50% (95% CI, 37.8%–62.3%) (Figure 1D). Here, 40 out of 50 viremic patients (80%) in the nonsurvivor group exceeded this value at least once during the ICU stay, compared with 32 out of 64 viremic patients (50%) in the survivor group ($P = .0016$). Consistently, in 29/33 (88%) of the nonsurvivors with failure of virus clearance, this cutoff value was constantly exceeded, compared with 2/12 (17%) of the survivors with failure of virus clearance.

DISCUSSION

In the present study, we evaluated the prognostic value of SARS-CoV-2 RNA levels and kinetics in blood and upper and lower respiratory tract samples by daily molecular analyses of all 3 compartments in 170 critically ill patients. This in-depth look at the course of virological data contrasts with previous studies that analyzed single-point measurements [1, 2, 4, 5, 7] or only focused on individual compartments without quantifying viral loads [7, 17]. Two studies on blood SARS-CoV-2 RNA loads found increased mortality in viremic patients, yet their findings were based on smaller cohorts and lower sampling frequencies [10, 11]. A recent large study focused on respiratory specimens and highlighted the association of viral RNA load and infectivity in outpatients compared with inpatients, but without addressing their predictive value for mortality and disease progression [18].

Notably, and consistent with previous studies [6, 10, 11], blood RNA loads on admission to the ICU were significantly elevated in patients with fatal outcomes ($P = .0009$), while no significant difference in admission viral loads could be shown for the lower respiratory tract. Furthermore, the maximum blood and LRT viral RNA loads during the disease were significantly higher in nonsurvivors compared with survivors. Again, no

Figure 1. A, SARS-CoV-2 RNA loads at the time of ICU admission in blood, LRT, and URT. B, Maximum loads of SARS-CoV-2 RNA in blood, LRT, and URT during the course of disease differ significantly between groups. Samples < LOD were set to 1×10^1, and samples < LOQ were set to 1×10^2 to allow for logarithmic presentation. C, Kaplan-Meier curves illustrate the probability of virus RNA elimination in the blood, LRT, and URT. The y-axis displays the proportion of patients with continuous detection of SARS-CoV-2 RNA. Successful RNA clearance was assumed at negative RT-qPCR results >3 days. D, Multivariate analysis (generalized linear logistic mixed model) (Table 1) reveals blood RNA level rather than LRT/URT RNA level as a strong predictor of outcome, with a 50.0% probability of death at blood RNA levels exceeding 2.51×10^3 (=3.40 $\log_{10}$) copies/mL. The red line represents the estimated effect, and the dotted black lines represent the 95% CI. Model estimators for the GLMM (blood) are AIC, 393.28; c-c, 0.86, for the GLMM (LRT) AIC, 923.81; c-c, 0.84, and for the GLMM (URT) AIC, 337.44; c-c, 0.92. *P* values are displayed as follows: $^{****}P < .0001$; $^{***}P = .0002$; $^{**}P = .0021$; $^{*}P = .0332$; ns = 0.1234. Abbreviations: AIC, Akaike Information Criterion; GLMM, generalized linear mixed model; ICU, intensive care unit; LOD, limit of detection; LOQ, limit of quantification; LRT, lower respiratory tract; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; URT, upper respiratory tract.

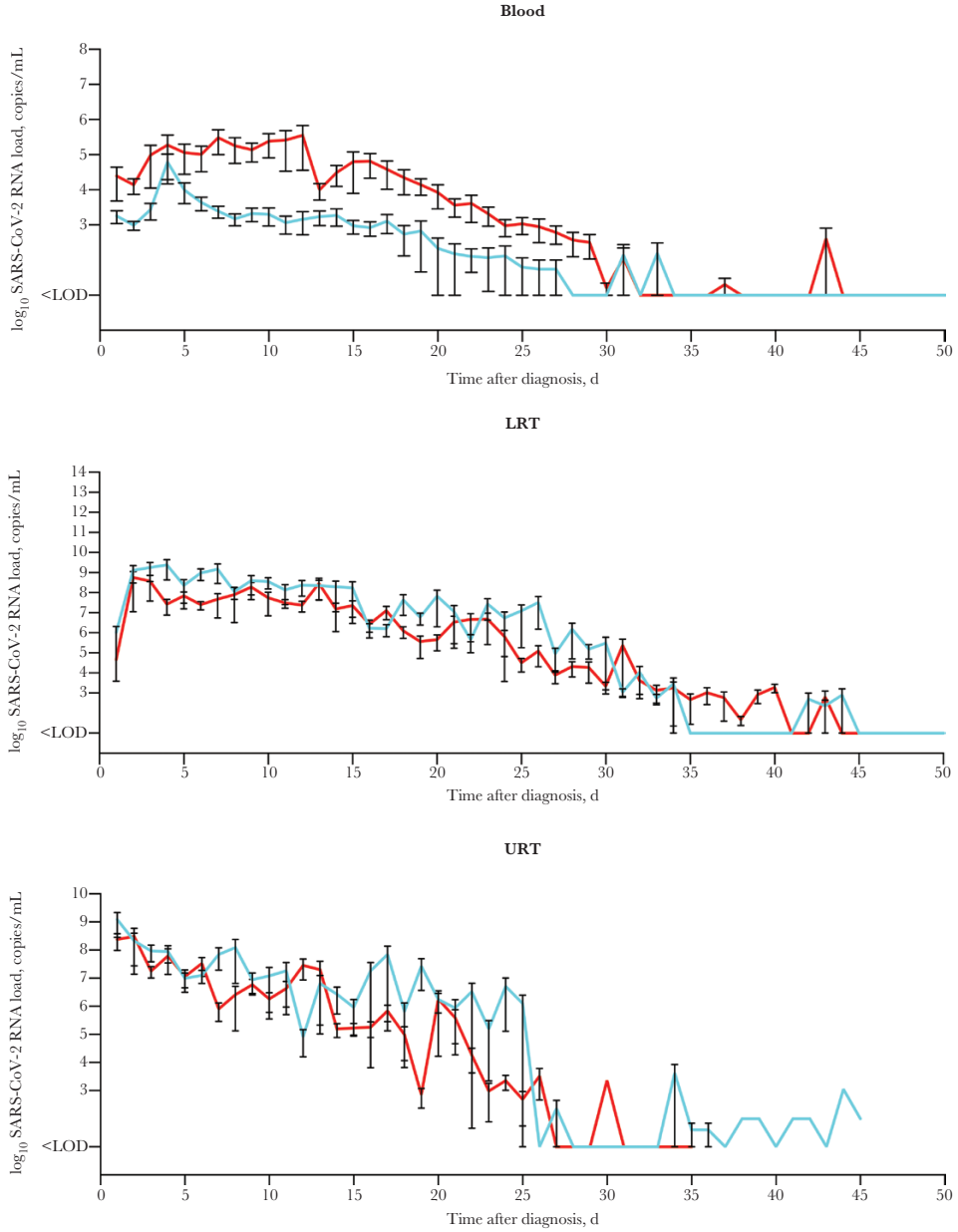


Figure 2. Courses of the SARS-CoV-2 RNA loads as determined by RT-qPCR throughout the disease from the time of diagnosis in blood (A), LRT (B), and URT (C). The respective mean and SEM of SARS-CoV-2 RNA loads are illustrated. Red lines refer to nonsurvivors; light blue lines refer to survivors. Samples < LOD were set to 1×10^1 copies/mL, and samples < threshold of 1×10^3 copies/mL were set to 1×10^2 copies/mL to allow for logarithmic presentation. Abbreviations: LOD, limit of detection; LOQ, limit of quantification; LRT, lower respiratory tract; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SEM, standard error of the mean; URT, upper respiratory tract.

Table 2. Multivariate Analysis

	Generalized Linear Logistic Mixed Model								
	Blood			LRT			URT		
Parameter	Estimator	SE	*P* Value	Estimator	SE	*P* Value	Estimator	SE	*P* Value
Intercept	8.82	1.88	<.0001	6.84	1.81	.0002	10.63	2.27	<.0001
Age, y	–0.06	0.02	<.0001	–0.07	0.01	<.0001	–0.15	0.02	<.0001
Sex (ref: Male)	0.78	0.32	.01	1.01	0.21	<.0001	1.15	0.40	.004
BMI, kg/m^2	0.11	0.02	<.0001	0.10	0.01	<.0001	0.25	0.06	<.0001
Charlson comorbidity index (1–13)	–0.40	0.10	.0001	–0.22	0.05	<.0001	–0.07	0.09	.45
SARS-CoV-2 RNA load (unit: 1 $\log_{10}$ level), copies/mL	–1.49	0.31	<.0001	–0.29	0.07	<.0001	–0.64	0.14	<.0001
Clinically diagnosed ARDS	–2.17	1.75	.21	–0.57	0.97	.56	0.85	0.64	.18
Mechanical ventilation	0.74	1.84	.69	–1.12	1.88	.55	–3.10	0.82	.0001
ECMO	–2.87	0.49	<.0001	–3.41	0.35	<.0001	–5.77	1.15	<.0001
Viremia (ref: none)	[a]	[a]	[a]	–1.52	0.25	<.0001	–1.90	0.45	<.0001

The patients' 90-day survival status served as the dependent variable. Multivariate analysis (generalized linear logistic mixed model) reveals blood and LRT RNA levels as predictors of adverse outcomes. The patient (correlation structure: compound symmetry) and time (correlation structure: first order autoregression) were set as random factors to account for repeated measurements. Firth approximation was used to improve the model's fitness. Model estimators for the GLMM (blood) were AIC, 393.28; c-c, 0.86. For the GLMM (LRT), they were AIC, 923.81; c-c, 0.84. For GLMM (LRT), they were AIC, 337.44; c-c, 0.92.

Abbreviations: AIC, Akaike Information Criterion; ARDS, acute respiratory distress syndrome; BMI, body mass index; ECMO, extracorporeal membrane oxygenation; GLMM, generalized linear mixed model; LRT, lower respiratory tract; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; URT, upper respiratory tract.

[a]Not applicable.

such differences were observed for maximum URT viral RNA levels during the course of the disease.

Clearance of viral RNA from the bloodstream occurred more frequently ($P < .0001$) and more rapidly (median time, 9 days) in survivors than nonsurvivors. Likewise, and similar to recently published data [8, 9, 11, 17], survivors were able to clear the virus successfully from the respiratory tract, yet time to clearance took considerably longer. These data indicate that persistently elevated blood RNA levels can serve as an early indicator of severe courses of the disease.

Most essentially, the multivariate analysis confirmed blood SARS-CoV-2 load to be an important outcome predictor independent of other clinically relevant covariables such as primary sociodemographic data, comorbidities, the presence of ARDS, and the need for mechanical ventilation or extracorporeal membrane oxygenation. Importantly, the blood model revealed a significantly higher effect for survival if compared with the URT and LRT models (blood: OR, 0.23; 95% CI, 0.12–0.42; LRT: OR, 0.75; 95% CI, 0.66–0.85; and URT: OR, 0.53; 95% CI, 0.40–0.69).

According to the model presented here, blood RNA levels exceeding 2.51×10^3 SARS-CoV-2 RNA copies/mL reflect a 50% probability of death. Considering this blood RNA load as a critical cutoff value, half of all patients in the survivor group compared with the majority in the nonsurvivor group exceeded this value at least once during the ICU stay. Notably, in 88% of the nonsurvivors, blood RNA loads remained constantly elevated above that cutoff value, while in all but 2 of the survivors, blood RNA loads declined below that threshold during the course of the disease.

The proportion of patients receiving COVID-19-related antiviral therapy (dexamethasone, remdesivir, or monoclonal antibodies) was comparable in survivors and nonsurvivors, though monoclonal antibody therapy was initiated in 4 of the nonsurvivors and none of the survivors. Altogether, the difference in successful viral blood clearance seems not to be attributable to specific therapeutic interventions.

However, the proportion of immunocompromised patients was higher among nonsurvivors in our study. Thus, our data prove evidence of an increased risk of SARS-CoV-2 viremia and associated mortality in this particular patient population [8–11]. However, it is not yet possible to conclude whether immunosuppression promotes viremia or whether, conversely, viremia exacerbates immunosuppression in critically ill patients.

Previous studies have shown that SARS-CoV-2 affects different organs besides the respiratory tract and that high viral loads in the affected organs correlate with increased mortality [19].

Although our study does not identify viremia itself as the cause of death, our data indicate that patients with high levels of viremia and delayed virus clearance represent a vulnerable subgroup. This subgroup might particularly benefit from specific therapy such as monoclonal antibodies or direct antiviral substances. Moreover, monitoring of viremia could thus be useful for future patient management in the ICU.

However, it is currently difficult for diagnostic laboratories to offer reliable quantitative molecular SARS-CoV-2 diagnostics for specimens other than respiratory tract samples. Food and Drug Administration (FDA)– or Conformitè Europëenne (CE)-approved molecular assays are missing for this purpose. As the importance of virologic blood diagnostics is highlighted, indicating a high prognostic value for patient outcomes in this study, such diagnostics might help clinicians in patient management, in assessing each patient's prognosis. Thus, there is an urgent need for the rapid evaluation and approval of blood RT-qPCRs for SARS-CoV-2 [10, 15].

We are aware that our study has limitations. The intermittent shortage of reagents and supplies led to the use of a variety of assays for quantification. Blood samples were almost exclusively analyzed with the cobas 6800 system, but slight deviations in the quantification in URT and LRT cannot be completely ruled out given the multitude of assays. Also, the URT sampling frequency is lower than for LRT and blood because nasopharyngeal swabs were waived in some of the severely ill mechanically ventilated patients. RNA quantification in respiratory tract samples has significant variability, and therefore its clinical implementation is challenging. Swab samples, in particular, are dependent on the collection technique and intra-individual fluctuations (eg, the detection of false high RNA loads by coughing up RNA-positive material in the URT). However, in this study, RNA load variabilities should largely be compensated by close longitudinal sampling. Furthermore, according to the epidemiological situation at patient enrollment, no patient in our study was infected with a recently emerging spike mutant variant (VOC); virus RNA loads in VOC-infected patients might have exceeded the loads measured here.

In summary, our data indicate that SARS-CoV-2 viremia is a better predictor of outcome than respiratory tract viral RNA load, and clearance of SARS-CoV-2 RNA from the bloodstream is strongly associated with survival. Thus, reliable quantification of SARS-CoV-2 RNA in the blood as part of clinical practice seems mandatory to assess patients' risk of fatal outcomes. Moreover, monitoring of viremia could be an important surrogate marker of the effectiveness of antiviral therapies. FDA-approved assays are required for this purpose.

Acknowledgments

Financial support. This study did not receive external funding.

Potential conflicts of interest. M.L. received speaker honoraria and related travel expenses from Roche Diagnostics. S.K. reports grants and personal fees from Pfizer, personal fees from Biotest, personal fees from Cytosorbents, personal fees from Gilead, personal fees from MSD, personal fees from Bayer, personal fees from Astellas, personal fees from Baxter, and personal fees from Fresenius, outside the submitted work. All other authors declare that they have no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Patient consent. The Ethics Committee of the Hamburg Chamber of Physicians was informed about the study. Due to the retrospective nature of the study, the need for informed consent was waived (WF-094/21).

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Letter of acceptance –
SARS-CoV-2 Blood RNA Load Predicts Outcome in Critically Ill COVID-19 Patients

From: **Susanne Pfefferle** s.pfefferle@uke.de
Subject: Manuskript zur SARS-CoV-2 Virämie accepted
Date: 29 September 2021 at 09:06
To: d.wichmann@uke.de, skluge@uke.de, fa.heinrich@uke.de, m.aepfelbacher@uke.de, k.roedl@uke.de, m.nentwich@uke.de, d.noerz@uke.de, mluetgeh@uke.de, ar.hoffmann@uke.de, e.bibiza@uke.de, m.christner@uke.de , f.olearo@uke.de

SP

Liebe Kolleg:innen,

Ich freue mich sehr (endlich) mitteilen zu können, dass unser gemeinsames Manuskript zur Virämie bei schwer kranken COVID-19 Patienten nun bei OFID akzeptiert wurde.
Ich möchte mich bei allen für die tolle Zusammenarbeit bedanken!

Herzliche Grüße,

Susanne Pfefferle

Ref.: Ms. No. OFID-D-21-00950R1
SARS-CoV-2 blood RNA load predicts outcome in critically ill COVID-19 patients
Open Forum Infectious Diseases

Dear Dr Pfefferle,

We are pleased to tell you that your work has now been accepted for publication in Open Forum Infectious Diseases and that we will soon be sending the manuscript files to Oxford Journals for processing and immediate publication on Advance Access.

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research such as yours, we are dependent on the experts who generously contribute their time to our peer review process. We will likely be sending you several manuscripts in your field of expertise over the coming year and ask that if you are not able to review a manuscript yourself, you supply us with the names of colleagues or trainees who might be willing to do so. Only with this kind of collaboration and good citizenship can our journal continue its mission.

With kind regards,

Dr Paul Edward Sax, MD, FIDSA
Editor-in-chief

Dr Jonathan Li, MD, FIDSA, MMSc
Deputy Editor

Dr Roger Bedimo
Associate Editor
Open Forum Infectious Diseases

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Address for correspondence: Wan Beom Park or Myoung-don Oh, Department of Internal Medicine, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, South Korea; email: wbpakr1@snu.ac.kr or mdohmd@snu.ac.kr

Postmortem Stability of SARS-CoV-2 in Nasopharyngeal Mucosa

Fabian Heinrich, Kira Meißner, Felicia Langenwalder, Klaus Püschel, Dominik Nörz, Armin Hoffmann, Marc Lütgehetmann, Martin Aepfelbacher, Eric Bibiza-Freiwald, Susanne Pfefferle, Axel Heinemann

Author affiliation: University Medical Center Hamburg-Eppendorf, Hamburg, Germany

DOI: https://doi.org/10.3201/eid2701.203112

Analyses of infection chains have demonstrated that severe acute respiratory syndrome coronavirus 2 is highly transmissive. However, data on postmortem stability and infectivity are lacking. Our finding of nasopharyngeal viral RNA stability in 79 corpses showed no time-dependent decrease. Maintained infectivity is supported by virus isolation up to 35 hours postmortem.

Detailed analyses of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission have shown the virus to be highly transmissible through droplet and contact-transmitted viral spreading; reproduction indices were 2.2–3.6 (*1*). Amid the coronavirus disease (COVID-19) pandemic, case-fatality rates of up to 9.26% occur in areas hard-struck by SARS-CoV-2 (*2*). The likelihood of virus transmission through deceased persons remains unclear. However, in recent pandemics of influenza, high and sustainable virus stability and infectivity within corpses were demonstrated (*3*,*4*), necessitating careful and conscious handling. To determine the possibility of SARS-CoV-2 transmission through deceased persons, we conducted a study of postmortem viral RNA stability.

The federal state of Hamburg, Germany, has mandated autopsies since March 2020 in accordance with the German Infection Protection Act for all patients with reverse transcription PCR (RT-PCR)–confirmed SARS-CoV-2 infection. Data and sample acquisition for the study were performed during March 22–May 1, 2020. To confirm the initial diagnosis and quantify the viral load in the corpses, nasopharyngeal swab samples (ESwab; Copan, https://products.copangroup.com) were taken at patient admission to the Department of Legal Medicine (University Medical Center Hamburg-Eppendorf). Corpses were stored at 4°C in the refrigerator. Antemortem and postmortem nasopharyngeal swab samples were taken according to recent standards (*5*) by trained, medically qualified personnel to ensure maximum reliability and consistent quality. Samples were analyzed for SARS-CoV-2 RNA as described previously (*6*).

The Ethics Committee of the Hamburg Chamber of Physicians approved the study (no. PV7311). The local clinical institutional review board, complying with the Declaration of Helsinki, also approved the study.

Antemortem nasopharyngeal swab samples (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/1/20-3112-App1.pdf) were collected by medical staff at the intensive care unit of the University Medical Center Hamburg and by general practitioners from on-call duty at a median of 6 days (range 2–14 [interquartile range (IQR) 6.3]) before death (n = 10). Using a Wilcoxon test for paired data, we did not detect any effect of the event of death on the SARS-CoV-2 RNA load (U= −5; p = 0.85). We found no correlation between the postmortem interval (time of death until cooling at 4°C; median 17.8 [range 2.7–482.6]) hours and the viral RNA loads of corpses, as indicated by Spearman correlation of 79 matched datasets (Figure, panel A).

To analyze postmortem stability of SARS-CoV-2 RNA, we selected 11 corpses with short postmortem intervals for a detailed observation over 7 days (168 hours) (Table). The median postmortem interval was 5.7 (range 2.9–32.0 [IQR 6.9]) hours. The median cycle threshold (C_t) of SARS-CoV-2 RNA in swab samples taken at admission was 29.52 (range 15.2–50.0 [IQR 22.5]) (Figure, panel A). We determined viral load in a series of 9 sequential pharyngeal swab samples (time points 0, 12, 24, 36, 48, 60, 72, 96, and 168 hours after admission). We consistently detected SARS-CoV-2 RNA at constant levels at all time points analyzed (Figure, panel B), except for patient 7 at 0, 12, and 24 hours after admission and patient 8 at admission. Because subsequent samples were positive for all corpses, we attributed those

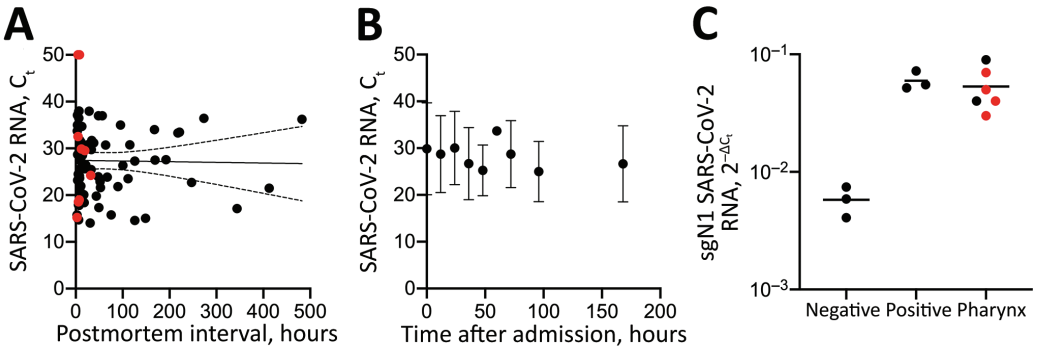

Figure. Postmortem stability of SARS-CoV-2 in nasopharyngeal mucosa. A) Correlation of SARS-CoV-2 RNA loads of the pharynx (at corpse admission to the Department of Legal Medicine) with the postmortem interval (time of death until cooling at 4°C) in 79 matched datasets. Red indicates patients in the longitudinal cohort. Spearman R = –0.07; 2-tailed p = 0.5. B) Median SARS-CoV-2 RNA loads with 95% CIs (error bars) in a series of 9 sequential pharyngeal swab samples (time points 0, 12, 24, 36, 48, 60, 72, 96, and 168 hours after admission) for 11 corpses. C) sgN1 RNA loads of SARS-CoV-2 in pharyngeal tissue of 6 corpses. Negative and positive controls from SARS-CoV-2 cell cultures. Red indicates samples with successful virus isolation from pharyngeal tissue (S. Pfefferle, unpub. data, https://doi.org/10.1101/2020.10.10.334458). Negative results are reflected by C_t 50. Ct, cycle threshold; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sgN1 RNA, subgenomic RNA loads of the N1-gene.

discrepancies to deviations in the sample collection. A general mixed model found no time-dependent effect on SARS-CoV-2 RNA loads (estimate −0.06, SE 0.01; p = 0.58) (*7*). Because of impaired interval-scaling of metric variables, we excluded negative C_t values from the statistical analysis. Intriguingly, the estimate suggests an increase of the viral load without revealing significant results (0.6%/hour).

Six patients in this study (patients 11–16) previously were part of a study in which virus growth from different tissues (including pharynx) of patients dying of RT-PCR–confirmed SARS-CoV-2 infection was investigated (S. Pfefferle, unpub. data, https://doi.org/10.1101/2020.10.10.334458) (Table). That study showed that replicating virus was detected in the throat of patients up to 35.8 hours after death. Both the

Table. Basic clinical information about and autopsy findings of patients in a longitudinal follow-up cohort and for virus isolation, Department of Legal Medicine, Hamburg, Germany, 2020*

Patient no.	Age, y/sex	BMI, kg/m^2	Main autopsy finding	Disease duration, d	Postmortem interval, h†	Postmortem SARS-CoV-2 RNA load at admission, C_t‡
1§	54/F	29.6	Pneumonia	5	11.92	29.86
2§	66/M	25.3	Pneumonia	ND	32.03	24.22
3§	63/M	37.3	Pulmonary embolism, pneumonia	6	5.03	32.55
4§	70/M	22.2	Pneumonia, bronchitis, respiratory failure	6	7.48	18.97
5§	52/M	38.8	Pulmonary embolism	10	5.32	ND
6§	90/F	24.9	Pneumonia, aspiration	13	19.35	29.52
7§	71/M	ND	Pneumonia, MODS	ND	7.87	50
8§	77/M	33.2	Pneumonia	18	5.08	50
9§	61/M	32.3	Intracerebral hemorrhage, pneumonia	ND	4.37	ND
10§	76/M	37.7	Pneumonia, MODS, endocarditis, leukemia	ND	2.85	15.22
11§,¶	59/F	22.2	Pneumonia, multiple myeloma	18	5.67	18.55
12¶	83/F	26.0	Pneumonia, non-Hodgkin lymphoma	25	6.83	ND
13¶	80/M	28.5	Pulmonary embolism, pneumonia, myelofibrosis	12	6.5	ND
14¶	71/F	29.0	Pneumonia, myelofibrosis	25	12.1	ND
15¶	84/F	21.4	Pneumonia	ND	35.75	ND
16¶	31/M	20.6	Pneumonia, germ cell tumor	ND	9.08	ND

*C_t, cycle threshold; MODS, multiple organ dysfunction syndrome; ND, not determined; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
†Time of death until cooling at 4°C.
‡Negative results are reflected by C_t 50.
§Longitudinal cohort.
¶Virus isolation cohort (S. Pfefferle, unpub. data, https://doi.org/10.1101/2020.10.10.334458).

detection of subgenomic RNA (sgRNA) by next-generation sequencing and virus growth could be shown in those throat samples. We also detected sgRNA by RT-PCR in throat tissue samples of these 6 previously published patients (*8*–*10*) (Figure, panel C); samples in which virus could be cultivated (S. Pfefferle, unpub. data, https://doi.org/10.1101/2020.10.10.334458) are highlighted in red.

We demonstrated maintained infectivity of SARS-CoV-2 in tissues of deceased patients. SARS-CoV-2 RNA persisted over time at constantly high titers. Taken together, our data indicate potentially high infectivity of human corpses, requiring hazard assessments in professional fields concerned and careful and conscious handling.

Our infectivity study relies on a limited number of cases and patients with severe immunosuppression. Further research should investigate viral persistence in corpses with longer postmortem intervals (>1 week) and corpses exhibiting lower initial viral loads. We recommend all work on corpses be conducted according to guidelines recently published by the World Health Organization, especially in the framework of widespread death in pandemics (https://apps.who.int/iris/rest/bitstreams/1300088/retrieve).

Acknowledgments

We offer condolences to the families and friends of all the patients whose deaths were attributable to COVID-19.

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About the Author

Mr. Heinrich is a medical student employed at the Institute of Legal Medicine, University Medical Center Hamburg-Eppendorf. His primary research interests include infectiologic and immunologic research.

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Address for correspondence: Fabian Heinrich, Institute of Legal Medicine, University Medical Center Hamburg Eppendorf, Butenfeld 34, 20259 Hamburg, Germany; email: fa.heinrich@uke.de

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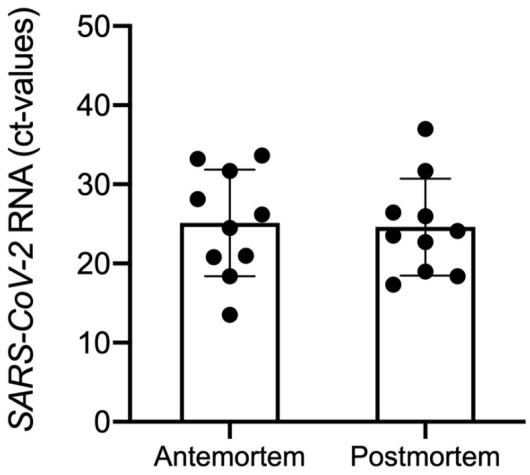
Postmortem Stability of SARS-CoV-2 in Nasopharyngeal Mucosa

Appendix



Appendix Figure. SARS-CoV-2 RNA loads (cycle threshold values) from matched antemortem and postmortem nasopharyngeal swab samples, illustrating 10 corpses from Figure 1, panel A, in the main text.

Letter of acceptance –
Postmortem stability of SARS-CoV-2 in Nasopharyngeal Mucosa

From: **Emerging Infectious Diseases** onbehalfof@manuscriptcentral.com
Subject: Your EIC Manuscript # EID-20-3112.R1
Date: 29 September 2020 at 19:55
To: fa.heinrich@uke.de, fabian.heinrich@gmx.com

ED

29-Sep-2020

Dear Mr. Heinrich:

We are pleased to inform you that your manuscript "Postmortem stability of SARS-CoV-2 in the nasopharyngeal mucosa" has been accepted for rapid publication online in Emerging Infectious Diseases.

The letter and its title will undergo substantive editing for length, grammatical correctness, and journal style. The galleys will be sent to you for approval.

Thank you for sending us your article. We look forward to working with you in the weeks to come.

Dr D. Peter Drotman
Editor-in-Chief
Emerging Infectious Diseases
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404-639-1960 (phone)

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Reviewer: 3
Comments to the Author
The authors have adequately addressed this Reviewer's comments. The manuscript offers a valuable contribution to the scientific literature on SARS-CoV-2.

Keep Figure 1A in the main paper (not just in response to reviewers letter). This is the important data. Figure 2 is not necessary for the paper. It shows that the assay used was comparable to the assay cited.

Reviewer: 2
Comments to the Author
Manuscript with great improvements.

EID Editorial Board Comments to the Author:
The review comments will be addressed in the copyediting stage, particularly with respect to Reviewer 3's request to keep figure 1A in the paper, perhaps as an appendix.

About the Author

Dr. Petersen is an associate professor at the University of the Faroe Islands, a senior researcher at the Faroese Hospital System, and head of the Centre of Health Sciences, Tórshavn, Faroe Islands. Her primary research interests include epidemiological research, and she has initiated and conducted multiple COVID-19 health science research projects in the Faroe Islands.

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Address for correspondence: Maria Skaalum Petersen, Department of Occupational Medicine and Public Health, The Faroese Hospital System, Sigmundargøta 5, 100 Tórshavn, Faroe Islands; email: maria@health.fo

Postmortem Antigen-Detecting Rapid Diagnostic Tests to Predict Infectivity of SARS-CoV-2–Associated Deaths

Fabian Heinrich, Ann Sophie Schröder, Anna-Lina Gerberding, Moritz Gerling, Felicia Langenwalder, Philine Lange, Axel Heinemann, Eric Bibiza-Freiwald, Dominik Sebastian Nörz, Martin Aepfelbacher, Susanne Pfefferle,[1] Benjamin Ondruschka,[1] Marc Lütgehetmann[1]

Author affiliation: University Medical Center Hamburg-Eppendorf, Hamburg, Germany

We investigated the infectivity of 128 severe acute respiratory disease coronavirus 2–associated deaths and evaluated predictive values of standard diagnostic procedures. Maintained infectivity (20%) did not correlate with viral RNA loads but correlated well with anti-S antibody levels. Sensitivity >90% for antigen-detecting rapid diagnostic tests supports their usefulness for assessment.

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Deaths associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have raised concerns that contact with the corpses of deceased persons might pose a risk for transmitting infection (*1*). Nasopharyngeal SARS-CoV-2 RNA loads were shown to remain stable up to 20 days postmortem (*2*), and the maintained infectivity of corpses has sporadically been examined (*2*–*4*). In contrast, body surfaces of corpses have been considered noninfectious (*5*). Systematic studies on the infectivity of corpses and predictive values of standard diagnostic procedures remain scarce.

For this study, we prospectively collected nasopharyngeal swab specimens from 128 SARS-CoV-2 RNA-positive and 72 RNA-negative corpses ≤14 days postmortem to assess infectivity and predictive values of virologic parameters (Table). We excluded corpses exhibiting advanced putrefaction. For initial assessment, we determined RNA loads using quantitative reverse transcription PCR (qRT-PCR) (Appendix, https://wwwnc.cdc.gov/EID/article/28/1/21-1749-App1.pdf).

We found SARS-CoV-2 RNA up to 325 hours postmortem, but RNA loads did not correlate with

[1]These senior authors contributed equally to this article.

Table. Baseline characteristics of corpses received by the Institute of Legal Medicine, Hamburg, Germany, 2020–2021*

Characteristic	SARS-CoV-2 RNA positive,† n = 128	SARS-CoV-2 RNA negative,† n = 72	Total, n = 200
Age, y, median (IQR)	83.5 (71.5–89.1)	81.0 (73.0–87.0)	82.3 (72.9–88.5)
Sex			
M	71 (55.5)	36 (50.0)	107 (53.5)
F	57 (44.5)	36 (50.0)	93 (46.5)
Place of death			
Home	28 (22.0)	30 (41.7)	58 (29.1)
Nursing home	38 (29.9)	3 (4.2)	41 (20.6)
Hospital	39 (30.7)	25 (34.7)	64 (32.2)
ICU	20 (15.7)	10 (13.9)	30 (15.1)
Other	2 (1.6)	4 (5.6)	6 (3.0)
Postmortem interval,‡ h, median (IQR)	8.7 (5.3–82.6)	4.9 (3.5–8.8)	7.0 (4.3–49.9)
Putrefactive changes	11 (8.9)	1 (1.4)	12 (6.1)
SARS-CoV-2 RNA load,¶ copies/mL, median (IQR)	7.0 x 10^6 (5.5 × 10^4–5.2 x 10^7)	Below LOD	Not applicable

*Values are no. (%) except as indicated. In case of missing data points, valid percentages are indicated. ICU, Intensive care unit; LOD, limit of detection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2
†B.1.1.7 variants (2/128) identified by multiplex-typing PCR (*5*). SARS-CoV-2–associated deaths were tested in a multiplex typing PCR for SARS-CoV-2 spike variants.
‡Interval from time of death until initial sampling and cooling at 4°C.

the postmortem interval (PMI; r = 0.003, p >0.99) (Figure, panel A). RNA loads were comparatively high (median 7.0 × 10^6 copies/mL, interquartile range [IQR] 5.5 × 10^4–5.2 × 10^7 copies/mL) (Figure, panel B) and in some cases exceeded loads in the acute phase of the disease (*6*), possibly because of postmortem mucosal softening and higher exfoliation of tissue during sample collection.

Virus isolation proved infectivity was maintained in 26/128 (20%) corpses (Appendix). PMI (median 13

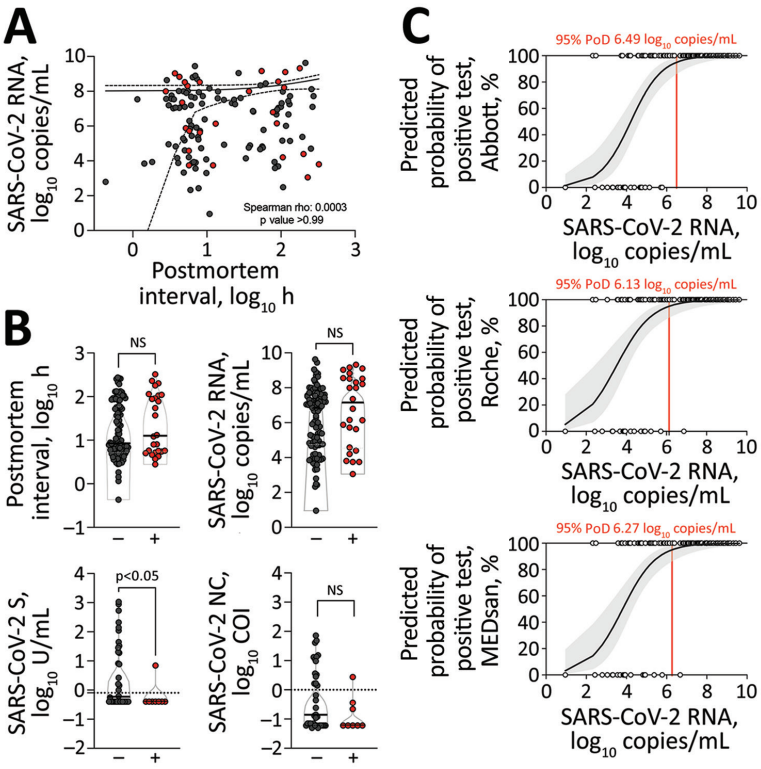

Figure. Overview of 128 consecutive records of SARS-CoV-2–associated deaths received by the Institute of Legal Medicine, Hamburg, Germany, 2020–2021. A) SARS-CoV-2 RNA loads by postmortem intervals. Spearman correlation was performed; estimates and 95% CI are shown. B) Postmortem intervals, viral RNA loads, quantitative (S), and qualitative (NC) antibody levels compared among culture-positive (+) and culture-negative (–) corpses. Comparisons were performed using Mann-Whitney-U or χ^2 testing, as appropriate. Median and interquartile ranges are shown. Horizontal dotted lines indicate cutoff value. C) Probability of positive antigen-detecting rapid diagnostic test results depending on viral RNA loads calculated by binomial logistic regression. Robust estimates with 95% CI are shown. Vertical red line indicates 95% PoD with the corresponding viral RNA load. Ag-RDT, antigen-detecting rapid antigen test; COI, cut-off index; NC, nucleocapsid; NS, not significant; PoD, probability of detection; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

hours, range 3–325 hours) and SARS-CoV-2 RNA load (1.4×10^7 copies/mL, IQR 3.7×10^4–3.3×10^8) among culture-positive corpses did not differ significantly from PMI (median 8 hour, range 0–275 hour; p = 0.38) and RNA loads (7.0×10^6 copies/mL, IQR 5.8×10^4–3.9×10^7 copies/mL; p = 0.14) among culture-negative corpses (Figure, panel B). We successfully isolated virus from samples with comparatively low amounts of RNA ($<1 \times 10^4$ copies/mL), in contrast with previous findings among living patients (*6*). We observed putrefactive changes in no culture-positive corpses compared with in 11/98 (11%) culture-negative corpses ($\chi^2 = 3.20$; p = 0.11), indicative of potentially decreased infectivity.

We confirmed seroconversion in 18/44 (41%) blood samples, 15/43 (35%) anti-nucleocapsid positive and 17/44 (39%) anti-spike positive (range <0.4–1066.0 U/mL; Appendix). Levels of anti-spike antibodies, representing neutralizing antibody levels (*7*), were not significantly correlated with PMI (r = 0.07; p = 0.64), but were well correlated with viral RNA levels (r = –0.70; p <0.0001). Anti-nucleocapsid antibodies were found in only 1/8 (13%) culture-positive compared with 14/35 (40%) culture-negative corpses ($\chi^2 = 2.17$; p = 0.23) (Figure, panel C). Moreover, anti-spike antibody levels differed significantly (p = 0.04) between culture-positive (1.22 U/mL, SD 2.32) and culture-negative (86.85 U/mL, SD 240.56) corpses, indicative of inverse association of SARS-CoV-2–specific antibody levels with infectivity (Figure, panel C).

Antigen-detecting rapid diagnostic tests (Ag-RDTs) are considered adequate alternative swift diagnostic tools in living patients (*8*,*9*), but knowledge about their postmortem applicability and reliability remains scarce. We tested Ag-RDTs from 3 manufacturers and found excellent performance for postmortem use (Appendix Table 1). Compared with qRT-PCR results, for the Panbio COVID-19 Ag Rapid Test Device (Abbott, https://www.abbott.com), sensitivity was 80.3% (95% CI 72.3%–86.4%) and specificity 100.0% (95% CI 95.0%–100.0%); for the SARS-CoV-2 Rapid Antigen Test (Roche https://www.roche.com), sensitivity was 86.4% (95% CI 79.1%–91.9%) and specificity 98.6% (95% CI 93.0%–100.0%); and for the SARS-CoV-2 Antigen Rapid Test (MEDsan https://www.medsan.eu), sensitivity was 84.1% (95% CI 76.6%–90.0%) and specificity 95.8% (95% CI 88.0%–99.0%) (Appendix Figures 1, 2).

We found SARS-CoV-2 RNA load correlated with Ag-RDT positivity in univariate and multivariate analyses (p<0.001), thereby confirming their predictive value (Figure, panel C; Appendix Table 2). Subgroup analyses of corpses with $>1 \times 10^6$ RNA copies/mL (n = 74) revealed 100% (95% CI 95.1%–100.0%) sensitivity in Abbott (n = 74) and Roche and MEDsan (n = 73 each) assays. In contrast, neither PMI (p = 0.34) nor putrefactive changes (p = 0.90) were predictive for testing positive in Ag-RDTs (exemplarily for the MEDsan assay; Appendix Table 2). Ag-RDT sensitivity in infectious corpses was 92.3% (95% CI 74.9%–99.1%) for Abbott, 96.2% (95% CI 80.4%–99.9%) for Roche, and 96.2% (95% CI 80.4%–99.9%) for MEDsan. We detected 2 SARS-CoV-2 variants of concern despite relatively low viral RNA loads (4.83 $\log_{10}$); the 2 samples tested positive by Abbott and Roche but were missed by MEDsan.

The first limitation of our study is that blood was not available from all corpses, and the serologic assays and Ag-RDTs used are not approved for cadaveric samples. Furthermore, because of a shortage of reagents and supplies, we had to use different tests to quantify RNA, and slight deviations cannot be ruled out.

In summary, we show that cadavers from SARS-CoV-2–associated deaths remain infectious long after death in a considerable proportion of cases. Postmortem infectivity does not correlate with PMI or viral RNA load but correlates with the absence of virus-specific antibodies. Ag-RDTs performed well, enabling rapid on-site detection. Because previous studies among living patients indicate that Ag-RDTs reliably detect all SARS-CoV-2 variants (*10*), we believe that our results on postmortem Ag-RDTs use can contribute to crisis management in severely affected regions and increase safety in the medical sector worldwide.

Acknowledgments

We thank Jessica Vering for her technical support and Daniela Fröb for managing records of SARS-CoV-2–associated deaths within the Institute of Legal Medicine. We offer condolences to the families and friends of all patients whose deaths were attributable to COVID-19.

The ethics committee of the Hamburg Chamber of Physicians approved this study (reference no. 2020-10353-BO-ff and PV7311).

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About the Author

Mr. Heinrich is a medical student employed at the Institute of Legal Medicine, University Medical Center Hamburg-Eppendorf. His primary research interests include infectiologic and immunologic research.

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Addresses for correspondence: Fabian Heinrich, Institute of Legal Medicine, University Medical Center Hamburg Eppendorf, Butenfeld 34, 20259 Hamburg, Germany; email: fa.heinrich@uke.de; Susanne Pfefferle, Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg Eppendorf, Martinistraße 52, 22529 Hamburg, Germany; email: s.pfefferle@uke.de

Monitoring International Travelers Arriving in Hong Kong for Genomic Surveillance of SARS-CoV-2

Haogao Gu, Samuel S.M. Cheng, Pavithra Krishnan, Daisy Y.M. Ng, Lydia D.J Chang, Gigi Y.Z. Liu, Sammi S.Y. Cheuk, Mani M.Y. Hui, Mathew C.Y. Fan, Jacob H.L. Wan, Leo H.K. Lau, Daniel K.W. Chu, Vijaykrishna Dhanasekaran, Malik Peiris, Leo L.M. Poon

Author affiliations: University of Hong Kong School of Public Health, Hong Kong, China (H. Gu, S.S.M. Cheng, P. Krishnan, D.Y.M. Ng, L.D.J. Chang, G.Y.Z. Liu, S.S.Y. Cheuk, M.M.Y. Hui, M.C.Y. Fan, J.H.L. Wan, L.H.K. Lau, D.K.W. Chu, V. Dhanasekaran, M. Peiris, L.L.M. Poon); HKU-Pasteur Research Pole, University of Hong Kong, Hong Kong (V. Dhanasekaran, M. Peiris, L.L.M. Poon); Centre for Immunology and Infection, Hong Kong (M. Peiris, L.L.M. Poon)

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We sequenced ≈50% of coronavirus disease cases imported to Hong Kong during March–July 2021 and identified 70 cases caused by Delta variants of severe acute respiratory syndrome coronavirus 2. The genomic diversity detected in Hong Kong was similar to global diversity, suggesting travel hubs can play a substantial role in surveillance.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineage B.1.617 (*1*) and 3 of its sublineages, B.1.617.1 (Kappa), B.1.617.2 (Delta), and B.1.617.3, were first detected in India. The Delta variant started circulating widely in different continents beginning in late March 2021 (*2*,*3*). It was initially classified as a variant of interest in April 2021 and then reclassified as a variant of concern in May 2021.

Hong Kong adopted an elimination strategy to control coronavirus disease (COVID-19). A previous study reported the use of stringent measures (e.g., mandatory COVID-19 testing, travel restrictions) to detect and prevent SARS-CoV-2 importation by COVID-19–positive travelers (*4*), thereby reducing the risk of new SARS-CoV-2 introductions, and also showed that regional and international airports could be useful sentinel surveillance sites to monitor SARS-CoV-2 circulation. In this study, we tested the feasibility of using surveillance strategies similar to those used in that study to monitor sequence diversity of Delta variant

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Postmortem Antigen-Detecting Rapid Diagnostic Tests to Predict Infectivity of SARS-CoV-2–Associated Deaths

Appendix

Methods

Patients and Ethics

For the study, we prospectively included a total of 200 corpses received at the Institute of Legal Medicine (University Medical Center Hamburg-Eppendorf, Hamburg, Germany); we excluded corpses exhibiting advanced putrefactive changes (marbling and mummification). All corpses were stored at 4°C upon receipt; we defined postmortem interval as the time from death until cooling. Informed consent was obtained from relatives or legal representatives. We performed data and sample acquisition from November 1, 2020–February 28, 2021. For initial assessment for SARS-CoV-2 RNA, quantitative reverse transcription (qRT-) PCR from nasopharyngeal swab samples was performed as part of routine diagnostics at the Institute of Microbiology, Virology and Hygiene (University Medical Center Hamburg-Eppendorf, Hamburg, Germany). In total, 128/200 corpses were SARS-CoV-2 RNA positive, and 72/200 were SARS-CoV-2 RNA negative. Notably, none of the 72 SARS-CoV-2 RNA–negative deceased patients had had a diagnosis of COVID-19 during their lifetime nor did they have a diagnosed or suspected case of SARS-CoV-2 or COVID-19 at the time of death.

Sampling and Molecular Diagnostic

We performed an initial assessment for the presence of SARS-CoV-2 RNA in all corpses received at the Institute of Legal Medicine by qRT-PCR. Following receipt of the initial results (usually <24 h later), we performed, 4 subsequent nasopharyngeal swabs, 1 tested using universal transport medium (MANTACC, https://www.mantacc.com) for qRT-PCR and virus isolation, and 3 for antigen-detecting rapid diagnostic tests using the swab supplied with the kit. For quantitative SARS-CoV-2 RNA detection, we used commercially available assays, such as

Cepheid Xpert Xpress SARS-CoV-2 (https://www.cepheid.com), Roche cobas SARS-CoV-2 (https://www.roche.com), and lab-developed assays (*1*,*2*). We used standard RNA reference material (obtained from INSTAND eV, https://www.instand-ev.de) for quantification. To calculate $\log_{10}$ RNA copies/mL (y) based on Ct-values (x), targets and conversion formulae were used: Cepheid Xpert Xpress SARS-CoV-2: y = –0.29x+12.83 (target E2); Roche cobas SARS-CoV-2: y = –0.308x+13.81 (target T2); SARS-CoV-2_UCT (utility channel test) LDT (lab-developed test): y = –0.291x+12.97 (target E-gene); NeuMoDx LDT: y = –0.425x+14.8 (https://www.neumodx.com; target E-gene), Roche LightCycler 480 II: y = –0.318x+13.32 (target E-gene). We did not consider the nonlinearity of RNA quantification within the analysis. We also analyzed all nasopharyngeal swab samples in a multiplex typing PCR (*3*), detecting del 69/70 and 501Y, enabling us to distinguish SARS-CoV-2 spike variants of concern, such as B.1.1.7 and B.1.351.

Cell Culture and Virus Isolation

We maintained and cultivated Vero E6 cells under standard conditions (*4*). For virus isolation, we used 500µL of each swab medium (universal transport medium) taken at the time of antigen-detecting rapid diagnostic (Ag-RDT) testing, and performed infection as described elsewhere (*5*). We analyzed virus growth after incubation at 37°C for 72h by qRT-PCR as described elsewhere (*1*).

Serologic Diagnostic

We obtained cadaveric blood from all corpses evaluated by full autopsy, 44/128 SARS-CoV-2 RNA–positive corpses. We used Roche Elecsys Anti-SARS-CoV-2-NC with the Roche cobas e411 according to manufacturer recommendations, for qualitative detection of SARS-CoV-2 nucleocapsid protein antibodies. We used Roche Elecsys Anti-SARS-CoV-2-S with the Roche cobas e411 according to manufacturer recommendations, for the quantitative detection of SARS-CoV-2 spike antibodies. We set cutoff values according to manufacturer recommendations: >1 COI (Elecsys Anti-SARS-CoV-2-NC) and >0.8 U/mL (Elecsys Anti-SARS-CoV-2-S).

Evaluation of Ag-RDTs

We performed Ag-RDTs from 3 different manufacturers (Appendix Table 1) according to manufacturer protocols: I) Abbott Panbio COVID-19 Ag Rapid Test Device

(https://www.abbott.com), II) Roche SARS-CoV-2 Rapid Antigen Test (https://www.roche.com), and III) MEDsan SARS-CoV-2 Antigen Rapid Test (https://www.medsan.eu). All 3 Ag-RDTs detect the SARS-CoV-2 nucleoprotein (N). All assays were listed by official authorities to meet the requirements for SARS-CoV-2 testing in Germany (*6*), but none of them was approved for use in the postmortem setting. Two independent examiners performed Ag-RDT readouts by visual inspection.

Statistical Analysis

We performed a sample size estimation for the number of cases included, assuming a significance level of $\alpha = 0.05$ and applying a margin of error of 0.05. We tested data distribution and variance equality by Q-Q plot and homoscedasticity plot. We used a Mann-Whitney-U test to compare differences between 2 independent groups in nonparametric distributed, unpaired datasets. We used χ^2 testing to compare proportions between groups. We calculated Spearman's rank correlation coefficients to assess the statistical correlation of nonparametric distributed variables. We used binary logistic regression and multivariate logistic regression for multivariate analyses. We included independent variables in the model on a clinical and scientific basis. We calculated Clopper-Pearson 95% confidence intervals for binomial proportions. P values <0.05 were considered statistically significant. We performed statistical analysis using IBM SPSS Statistics, version 27.0.0.0 (https://www.ibm.com), and STATA/MP, version 17.0 (https://www.stata.com). We used GraphPad Prism software version 9.1.1 (https://www.graphpad.com) for data illustration.

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Appendix Table 1. Antigen-detecting rapid diagnostic test specifications as provided by the manufacturer for all tests used in the study*

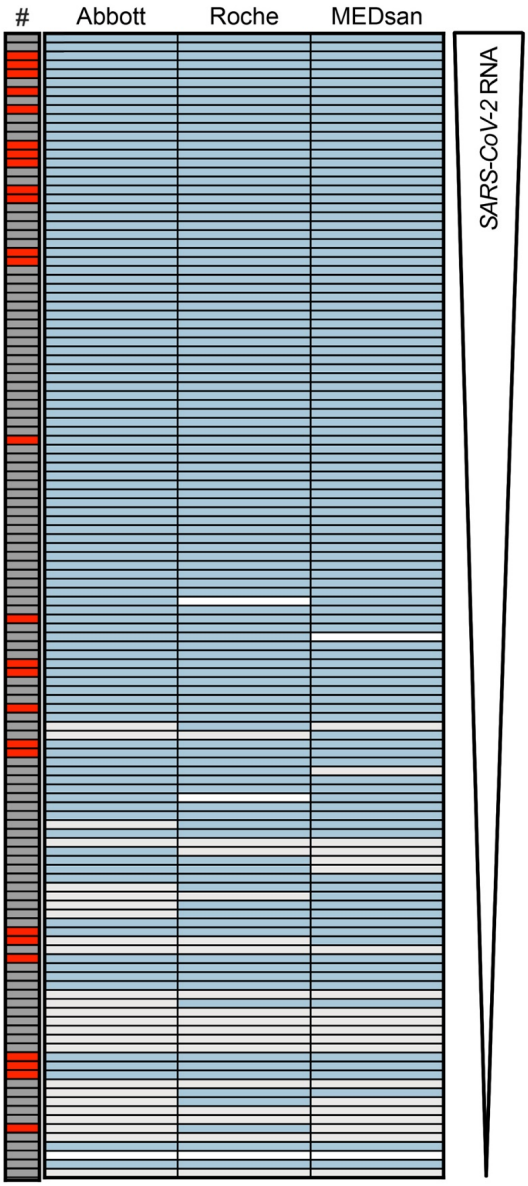
Test device name	Manufacturer	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Limit of detection, $TCID_{50}$/ mL
Panbio COVID-19 Ag Rapid Test Device	Abbott†	93.3 (83.8–98.2)	99.4 (97.0–100.0)	1.50×10^{2}
SARS-CoV-2 Rapid Antigen Test	Roche Diagnostics Deutschland GmbH‡	96.5 (91.3–99.0)	99.7 (98.2–99.9)	4.94×10^{2}
MEDsan SARS-CoV-2 Antigen Rapid Test	MEDsan¶	92.5 (86.2–96.5)	99.8 (98.9–99.9)	1.40×10^{1}

*$TCID_{50}$, 50% tissue culture infection dose.
†https://www.abbott.com
‡https://www.roche.com
¶https://www.medsan.eu

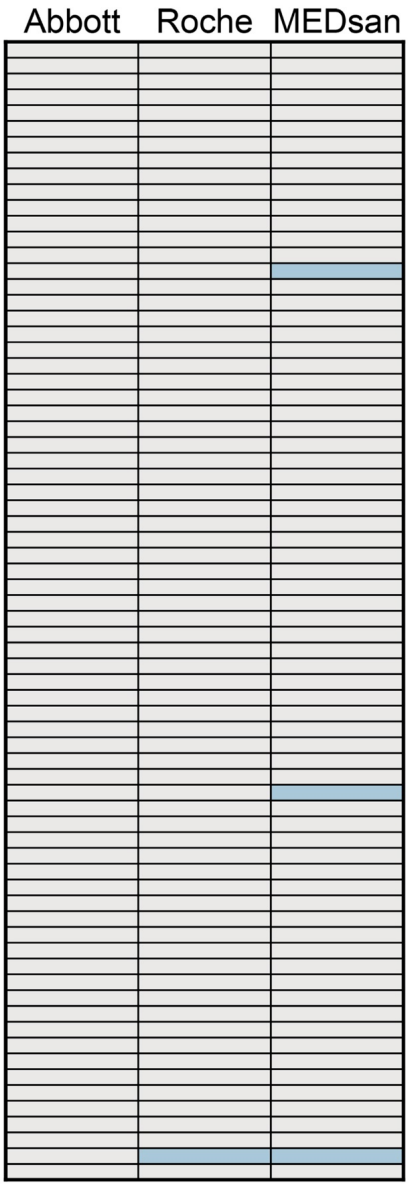
Appendix Table 2. Predictive factors for positive testing by antigen-detecting rapid diagnostic tests investigated in univariate and multivariate logistic regression analyses*,†

Parameter	Univariate analysis OR (95%CI)	Univariate analysis P value#	Multivariate analysis OR (95%CI)	Multivariate analysis P value#
Abbott assay‡				
Postmortem interval, /h	1.00 (0.99–1.00)	0.70	1.00 (0.99–1.01)	0.70
SARS-CoV-2 RNA load, log_{10}, copies/mL	3.65 (2.16–6.17)	<0.0001	3.65 (2.14–6.23)	<0.0001
Putrefactive changes	1.55 (1.03–2.33)	0.04	1.34 (0.78–2.31)	0.29
Roche assay¶				
Postmortem interval, /h	1.01 (1.00–1.02)	0.15	1.01 (1.00–1.03)	0.09
SARS-CoV-2 RNA load, log_{10}, copies/mL	3.09 (1.81–5.28)	<0.0001	3.49 (1.95–6.25)	<0.0001
Putrefactive changes	1.22 (0.71–1.79)	0.63	0.66 (0.33–1.31)	0.23
MEDsan assay§				
Postmortem interval, /h	1.00 (0.99–1.01)	0.49	1.00 (1.00–1.01)	0.34
SARS-CoV-2 RNA load, log_{10}, copies/mL	3.31 (1.94–5.64)	<0.0001	3.40 (1.97–5.86)	<0.0001
Putrefactive changes	1.32 (0.89–1.95)	0.17	0.96 (0.56–1.65)	0.90

*OR, odds ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2
†True-positive testing served as the dependent variable (compared with qRT-PCR). Independent variables in the model were included on a clinical and scientific basis.
‡https://www.abbott.com; model estimator: $\chi^2 = 56.11$, $p < 0.0001$.
¶https://www.roche.com; model estimator: $\chi^2 = 41.86$, $p < 0.0001$.
§https://www.medsan.eu; model estimator: $\chi^2 = 44.22$, $p < 0.0001$.
#P values <0.05 considered statistically significant.



Appendix Figure 1. Antigen-detecting rapid diagnostic test results are illustrated as positive (light blue) and negative (light gray). # indicates virus culture status; culture-positive corpses are marked red.



Appendix Figure 2. Specificity of antigen-detecting rapid diagnostic tests in the postmortem setting. Overview of test results in SARS-CoV-2–negative corpses (n = 72). Positive test results are marked in light blue and negative results in light gray.

Letter of acceptance –
Postmortem Antigen-Detecting Rapid Diagnostic Tests to Predict Infectivity of SARS-CoV-2–Associated Deaths

From: **Emerging Infectious Diseases** onbehalfof@manuscriptcentral.com
Subject: Your EIC Manuscript # EID-21-1749.R1
Date: 12 October 2021 at 23:54
To: fa.heinrich@uke.de, fabian.heinrich@gmx.com

ED

12-Oct-2021

Dear Mr. Heinrich:

We are pleased to inform you that your Letter on "Postmortem infectivity of SARS-CoV-2 associated deaths and predictive value of standard diagnostic procedures" has been accepted for rapid publication online in Emerging Infectious Diseases.

The letter will undergo substantive editing for length, grammatical correctness, and journal style. The galleys will be sent to you for approval.

Thank you for sending us your letter. We look forward to working with you in the weeks to come.

Dr D. Peter Drotman
Editor-in-Chief
Emerging Infectious Diseases
eidchief@cdc.gov
404-639-1960 (phone)

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Reviewer: 2
Comments to the Author
Heinrich et al have thoroughly addressed reviewer comments. I have some small clarifications below.

1. X axis labels should be added to figure 1BC for 'virus culture' -/+.
2. Was the 44 blood draw donors randomly selected? Define how they were sampled.
3. Define that all the Ag-RDT are Nucleoprotein based, and define the term first (acronym missing in main text).

Reviewer: 1
Comments to the Author
The authors have appropriately addressed all of my comments in the revised version and I therefore recommend to accept the manuscript.

EID Editorial Board Comments to the Author:
The reviewer comments will be addressed in the copyediting stage.

Presentation of the publication and bibliography

Introduction

Experiences of the coronavirus disease 2019 (COVID-19) pandemic potently focused global attention on the ongoing risks around emergent pathogens and the challenges related to controlling transmission. The rapid spread of severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) early in the pandemic strained healthcare systems, and countries in severely affected areas implemented crisis care standards and triage protocols[1]. The strain was not limited to the care of ill but still-living patients. It has been estimated that, to date, SARS-CoV-2 infections have resulted in 14.83 million excess deaths globally, and the high numbers of deaths posed immense challenges for healthcare systems, overwhelming facilities and funeral services and leading to the establishment of temporary morgues in some areas[2,3]. Families could not bid farewell to their deceased relatives in many places. In other places, earth burials were prohibited due to concerns about soil contamination, resulting in all deceased being mass cremated despite family objections[4-6].

For SARS-COV-2 infection, a mean incubation period of five days is typically described, ranging between 1 and 14 days across studies[7,8]. Viral loads and the probabilities of positive viral cultures peak, on average, on day four after symptom onset[9,10]. Accordingly, the risk of transmission appears to be highest within the first five days after symptom onset, although transmission can occur broadly around this point, including in the pre-symptomatic phase of infection or from patients who remain asymptomatic[11-16]. While viral RNA can be detected for up to 2 weeks in asymptomatic individuals and for up to 4 weeks or longer in individuals with more severe COVID-19 or those on immunosuppression, replicable virus persistence is more short-lived, with most studies demonstrating a robust and often rapid decline in replicable virus within the first week of infection[9,12,17-30]. That said, longer timelines might apply for immunocompromised individuals where replicable virus was detected for extended periods[24,30]. For most patients, seroconversion was described to occur within the first 14 days of infection[9,31,32]. There is a strong positive association between RNA loads and replicable virus detection, with some studies describing 1 x 10^6 copies/ml associated with a 95% sensitivity and 72% specificity to predict virus culture positivity[9,30,33-35]. Furthermore, a strong correlation between neutralising antibody titers and replicable virus detection has been observed[9,30,36,37]. A study using human virus challenge in young adults found that rapid antigen test results were strongly associated with detecting replicable viruses in mild to moderately symptomatic individuals[27]. In contrast to extensive investigation on the trajectories of viral RNA loads, antibody levels, the detection and persistence of viral proteins, and infectiousness in live patients, these features of SARS-CoV-2 have not been studied in corpses.

Assessing the risk of severe disease and death in COVID-19 patients, especially in intensive care units, was challenging at the time of the herein presented study because it was still unclear which disease-specific factors were predictive of COVID-19 severity and outcomes. A range of patient-specific factors, such as sociodemographic features and pre-existing medical conditions, including age, sex, and comorbidities, such as chronic lung diseases, cancer, cerebrovascular diseases, and chronic kidney diseases, were reported to be associated with COVID-19 severity and outcome[38]. In terms of disease-specific factors, there was first evidence that SARS-CoV-2 RNA loads in the upper respiratory tract at the time of diagnosis were associated with COVID-19 severity, and descriptive data from a cohort study of 76 hospitalised patients indicating that upper respiratory tract SARS-CoV-2 RNA loads throughout the course were likewise associated with COVID-19 severity[39,40]. Similarly, SARS-CoV-2 RNA loads at single-point measurements in the lower respiratory tract – obtained between 14 days before and seven days after ICU admission – were associated with COVID-19 severity[41]. A delay in virus clearance from the lower respiratory tract was reported to be associated with all-cause mortality[41]. However, the analysis did not account for the competing risk of all-cause mortality. The presence of viremia and viral loads in plasma at the time of admission also have been associated with COVID-19 severity and death[42-44]. In a small cohort of 24 ICU patients with hematologic and oncologic diseases, failure to clear SARS-CoV-2 RNA from the bloodstream was associated with a higher risk of all-cause mortality[45], although again, this analysis did not account for the competing risk of all-cause mortality, yielding upward-biased estimates of cause-specific failure probabilities. An important limitation of these studies examining the link between SARS-CoV-2 RNA loads in different compartments and all-cause mortality is their limitation to single-point measurements without consideration of the trajectory of SARS-CoV-2 RNA loads throughout the disease course.

Accurate diagnostic testing for SARS-CoV-2 was recognised early in the pandemic as an essential cornerstone of response efforts[46] to help identify and isolate infected individuals, inform clinical management and treatment decisions, and provide data for public health interventions and policy[46]. In this context, there was an urgent need to understand the strengths and limitations of different testing methodologies, including reverse transcription-quantitative polymerase chain reaction (RT-qPCR), antigen-detecting rapid diagnostic tests (AgRDTs), enzyme-linked immunosorbent assays (ELISAs), and virus culture in terms of sensitivity, specificity, predictive values, and overall reliabilities[47,48]. While the gold standard of SARS-CoV-2 diagnosis remains viral RNA detection by nucleic acid amplification tests

(NAATs), this modality is logistically challenging with relatively long diagnosis times[49]. In contrast, virus-specific and neutralising antibodies, as measured by ELISA, have been used to measure correlates of acquired or vaccine-related immunity, and recently, AgRDTs have been suggested as a sufficient and fast alternative diagnostic tool for SARS-CoV-2 infections in some settings[50-52]. In living patients, the interplay between viral loads (detected by NAAT), shed and surface-bound viral proteins (detected by AgRDTs), virus-specific antibodies (detected by ELISA), and replicable viruses (detected by virus culture) has been exhaustively described[9,10,27,30,33-35,37].

Testing strategies were developed and recommended during the pandemic for different situations, including the postmortem period[53-55]. However, differential stability of viral components in the postmortem setting and failure to mount sufficient antibody responses in people dying from COVID-19–might lead to differential results in the postmortem setting, and it remains to be seen how well standard diagnostic procedures predict sustained postmortem infectivity. Generally, it has been unclear how to deal with the deceased and whether corpses still pose a risk of transmission of SARS-CoV-2[3]. While there is robust evidence that SARS-CoV-2 is mainly transmitted through direct contact and droplets in live people, the predominant mode of transmission in corpses is less well understood[56,57]. Recommendations for corpse handling rely solely on theoretical considerations, and systematic studies investigating corpse infectivity have been lacking, although corpses have long been considered potentially infectious under the assumption that viruses remain stable across various conditions[58-62]. For example, small cohort studies of HIV-related deaths have inferred sustained infectivity of HIV[62]. Viral perseverance can mechanistically result from partial postmortem durability of the host environment (i.e., variabilities in the kinetics of cellular death)[63]. The standardised cooling of corpses in the routine practices of forensic and pathological institutes and funeral homes can also contribute to sustained stability. Moreover, the solid environmental stability of some viruses might lead to viral perseverance without the colonisation of a viable host[64-67].

This doctoral thesis focuses on two critical aspects of SARS-CoV-2 diagnostic testing that require specific attention to improve current and future responses to dangerous and highly infectious pathogens. The first critical aspect of testing is the predictive value of RT-qPCR for patient outcomes in intensive care units, aiming to identify patients at higher risk of severe illness or death. Therefore, the first part of the doctoral thesis evaluated the association between diagnostic testing results and outcomes (Heinrich et al., 2021). Admission RNA loads, peak RNA loads, and RNA loads over time in the first 30 days after ICU admission assessed in the upper respiratory tract, the lower respiratory tract, and blood were correlated with 90-day all-cause mortality. For that work, a generalised linear mixed model investigated the association over time in the first 30 days between SARS-CoV-2 RNA loads in each compartment and 90-day all-cause mortality while adjusting for important confounding variables. The second part of this doctoral thesis evaluated the timeline of viral persistence among corpses, followed by a measurement of the predictive value of different diagnostic tests, including RT-qPCR, AgRDT, and ELISA, for determining the infectiousness of deceased COVID-19 patients, with the aim to determine the postmortem stability of SARS-CoV-2 and infectivity of deceased COVID-19 patients. First, the postmortem stability of SARS-CoV-2 RNA was investigated by examining the correlation of admission nasopharyngeal SARS-CoV-2 RNA loads with postmortem intervals, where the kinetics of nasopharyngeal SARS-CoV-2 RNA loads over time from admission until testing were investigated in SARS-CoV-2-positive corpses, and in a subset of these, sustained postmortem infectivity was determined using subgenomic RNA loads and virus culture (Heinrich et al., 2021)[1]. Following this, the postmortem sensitivity and specificity of different AgRDTs were examined in a cohort of SARS-CoV-2 RNA-positive and SARS-CoV-2 RNA-negative corpses (Heinrich et al., 2022). Data from both investigations were used to determine a refined estimate of postmortem infectivity of SARS-CoV-2 RNA-positive corpses and allowing an investigation of the predictive value of standard diagnostic procedures for sustained postmortem infectivity. This doctoral thesis set out to help determine how standard diagnostic procedures can aid clinical decision-making, risk assessment, and infection control measures for living and deceased infected patients. The results contribute to more effective testing strategies, improved patient care, and better-informed public health policies not only for ongoing efforts related to COVID-19 but also for potential future pandemics.

Results

Results for live COVID-19 patients

In total, 170 patients admitted to the intensive care units at the University Medical Center Hamburg-Eppendorf due to COVID-19 were enrolled in the study to investigate the association between SARS-CoV-2 RNA loads in different compartments with all-cause mortality. The mean patient age was 63 years (IQR 55 to 73), and 35% were females (n=59). In total, 70 patients were dead (41%), and 100 were alive (59%) at the end of the 90-day follow-up. The median follow-up time was 33 days (IQR 30 to 43) using the reverse

[1] As currently understood, subgenomic RNA loads do not imply active replication of SARS-CoV-2 (Alexandersen S, Chamings A, Bhatta TR. SARS-CoV-2 genomic and subgenomic RNAs in diagnostic samples are not an indicator of active replication. Nature Communications 2020; 11(1): 6059).

Kaplan-Meier estimator[2]. Detailed patient characteristics are depicted in Supplementary Table 1. Kaplan-Meier estimated survivor curves are illustrated in Supplementary Figures 1 to 4. Visually, there was no evidence for a difference in survival by most comorbidities, but a difference in survival was observed for disease severity at ICU admission as assessed by SAPS II scores. The log-rank test was compatible with the null hypothesis for a difference in survival by most comorbidities and provided strong evidence for a difference in survival by the disease severity at ICU admission (p=0.01).

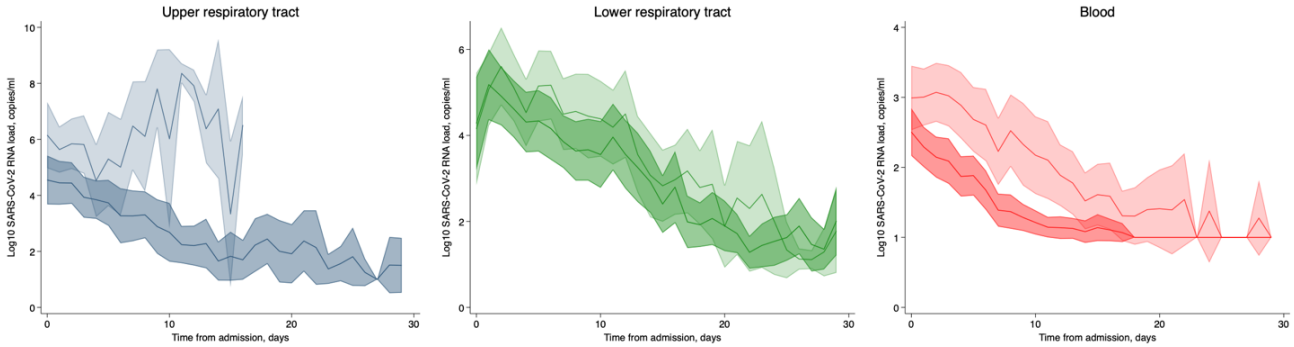
On average, patients had four samples of SARS-CoV-2 RNA loads taken from the upper respiratory tract (SD: 4.45), nine samples of SARS-CoV-2 RNA loads taken from the lower respiratory tract (SD: 9.18), and 12 SARS-CoV-2 RNA loads in the blood (SD: 9.22) available throughout the observation period. When investigating the pattern of missingness, the pattern was inversely monotone, with most patients having initial but not later observations missing. Missingness was assumed completely at random for the analyses. More details can be found in Supplementary Material 2. Missingness proportions in independent covariables are depicted in Supplementary Table 2.

To assess whether viral loads at admission predict outcomes, median admission SARS-CoV-2 RNA loads in the different compartments were compared between COVID-19 survivors and non-survivors. The mean SARS-COV-2 RNA load in the upper respiratory tract at the time of admission was 5.22 $\log_{10}$ copies/ml (SD 2.59), with higher viral loads (6.15 $\log_{10}$ copies/ml, SD 2.69) in individuals who had died at the end of the follow-up than in individuals who survived (4.55 $\log_{10}$ copies/ml, SD 2.35)[3]. Mann-Whitney U testing was used to compare median upper respiratory tract SARS-CoV-2 RNA loads at admission between patients who died and those who survived, and moderate evidence against the null hypothesis was observed (p=0.03). The mean and normal approximated 95% confidence interval for SARS-CoV-2 RNA loads in the upper respiratory tract over time from admission in patients who had died and those who survived to the end of follow-up can be found in Figure 1.

In the lower respiratory tract, the mean SARS-COV-2 RNA load at the time of admission was 4.23 $\log_{10}$ copies/ml (SD 2.33), with comparable viral loads (4.15 $\log_{10}$ copies/ml, SD 2.30) in individuals who had died and survived to the end of follow-up (4.28 $\log_{10}$ copies/ml, SD 2.41)[c]. Mann-Whitney U testing was used to compare median lower respiratory tract SARS-CoV-2 RNA loads at admission between patients who had died or survived to the end of follow-up, and no evidence against the null hypothesis was observed (p=0.76). The mean SARS-CoV-2 RNA load in the lower respiratory tract over time from admission in individuals who had died compared to those who survived to the end of follow-up can be found in Figure 1.

In the blood, the mean SARS-COV-2 RNA load at the time of admission was 2.72 $\log_{10}$ copies/ml (SD 1.44), with higher viral loads (2.99 $\log_{10}$ copies/ml, SD 1.57) in individuals who had died at the end of the follow-up than in individuals who had not died (2.50 $\log_{10}$ copies/ml, SD 1.31) until the end of the follow-up[c]. Mann-Whitney U testing was used to compare median blood SARS-CoV-2 RNA loads at admission between patients who had died and those who survived to the end of the follow-up, and weak evidence against the null hypothesis was observed (p=0.05). The mean SARS-CoV-2 RNA load in the blood over time from admission in individuals who had died compared to those who survived to the end of follow-up can be found in Figure 1. Individual trajectories suggest a strong degree of tracking and fanning (Supplementary Figure 5).

Figure 1. Mean and normal-approximated 95% confidence intervals for $\log_{10}$ SARS-CoV-2 RNA loads in the upper and lower respiratory tract and blood by all-cause mortality are shown. Data from patients who had died by the end of the follow-up period are shown in opaque colour.



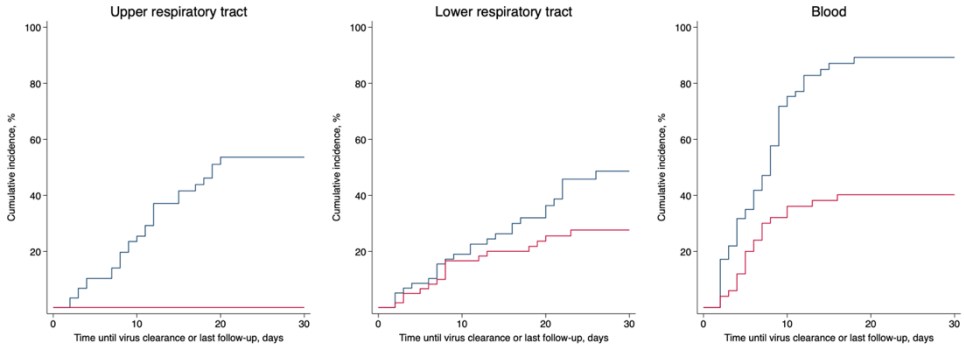
The peak SARS-COV-2 RNA load in the upper respiratory tract was 5.22 $\log_{10}$ copies/ml (SD 2.59), with higher viral loads (6.15 $\log_{10}$ copies/ml, SD 2.69) in individuals who had died at the end of the follow-up

[2] In the main manuscript, median follow-up time was based on all subjects in the study, with the follow-up time from the start to the time the last subject has an event or is censored.
[3] Admission measurements in the main manuscript were defined as the first non-missing measurement within the first four days after ICU admission.

compared to those who had survived (4.55 log_{10} copies/ml, SD 2.35)[4]. The peak SARS-COV-2 RNA load in the lower respiratory tract was 5.22 log_{10} copies/ml (SD 2.59), with higher viral loads (6.15 log_{10} copies/ml, SD 2.69) in individuals who had died at the end of the follow-up compared to those who had survived (4.55 log_{10} copies/ml, SD 2.35)[d]. The peak SARS-COV-2 RNA load in the blood was 5.22 log_{10} copies/ml (SD 2.59), with higher viral loads (6.15 log_{10} copies/ml, SD 2.69) in individuals who had died at the end of the follow-up compared to those who had survived (4.55 log_{10} copies/ml, SD 2.35)[d]. Using Mann-Whitney U testing, median peak viral loads were compared from the upper and lower respiratory tract and blood compartments for patients who had died versus survived to the end of the follow-up period; strong evidence for a difference in median peak viral loads was found for the upper respiratory tract (p=0.0002) and blood (p=0.001) compartments, but weak evidence for a difference in median peak viral loads was found for the lower respiratory tract (p=0.09).

Then, the time from virus positivity until virus clearance was compared for survivors and non-survivors. The main manuscript presents Kaplan-Meier estimated survivor curves and log-rank tests. As these estimated survivor curves yield downward-biased estimates of cause-specific survival probabilities due to competing risks, cumulative incidence functions were calculated and illustrated here (Figure 2). The weighted log-rank test provided weak evidence for a difference in virus clearance by survival status in the upper respiratory tract (p=0.05) and very strong evidence for a difference in virus clearance by survival status in the lower respiratory tract (p<0.001) and blood (p<0.0001).

Figure 2. Cumulative incidence function of virus clearance, by compartment, in patients with virus detected at any time. The time origin was virus detection, and the time scale was days from virus detection to clearance, last follow-up, or death. LOCF was employed for missing values. Patients who died at the end of the follow-up are shown in red, and those who survived are shown in blue.



Next, a modelling approach was employed to examine whether viral loads over time predict outcomes. A generalised linear mixed model with a random patient intercept and random slope for time was used to examine the association over time between serial SARS-CoV-2 RNA loads in each compartment in the first 30 days and 90-day all-cause mortality (Table 1). For the upper respiratory tract, every 10-fold increase in SARS-CoV-2 RNA load within one patient over time in the first 30 days was associated with 0.53 times the odds of 90-day all-cause survival (95% CI: 0.40 to 0.69) when adjusting for other covariables and viremia status in the model. There was no evidence for a change in the odds of 90-day all-cause survival with every 10-fold increase in upper respiratory tract SARS-CoV-2 RNA loads over time in the first 30 days when adjusting for other covariables in the model (p=0.14). In the lower respiratory tract, every 10-fold increase in SARS-CoV-2 RNA load within one patient over time in the first 30 days was associated with 0.75 times the odds of 90-day all-cause survival (95% CI: 0.65 to 0.85) when adjusting for other covariables and viremia status in the model. There was very strong evidence for a change in the odds of all-cause survival with every 10-fold increase in lower respiratory tract SARS-CoV-2 RNA loads over time in the first 30 days when adjusting for other covariables in the model (p<0.0001). In the blood, every 10-fold increase in SARS-CoV-2 RNA load within one patient over time in the first 30 days was associated with 0.23 times the odds of 90-day all-cause survival (95% CI: 0.12 to 0.41) when adjusting for other covariables in the model. There was very strong evidence for a change in the odds of 90-day all-cause survival with every 10-fold increase in blood SARS-CoV-2 RNA loads over time in the first 30 days when adjusting for other covariables in the model (p<0.0001). The model-predicted probabilities of survival for the average patient are illustrated in the main manuscript (Figure 1D)[5].

[4] Peak measurements in the main manuscript were single-imputed with the lower limit of detection.
[5] The average population was defined based on the means of continuous and categorical variables in the analytical sample for each model. Because a complete case analysis was employed, the average population slightly differed between the different models.

Table 1. Odds ratios and 95% CIs from generalised linear mixed models are presented in the main manuscript.

	Compartment								
	Upper respiratory tract*			Lower respiratory tract*			Blood†		
	OR	95%CI	P-val.	OR	95%CI	P-val.	OR	95%CI	P-val.
SARS-CoV-2 RNA load, log_{10} copies/ml	0.52	0.40-0.69	0.14	0.75	0.65-0.85	<0.0001	0.23	0.12-0.41	<0.0001

*Adjusted for age, sex, body mass index, Charlson comorbidity index, acute respiratory distress syndrome, mechanical ventilation, extracorporeal membrane oxygenation, and viremia. †Adjusted for age, sex, body mass index, Charlson comorbidity index, acute respiratory distress syndrome, mechanical ventilation, and extracorporeal membrane oxygenation. **Abbreviations:** OR, odds ratios; CI, confidence interval; P-val., p-value.

Results for deceased COVID-19 patients

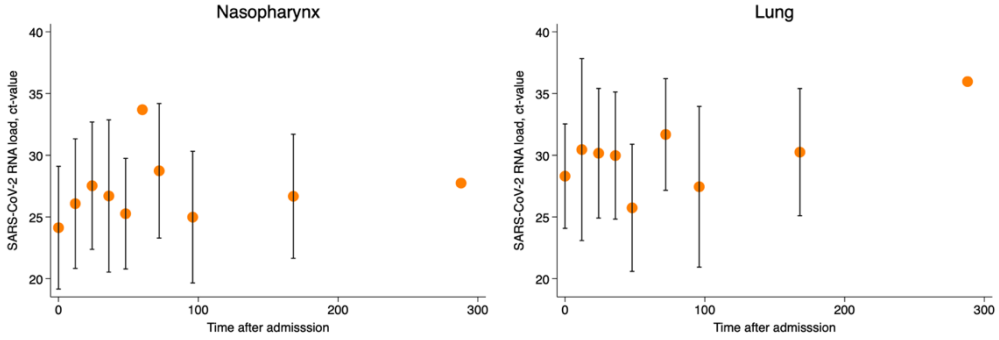
To evaluate the postmortem stability of SARS-CoV-2 RNA, the correlation between admission semiquantitative SARS-CoV-2 RNA loads and the postmortem interval was examined in a consecutive sample of SARS-CoV-2 RNA-positive corpses (n=79). Spearman's correlation coefficient was used to investigate this relationship. No evidence for a monotonic non-linear relationship between admission SARS-CoV-2 RNA loads and the postmortem interval was observed (p=0.50). A difference in the median between antemortem (defined as the most recent measurement available before death in the IMMVH databases) and postmortem (defined as the first measurement available after death in the IMMVH databases) SARS-CoV-2 RNA loads was examined in corpses with both loads available using a Wilcoxon signed-rank test (n=10). No evidence for a difference in median antemortem and postmortem SARS-CoV-2 RNA loads was observed (p=0.85).

A subset of SARS-CoV-2 RNA-positive corpses with short postmortem intervals was followed over time with sequential nasopharyngeal swabs to examine the trajectory of SARS-CoV-2 RNA loads in patients who succumbed to COVID-19 (n=11). Sample characteristics of these corpses are detailed in the publication of this work (Heinrich et al., 2021). The mean semiquantitative SARS-CoV-2 RNA loads and normal-approximated 95% confidence intervals over time from admission are illustrated in Figure 3. The individual trajectories suggest a strong degree of tracking and a moderate degree of fanning (Supplementary Figure 6).

Repeated measurements of semiquantitative SARS-COV-2 RNA loads were missing in 29.1% of patients (n=53/182), with 26.4% of repeated measurements missing in the lung (n=24/91) and 31.8% of repeated measurements missing in the nasopharynx (n=29/91). The missing pattern was arbitrary, with some parts fitting a monotone pattern. For the subsequent analyses, missing semiquantitative SARS-CoV-2 RNA loads were assumed to be missing completely at random, and a complete case analysis was conducted.

A linear mixed model explored trajectories of semiquantitative SARS-CoV-2 RNA loads in this subset of corpses. As suggested from the individual trajectories, a random-intercept model provided an improved fit to the data compared to a model without random intercepts (p<0.0001[6]; Supplementary Figure 6). The final model is given in Table 2. With every hour increase in time from admission to testing, a mean difference of -0.005 (95% CI: -0.02 to 0.01) in ct-values was found when adjusting for other covariables in the model, with no evidence for a difference in ct-values with every hourly increase when adjusting for covariables in the model (p=0.58). More detailed descriptions of the linear mixed model results can be found in Supplementary Material 3.

Figure 3. Longitudinal mean and normal approximated 95% confidence intervals for the semiquantitative nasopharyngeal and pulmonary SARS-CoV-2 RNA load over time from admission, namely 0, 12, 24, 36, 48, 60, 72, 96, and 168 hours after admission. Measurements below the lower limit of detection were excluded for illustration purposes.



[6] Likelihood ratio test of nested models.

At baseline, 95% of the patient intercepts were modelled between ct-values of 13.07 and 38.14. A linear mixed model was calculated without excluding measurements below the lower limit of detection for sensitivity analyses, as this might introduce selection bias towards deceased individuals with higher viral loads, but found the effect estimate and inferences for the time from admission were unchanged (Supplementary Table 3). Moreover, sensitivity analyses using scaled F-test statistics, as suggested by Kenward and Roger to account for the small-sample bias with unbalanced datasets, are presented in Supplementary Tables 4 and 5. No substantial change in inference was observed when accounting for the small-sample bias.

Table 2. Linear mixed model with semiquantitative SARS-CoV-2 RNA loads throughout the study included as the dependent variable in the model. SARS-CoV-2 RNA loads below the limit of detection were excluded from the analysis.

	Average mean difference	**95% CI**	**P-value**
Time after admission until testing	-0.005	-0.02 to 0.01	0.58
Location (Ref: Nasopharynx)	7.41	4.51 to 10.32	<0.001
PMI, hours	0.05	-0.41 to 0.52	0.83
Time-varying conventional autopsy status	1.48	-1.31 to 4.27	0.30
Location x Time-varying conventional autopsy status	-4.89	-8.35 to -1.43	0.01
Random effects	**Coefficient***	**95% CI**	
Between patient variance			
Patient intercept	40.89	15.38 to 108.73	
Within patient variance			
Residual variance	16.45	12.32 to 21.96	

Abbreviations: CI, confidence interval; PMI, postmortem interval (defined as the time from death until morgue admission). "x" denotes interaction terms. *If not stated otherwise, coefficients refer to the variance.

Next, a second consecutively enrolled cohort of SARS-CoV-2 RNA-positive corpses was evaluated to determine maintained infectivity and the predictive values of standard diagnostic procedures for maintained infectivity (n=128). The median patient age was 83.5 years (IQR: 71.9 to 89.0), and 45% were female (n=57). Again, sample characteristics of these corpses are detailed in the publication of this work (Heinrich et al., 2022). Missing data proportions in baseline covariates were small, with four corpses with missing purification state (3.12%).

While a primary focus of this second study of corpses was to measure maintained infectivity, the cohort also served the ability to confirm the results regarding the association of viral loads at admission and the postmortem interval in a larger cohort. The median admission nasopharyngeal SARS-COV-2 RNA load was 7×10^6 copies/ml (IQR: 5.6×10^4 to 5.2×10^7). Spearman's correlation coefficient was again used to investigate the relationship between admission nasopharyngeal quantitative SARS-CoV-2 RNA loads and the postmortem interval. Again, no evidence for a monotonic non-linear relationship between admission nasopharyngeal quantitative SARS-CoV-2 RNA loads and the postmortem interval was observed (p>0.99).

A series of virus culture experiments were performed to evaluate the predictive value of standard diagnostic procedures for maintained infectivity. Virus culture was positive in 20% of SARS-CoV-2 RNA-positive corpses in this cohort (n=26). The median postmortem interval in corpses with positive virus cultures was 12.6 hours (IQR: 5.5 to 105.8) and 8.5 hours (IQR: 5.3 to 58) in corpses with negative virus culture results. A difference in median postmortem intervals between corpses with and without positive virus culture was examined using a Mann-Whitney U test; no evidence for a difference in median postmortem intervals was observed (p=0.38). The median nasopharyngeal quantitative SARS-CoV-2 RNA load in corpses with positive virus cultures was 1.43×10^7 copies/ml (IQR: 3.8×10^4 to 3.31×10^8) and 7.0×10^6 copies/ml (IQR: 5.8×10^4 to 3.95×10^7) in corpses with negative virus culture results. A difference in median nasopharyngeal quantitative SARS-CoV-2 RNA loads between corpses with and without positive virus culture was examined using a Mann-Whitney U test; no evidence for a difference in median nasopharyngeal quantitative SARS-CoV-2 RNA loads was observed (p=0.14). When comparing expected and observed proportions of putrefactive changes between corpses with and without positive virus culture, weak evidence was found for an association between putrefaction and positive virus culture (p=0.07)[7].

In a subset of corpses with blood samples available, seroconversion was examined as described above (n=44). Of these, 39% were positive for anti-spike antibodies (n=17) and 35% for anti-nucleocapsid antibodies (n=15). The median anti-spike antibody level was 0.4 U/ml (IQR: 0.4 to 13.5). 33% were positive for both anti-spike and anti-nucleocapsid antibodies (n=14), suggestive of infection, and 5% were only

[7] In the manuscript, a χ^2 test was used with expected cell counts below 5, which explains the discrepancies observed between the p-values (0.11 in the manuscript and 0.07 from Fisher's exact test).

positive for anti-spike antibodies (n=2), suggestive of vaccination only and pending seroconversion for the ongoing infection. 60% were negative for both spike and nucleocapsid antibodies (n=26). Spearman's correlation coefficient was used to investigate the relationship between anti-spike antibody levels and the postmortem interval. No evidence for a monotonic non-linear relationship between anti-spike antibody levels and the postmortem interval was observed (p=0.64). Likewise, Spearman's correlation coefficient was used to investigate the relationship between anti-spike antibody levels and the nasopharyngeal quantitative SARS-CoV-2 RNA loads. Very strong evidence of a monotonic non-linear relationship between anti-spike antibody levels and the nasopharyngeal quantitative SARS-CoV-2 RNA loads was observed (p<0.0001). When comparing expected and observed proportions of anti-nucleocapsid antibody conversion between corpses with and without positive virus culture, no evidence was found for an association between anti-nucleocapsid antibody conversion and positive virus culture results (p=0.14)[8]. When comparing expected and observed proportions of anti-spike antibody conversion between corpses with and without positive virus culture, weak evidence was found for an association between anti-spike antibody conversion and positive virus culture (p=0.10). The median anti-spike antibody level in corpses with positive virus culture was 0.4 U/ml (IQR: 0.4 to 0.4) and 0.6 U/ml (IQR: 0.4 to 24.3) in corpses with negative virus culture results. A difference in median anti-spike antibody levels between corpses with and without positive virus culture was examined using a Mann-Whitney U test. No evidence for a difference in median anti-spike antibody levels between corpses with and without positive virus culture results was observed (p=0.38).

Finally, the sensitivity and specificity of antigen-rapid diagnostic tests were examined in consecutive samples of SARS-CoV-2 RNA-positive (n=128) and negative (n=72) corpses for three antigen-detecting rapid diagnostic tests (AgRDTs) from different manufacturers. Test 1 (Panbio COVID-19 Ag Rapid Test Device) had a sensitivity of 80.3% (95% CI: 72.3 to 86.8), test 2 (SARS-CoV-2 Rapid Antigen Test) had a sensitivity of 86.4% (95% CI: 79.1 to 91.9), and test 3 (MEDsan SARS-CoV-2 Antigen Rapid Test) had a sensitivity of 84.1% (95% CI: 76.6 to 90.0) in individuals positive for the golden standard RT-qPCR. Specificity was determined and was 100.0% (95% CI: 95.0 to 100.0) for test 1, 98.6% (95% CI: 93.0 to 100.0) for test 2, and 95.8% (95% CI: 88.0 to 99.0) for test 3. Sensitivity in corpses with positive virus culture for test 1 was 92.3% (95% CI: 74.9 to 99.1), for test 2 was 96.2% (95% CI: 80.4 to 99.9), and for test 3 was 96.2% (95% CI: 80.4 to 99.9). AgRDT results were missing in 0.78% for Test 1 (n=1), 2.34% for Test 2 (n=3), and 1.56% for Test 3 (n=2). In univariable logistic regression, for all three tests, there was no evidence for a change in the odds of true positivity with every hour increase in the postmortem interval (Table 3). Likewise, for all three tests, there was no evidence for a change in the odds of true positivity when comparing individuals with and without incipient putrefaction changes. Every 10-fold increase in postmortem nasopharyngeal SARS-CoV-2 RNA loads was associated with 3.65 times the odds of true positivity (95% CI: 2.16 to 6.17; p<0.001) for test 1, 3.09 times the odds of true positivity (95% CI: 1.81 to 5.28; p<0.001) for test 2, and 3.31 times the odds of true positivity (95% CI: 1.94 to 5.64; p<0.001) for test 3 (Supplementary Figure 7). For all three tests, there was very strong evidence for a change in the odds of true positivity with every 10-fold increase in postmortem nasopharyngeal SARS-CoV-2 RNA loads. Compared to individuals with negative culture results, those with positive virus cultures had 3.54 times the odds of true positivity for test 1 (95% CI: 0.78; p=0.10), 4.82 times the odds for test 2 (95% CI: 0.61 to 38.16; p=0.14), and 5.86 times the odds for test 3 (95% CI: 0.75 to 46.02; p=0.09). For tests 1 and 3, there was weak evidence for a change in the odds of true positivity when comparing individuals with and without positive virus culture. Results from multivariable logistic regression results are listed in Table 3.

Table 3. Univariable and multivariable logistic regression with positive AgRDT testing in SARS-CoV-2 RNA positive corpses as each model's dependent variable. Multivariable logistic regression included 123 patients for test 1, 121 patients for test 2, and 122 patients for test 3.

	Univariable logistic regression		**Multivariable logistic regression**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Test 1				
PMI, h	1.00 (0.99 to 1.005)	0.70	1.00 (0.99 to 1.01)	0.74
Log_{10} SARS-CoV-2 RNA load, copies/ml	3.65 (2.16 to 6.17)	<0.001	3.50 (2.06 to 5.95)	<0.001
Putrefactive changes	0.54 (0.13 to 2.24)	0.40	0.65 (0.09 to 4.75)	0.67
Test 2				
PMI, h	1.01 (0.99 to 1.02)	0.15	1.01 (1.00 to 1.03)	0.10
Log_{10} SARS-CoV-2 RNA load, copies/ml	3.09 (1.81 to 5.28)	<0.001	3.36 (1.86 to 6.05)	<0.001
Putrefactive changes	0.66 (0.13 to 3.35)	0.61	0.80 (0.10 to 6.51)	0.83
Test 3				
PMI, h	1.00 (0.99 to 1.01)	0.49	1.00 (0.995 to 1.01)	0.37

[8] In the manuscript, a χ^2 test was used with expected cell counts below 5, which explains the discrepancies observed between the p-values (0.23 in the manuscript and 0.14 from Fisher's exact test).

Log_{10} SARS-CoV-2 RNA load, copies/ml	3.31 (1.94 to 5.64)	<0.001	3.22 (1.88 to 5.52)	<0.001
Putrefactive changes	0.42 (0.10 to 1.75)	0.23	0.41 (0.06 to 2.89)	0.37

Abbreviations: PMI, postmortem interval; SARS-CoV-2 RNA, Severe acute respiratory distress syndrome coronavirus-2 ribonucleic acid; 95% CI, 95% confidence interval.

Discussion

The findings from the presented studies provide valuable insights into the predictive values of standard diagnostic procedures for SARS-CoV-2 in live and dead COVID-19 patients and their implications for clinical practice and public health measures. Key findings include viral loads in lower respiratory tract and blood in the first 30 days are predictive of 90-day all-cause mortality, and in corpses, the persistence of SARS-CoV-2 RNA long after death, with no evidence of an association between admission nasopharyngeal SARS-CoV-2 RNA loads and the time after death, the ability of the current range of standard diagnostic tests to detect and predict postmortem infectivity and, relatedly, the sensitivity of antigen rapid tests in this population.

In living patients, the given study highlighted the predictive value of viral load measurements. The primary objective of this large cohort study of ICU patients was to explore the association between SARS-CoV-2 RNA loads over time in the first 30 days in different compartments and 90-day all-cause mortality. Previous studies investigating the association between SARS-CoV-2 RNA loads and COVID-19 outcomes have restricted their analyses to single-point measurements, thus limiting the ability to understand temporal dynamics and to better evaluate causality. Inferences cannot be made about nonspecific time points. In brief, very strong evidence for a 33% to 435% increase in the odds of 90-day all-cause death was observed with every 10-fold increase in lower respiratory tract and blood SARS-CoV-2 RNA loads in the first 30 days but not with every 10-fold increase in upper respiratory tract SARS-CoV-2 RNA loads in the first 30 days when adjusting for other covariables in the model. This suggests that SARS-CoV-2 RNA loads in the first 30 days after ICU admission are a strong and independent predictor of survival for ICU patients diagnosed with COVID-19 and that screening for SARS-CoV-2 RNA in the blood may help to identify individuals at risk of death. Individuals with high and persistent viral loads, especially in the blood, may particularly benefit from targeted interventions such as monoclonal antibody therapy or direct antiviral medications. Further research is needed to understand better the mechanism by which SARS-COV-2 RNA loads, particularly in the blood, mediate death.

Moderate evidence of a difference in median SARS-CoV-2 RNA loads was found in the upper respiratory tract and blood on the day of ICU admission between patients who had survived at the end of the follow-up and patients who had not. This aligns with data published later in the COVID-19 pandemic reporting evidence for a median difference in SARS-CoV-2 RNA loads at admission in the upper respiratory tract and blood between COVID-19 survivors and non-survivors[39,42,44,68-72]. Gutmann and colleagues found that patients presenting with viremia in the first six days after ICU admission had 2.05 times the instantaneous risk of 28-day mortality that patients without viremia had when adjusting for other variables in the model[73]. Another study investigated median differences in SARS-CoV-2 RNA loads in the blood and COVID-19 outcomes, although the time when samples were obtained was not specified[74]. The lack of evidence for a difference in median SARS-CoV-2 RNA loads in the lower respiratory tract on the day of ICU admission between patients who had survived to the end of the follow-up versus those who did not in the herein presented study may be explained by a wide 95% confidence interval for the -0.61 log_{10} median difference in SARS-CoV-2 RNA copies/ml (95% CI: -2.01 to 1.31)[9], and thus the study was possibly underpowered to detect a difference in median loads at the time of ICU admission. Moreover, patients with measurements from the lower respiratory tract available at admission might generally suffer more severe disease given the need for intervention to obtain the material suggesting missingness of viral loads at random conditional on disease severity. Other studies found very strong evidence for a mean difference in SARS-CoV-2 RNA loads at admission in the lower respiratory tract between individuals who had survived at the end of the follow-up and those who had not[41]. The main manuscript defines admission values as the first non-missing measurement within the first four days after ICU admission. This imputation assumed that the missingness of viral loads within the first four days is completely at random. Given that viral loads were obtained as part of the daily clinical routine and single-imputation is restricted to the first four days after ICU admission, this assumption can be plausible with no substantial change in the inferences drawn.

Similar to the findings of an association of median admission RNA loads and survival, there also was weak to strong evidence for a difference in median peak SARS-CoV-2 RNA loads between patients who survived to the end of the follow-up and patients who had not. In the main manuscript, missing peak measurements were single-imputed with the lower limit of detection. This approach assumed peak viral load missingness not at random, with missing values related to the underlying viral load. This assumption can be plausible as patients may not undergo interventions to obtain lower respiratory tract samples when clinicians expect

[9] The median difference and 95% confidence interval were calculated under the same assumptions used for the Mann-Whitney U test, with assumed equality of variance between patients who had died at the end of the follow-up and individuals who had not died.

low viral loads. Importantly, this single-imputation changed inferences from the original evaluation. In the analyses presented here, there was no evidence of a difference in median peak SARS-CoV-2 RNA loads in the upper respiratory tract between patients who had survived at the end of the follow-up and those who had not.

In terms of viral clearance, weak to very strong evidence for a difference in time from virus positivity to virus clearance from the upper respiratory tract, lower respiratory tract and blood was observed between patients who had died at the end of the follow-up and patients who had not. This aligns with a cohort study investigating virus clearance from the lower respiratory tract and two cohort studies investigating virus clearance from the blood in 52 and 24 COVID-19 patients admitted to the ICU[41,43,45,75]. In contrast to the work presented here, these prior studies did not account for the competing risk of all-cause mortality. Adjustment for competing risks resulted in weak evidence for a difference in the time from virus positivity until virus clearance from the upper respiratory tract. This finding is consistent with a cohort study investigating the time to virus clearance in 2,142 COVID-19 patients, although again, that analysis did not account for the competing risk of all-cause mortality[76]. Overall, the findings presented in this thesis suggest that sustained SARS-CoV-2 RNA loads could serve as an early indicator of subsequent adverse outcomes. From a statistical point of view, a sub-distribution hazard model such as a Fine and Grey model may be used to derive estimates directly associated with the cumulative incidence function and adjust for potential confounding. This might help to investigate whether a difference in viral clearance might relate to differences in treatment between the two groups. A last observation carried forward approach was used for missing viral loads in this context in the analysis reported here and in the main manuscript. The approach assumes missingness completely at random, equality of distributions in viral loads at the last and later observations, and no change in viral load over time. These can be considered quite strong and untestable assumptions, and using a last observation carried forward approach is generally not recommended[77]. As used in the later part of this dissertation, multiple imputations and generalised linear mixed models are recommended to better handle missing data in that context.

Notably, after adjusting for sociodemographic and medical characteristics, very strong evidence for a difference in the odds of 90-day all-cause death was observed with every 10-fold increase in the lower respiratory tract and blood SARS-CoV-2 RNA loads over time in the first 30 days, but no evidence for a difference in odds was observed with every 10-fold increase in upper respiratory tract SARS-CoV-2 RNA loads over time in the first 30 days. Depending on the compartment, a 33% to 435% increase in the odds of 90-day all-cause mortality was observed with every 10-fold increase in SARS-CoV-2 RNA loads within one patient conditional on the covariates in the model, with viral loads of 7.66 $\log_{10}$ copies/ml in the upper respiratory tract, viral loads of 5.63 $\log_{10}$ copies/ml in the lower respiratory tract, and viral loads of 3.40 $\log_{10}$ copies/ml in the blood in the first 30 days associated with a 50% probability of 90-day all-cause mortality. Aligning with these findings, Munker et al. also found that SARS-CoV-2 RNA loads in the lower respiratory tract over time were associated with disease severity in a cohort of 92 patients[22]. However, all-cause mortality was not assessed and the lack of longitudinal modelling risks inflated type I errors. In another study using multinomial logistic regression in a cohort of 121 adult patients hospitalised with COVID-19 and proven viremia, every one-day increase in the duration of viremia was associated with 1.40 (95% CI: 1.02 to 1.92) times increase in the odds of dying compared to continued hospitalisation, conditional on the outcome being death or continued hospitalisation[43]. While seemingly similar in meaning, the herein presented estimates for the impact of a 10-fold increase in SARS-CoV-2 RNA loads over the first 30 days cannot be directly compared between compartments due to the non-collapsibility of the odds ratio and the use of different adjustment sets for the model for the respiratory tracts and blood.

The presented model was adjusted for baseline age, sex, body mass index, Charlson comorbidity index, and any time ARDS, mechanical ventilation, and ECMO. Given the nature of these conditions, the model was adjusted for factors on the causal pathway between the exposure and the outcome being studied. Specifically, patients with higher viral loads are more likely to develop ARDS, making them more likely to require mechanical ventilation and ECMO. Moreover, the models that examined the association between SARS-CoV-2 RNA loads over time in the first 30 days in the upper respiratory tract and lower respiratory tract and 90-day all-cause mortality were adjusted for the viremia status of the patient. Higher viral loads in the upper and lower respiratory tract arguably result in a higher likelihood of viremia, as shown by Hagmann et al.[43]. When studying the connection between exposure and outcome, removing covariates based solely on model fit statistics, such as the Akaike information criterion, is generally not advisable. Nevertheless, based on clinical knowledge, residual confounding is unlikely to occur. Adjusting time-varying variables using an ever-event status may result in losing time-varying information. Additionally, it could introduce bias by overestimating or underestimating the effect of the covariates due to misclassification and incorrect effect attribution. A detailed discussion of the impact of missing data issues in this cohort can be found in Supplementary Material 4. The generalised linear mixed model does not account for the high degree of loss of follow-up observed in the study presented here, as this model would assume that everyone is followed to the end of the follow-up period. This assumption arguably does not hold here. Thus, the presented estimates would hold for (mainly artificial) conditions in which death can be prevented. Further

research should be done using time-to-event models such as semi-parametric models with the baseline hazard function unspecified and time-varying covariates to investigate the association between the exposure and outcome of interest and account for the loss of follow-up. Survival time was estimated with hospital admission rather than ICU admission being the time origin. Because the time from hospital admission to ICU admission varies among patients based on COVID-19 severity, immortal time bias occurs, causing patients with mild disease to appear to have longer survival times if they progress during admission. Due to intermittent shortages of reagents and supplies, various assays were used for quantification. While the Cobas 6800 system was consistently used to analyse blood samples, there may have been slight deviations in the quantification of upper and lower respiratory tract samples due to the variations in assay supplies. The significant variability in RNA quantification in respiratory tract samples makes its clinical implementation challenging. Swab samples, in particular, are influenced by collection techniques and individual fluctuations, such as the potential for false high RNA loads due to cough-induced mobilisation of RNA-positive material into the upper respiratory tract during sampling. However, efforts were made to mitigate RNA load variabilities through longitudinal sampling[78]. Where single-point measurements are susceptible to measurement errors, the longitudinal nature of the study allows for the averaging out of random errors, making the results more robust.

In summary, in live patients, SARS-CoV-2 RNA loads over the first 30 days in the lower respiratory tract and blood are a strong predictor of 90-day all-cause mortality after adjusting for confounder variables, suggesting that screening for and quantifying SARS-CoV-2 RNA in the blood as part of clinical practise may help to identify individuals at risk of death and guide patient management. Individuals with high and persistent viral loads, especially in the blood, may particularly benefit from targeted interventions such as monoclonal antibody therapy or direct antiviral medications. Moreover, monitoring of viremia could be an important surrogate marker of the effectiveness of antiviral treatments. Currently, diagnostic laboratories struggle to provide reliable quantitative molecular SARS-CoV-2 diagnostics for specimens other than respiratory tract samples due to the absence of approved molecular assays. Given the findings presented herein around the predictive value of virologic blood diagnostics, there is an urgent need for the rapid evaluation and approval of blood RT-qPCRs for SARS-CoV-2. Further research is necessary to understand better how SARS-CoV-2 RNA loads in the blood contribute to mortality and the optimal testing modalities to measure them.

In corpses, with no evidence of an association between admission nasopharyngeal SARS-CoV-2 RNA loads and the postmortem interval observed and no evidence of a decrease in nasopharyngeal viral RNA loads from admission up to 168 hours after death, the remarkable stability of viral RNA raises questions about potential transmission risks during the handling of corpses by medical personnel and undertakers. In the following, it was shown that a considerable proportion of SARS-CoV-2-associated deaths remain potentially infectious long after death, with viable viruses isolated by cell cultures from nasopharyngeal samples in 20% of patients up to 13 days postmortem. While no evidence of a difference in median postmortem intervals was observed between corpses with and without positive virus culture, weak evidence for a difference in expected and observed proportions of positive virus culture results between patients with and without putrefaction changes suggests that the degradation process affects infectivity in the postmortem setting. In terms of their predictive value of standard diagnostic tests, no evidence of an association between replicable virus presence and viral RNA loads was found. Weak evidence for an association between replicable virus presence and results of AgRDTs was observed for some of the tests. The presence of replicable viruses correlated with anti-spike antibody seroconversion and anti-spike antibody levels. Moreover, the performance of serological tests on cadaveric samples warranted further investigation in the postmortem setting. High sensitivity (80-86%) and specificity (92-100%) of AgRDTs was shown for detecting SARS-CoV-2 in postmortem nasopharyngeal samples compared to RT-PCR. This suggests that AgRDTs can be valuable tools for rapid on-site detection in postmortem settings, potentially guiding safety measures for handling bodies and conducting autopsies. To confirm the findings, longitudinal studies using cell culture experiments are needed to further investigate the persistence of infectivity in corpses over time.

No evidence was found for a nonlinear monotonic association between SARS-CoV-2 RNA loads and the postmortem interval. Likewise, in a subset of SARS-CoV-2 RNA-positive corpses with short postmortem intervals, no evidence for a difference in semiquantitative SARS-CoV-2 RNA loads with every hour increase in time from admission until testing was observed. With measurements up to 168 hours after admission and single measurements after 288 hours, it remains to be seen whether these finding extends to longer intervals. A mean difference of -0.005 ct-values was found with every hour increase in time from admission. It must be noted that corpses were stored at 4°C from admission until discharge and that viral kinetics were not examined at room temperature, under which viral degradation may occur more quickly. Likewise, no evidence for a difference in ct-values with every hour increase in time from death until admission was observed when adjusting for other covariables in the model. However, only corpses with short postmortem intervals below 35 hours were included, and the findings presented here might not extend to longer postmortem intervals. An additional consideration is the potential limitations in the statistical approach.

Potentially influential values were not explored using Cook's distance or leverage. With only single measurements available after 288 hours and given the small sample size, potentially influential values may profoundly impact the estimates and inferences from the linear mixed model presented here[79,80]. Given the small number of clusters, the normality assumption for their distribution might not hold and can lead to a downward bias in the between-patient variance[79,80]. Moreover, standard errors of the fixed effects also may be biased, leading to invalid inferences[79,80]. The scaled F-test statistic suggested by Kenward and Roger accounted for the small-sample bias with unbalanced datasets for herein presented sensitivity analyses[81]. No substantial change in the inference was observed. The exclusion of SARS-CoV-2 RNA negative cases introduced selection bias towards individuals with higher viral loads yet did not substantially change inferences for the average mean difference in time from admission, as determined by sensitivity analyses with single imputed ct-values in case of measurements below the limit of detection. Because the loss to follow-up of nasopharyngeal SARS-CoV-2 RNA loads of deceased individuals with natural causes of death is assumed to be unrelated to patient characteristics as well as viral loads, missingness was assumed to be completely at random, and while a complete case analysis results in a loss of power, it does not introduce bias. No missingness in independent covariables was observed.

In a large cohort of corpses, positive virus culture was observed in 20% of SARS-CoV-2 RNA-positive corpses (95% CI: 14 to 28), suggesting that replicable virus can be found postmortem and potentially transmitted from corpses to healthcare and mortuary staff. Replicable virus was found up to 14 days postmortem, with no evidence for a difference in median postmortem intervals observed between corpses with and without successful virus isolation. Positive virus culture results were not associated with quantitative SARS-CoV-2 RNA loads (OR: 1.17, 95% CI: 0.92 to 1.42, p=0.19)[10]. However, positive virus culture results were associated with seroconversion for anti-spike antibodies and anti-spike antibody levels in a subset of corpses with blood samples available. This aligns with recent studies in live patients that describe seroconversion as predictive of infectivity as determined by virus culture[9,30,37]. Because blood samples were only obtained for corpses with a postmortem interval <48 hours and excluding patients with high comorbidity burdens, the generalisability of these findings beyond 48 hours postmortem intervals remains unclear, and the postmortem degradation of viral components may progress differentially beyond 48 hours. Notably, when comparing expected and observed proportions of putrefactive changes between corpses with and without positive virus culture, weak evidence for an association between putrefaction and positive virus culture results was found. The latter suggests that the degradation process may indeed affect infectivity in the postmortem setting, with the degradation process depending on parameters other than time from death until admission, such as environmental factors, including ambient temperature and humidity. Considering that deceased individuals in both groups were admitted within a maximum postmortem interval of 14 days, it is uncertain whether these findings can be applied to later postmortem scenarios. In addition, the infectivity observed in cell cultures cannot be considered directly representative of in vivo infectiousness[82].

SARS-CoV-2 RNA has been found in corpses for a long time after death across different studies, and a systematic review described SARS-CoV-2 RNA for up to 1.3 months in the nasopharynx and four months in the lungs of COVID-19 deceased patients[83]. Similar to the presented results herein, other studies have not found evidence for a linear association between semiquantitative viral loads and time from death until admission; however, this study did not test for a non-linear relationship or account for within-patient correlation due to repeated measurements[84]. The herein presented finding of persistent infectivity in the postmortem setting aligns with other published data, with some reports describing even higher proportions of sustained infectivity in smaller cohorts (with unclear definitions of inclusion and exclusion criteria) with greater statistical uncertainty (55%, 95% CI: 23 to 83%, n=11). In this study, the composition of postmortem intervals was similar to the herein presented study, with no infectious virus found beyond 13 days postmortem, although in other smaller cohort studies, sustained infectivity in corpses was observed with postmortem intervals of up to 17 days despite visible signs of decomposition[85,86]. It is unclear how informative data from animal studies apply to human conditions: in mice, infectivity was reduced by 96% (95% CI: 92 to 100) by day 5 and 99% (95% CI: 98 to 100) by day 14[11], however, given the small body surface, their cooling kinetics are substantially different from humans given the small body surface[87].

Viral transmission between living patients is primarily caused by contact, droplet, and aerosol transmission. Among these modes of transmission, droplet and aerosol transmission are considered the major drivers of viral spread[88]. However, in the postmortem setting, where patients do not generate droplets or aerosols, contact transmission is the primary mode of viral spread. While the major focus of this thesis was on evaluating the performance of various standard diagnostic tests in various settings, including in live and dead patients, I also contributed to several projects examining the risk of actual viral transmission in

[10] This effect measure was calculated using univariable logistic regression for a better understanding of the association between nasopharyngeal quantitative SARS-CoV-2 RNA loads and successful virus culture. Nasopharyngeal quantitative SARS-CoV-2 RNA loads were included as the dependent variable in the model. While not presented in the main manuscript, it is advisable to present effect measures and 95% confidence intervals.

[11] Normal approximated 95% confidence intervals are presented, assuming binomial standard errors are given in the manuscript.

corpses. While the studies outlined herein studied viral loads and sustained infectivity in the nasopharynx of corpses, high viral loads on and around the body may better predict the risk of infection. Schröder et al. reported that nasopharyngeal viral loads predict virus detection and loads on and around deceased bodies, suggesting that the findings presented here might also be relevant to other body sites[89]. However, while SARS-CoV-2 RNA was present, no viable virus was found on the body's skin or bags[89]. This is surprising because the high environmental stability of SARS-CoV-2 has been reported even when the virus was deprived of a suitable host[64-67]. The findings reported herein, and the implied moderate risk of infection in the postmortem setting aligns with the low 4.0% (95% CI: 0.1 to 20.4) anti-nucleocapsid antibody prevalence observed in forensic doctors and their assistants in the first year of the pandemic[12,89]. Likewise, a low 2.3% (95% CI: 0.1 to 12.0) anti-nucleocapsid antibody prevalence was observed in undertakers between 2020 and 2021[h,90]. It is important to note that these findings were obtained in a setting with advanced personal protective equipment and with autopsy halls fitted with saws with suction devices and extra ventilation. Notably, some degree of aerosol and droplet generation is expected upon conventional autopsy due to electric saws[91]. SARS-CoV-2 RNA prevalence and positive virus culture have been reported in different organ systems (based on autopsy studies), such as the pulmonary, cardiovascular, and hepatobiliary systems[92]. The controversy remains regarding whether the detection of the virus and the isolation of live viruses from various organs indicate a significant organ tropism, as proposed by Puelles et al. and others, versus potential contamination from the blood[92,93]. Nevertheless, their findings suggest that the SARS-CoV-2 may persist in the human body.

High sensitivity and specificity of different AgRDTs was found in the postmortem setting. Test sensitivities exceeded 80%, and specificities exceeded 97% for two of the three tests in the postmortem setting, thus aligning with recommendations for AgRDT usage by the Paul-Ehrlich Institute[94]. Sensitivities were even higher in corpses with positive virus culture results. In line with previous reports, true positive testing was associated with quantitative SARS-CoV-2 RNA loads for all three tests[95]. In the postmortem setting, virus loads associated with a 95% predicted probability of true positive testing were even lower than in the antemortem setting. This might be attributable to postmortem mucosal softening and higher tissue exfoliation during sample collection[95]. Importantly, no evidence for a change in the odds of true positive testing with every hour increase in the postmortem interval or between individuals with and without incipient putrefaction changes was observed for either AgRDT. Again, it is essential to note that this finding cannot necessarily be generalised to individuals with postmortem intervals beyond 14 days.

Finally, sustained postmortem infectivity was demonstrated in corpses for up to 14 days postmortem. Infectivity was mainly determined by seroconversion rather than viral loads. While no evidence of a difference in median postmortem intervals was observed between corpses with and without positive virus culture on the population level, weak evidence for a difference in expected and observed proportions of positive virus culture results between patients with and without putrefactive changes suggests that the degradation process affects infectivity in the postmortem setting. Therefore, appropriate personal protective equipment and handling of corpses with suspected COVID-19 is required as recommended by the authorities concerned[96].

In conclusion, in aggregate, the data from the studies presented in this thesis indicate that, in severe COVID-19, standard diagnostic procedures like RT-qPCR quantifying viral RNA loads, particularly from the blood, might help identify patients at increased risk of death who may particularly benefit from targeted interventions such as monoclonal antibody therapy or direct antiviral medications. In addition, while standard diagnostic procedures like RT-qPCR remain the gold standard for viral testing, rapid antigen tests have demonstrated good performance in postmortem settings and can be valuable tools for quickly evaluating infectivity in these settings.

[12] Exact binomial 95% confidence intervals are presented.

English summary

ENGLISH: This doctoral thesis examines the predictive value of standard diagnostic procedures in live and dead COVID-19 patients. In a large cohort of COVID-19 patients admitted to ICU, an association was observed between SARS-CoV-2 RNA loads in different compartments over the first 30 days after ICU admission and 90-day all-cause mortality. Patients who died had higher viral loads in the upper respiratory tract (URT) at admission but not throughout the follow-up period (p=0.14). In the lower respiratory tract (LRT), no evidence for a difference in viral loads at admission was observed between survivors and non-survivors, but higher viral loads over time were observed among the patients who died: every 10-fold increase in SARS-COV-2 RNA loads in the LRT in the first 30 days was associated with a 33% increase in the odds of 90-day all-cause mortality (p<0.0001). In the blood, higher viral loads at admission and over time were observed among patients who died: every 10-fold increase in SARS-COV-2 RNA loads in blood in the first 30 days was associated with a 434% increase in the odds of 90-day all-cause mortality (p<0.0001). Given the difference in the time to virus clearance in patients surviving and not surviving, the persistence of a high viral load, especially in the blood, could be an early indicator of adverse outcomes. Then, the infectiousness of COVID-19 was examined in deceased patients and the predictive value of standard diagnostic procedures for infectiousness. Postmortem viral stability was demonstrated through a series of experiments, which demonstrated no evidence of an association between admission nasopharyngeal SARS-CoV-2 RNA loads and the postmortem interval; the persistence of viral loads with no evidence for a decrease in nasopharyngeal viral RNA loads over postmortem intervals up to 168 hours; and, the ability to isolate viable virus by cell culture from nasopharyngeal samples in 20% of patients up to 13 days postmortem. Evidence for a difference in proportions of positive virus cultures between patients with and without putrefactive changes suggests that the degradation process affects infectivity in the postmortem setting. No evidence of an association between viral RNA loads and the presence of infectious viruses was found in corpses. Cell culture results correlated with anti-spike antibody seroconversion and anti-spike antibody levels and weak evidence of an association between AgRDT results and the presence of infectious virus was observed. Antigen rapid tests showed high sensitivity (80-86%) and specificity (92-100%) for detecting SARS-CoV-2 in postmortem nasopharyngeal samples compared to RT-PCR.

German summary

GERMAN: Diese Doktorarbeit untersucht den prädiktiven Wert von diagnostischen Verfahren in lebenden und verstorbenen COVID-19 Patienten. In einer Kohorte von COVID-19 Patienten auf der Intensivstation wurde ein Zusammenhang zwischen den Viruslasten in verschiedenen Kompartimenten in den ersten 30 Tagen nach Aufnahme und der 90-Tage-Gesamtmortalität festgestellt. Für die oberen Atemwege (URT) zeigten sich bei Aufnahme höhere Viruslasten bei Patienten mit Exitus, jedoch nicht während des Nachbeobachtungszeitraums (p=0,14). Für die unteren Atemwege (LRT) ergaben sich keine Hinweise auf einen Viruslastunterschied zwischen überlebenden und versterbenden Patienten bei Aufnahme, jedoch wurden unter Patienten mit Exitus während des Nachbeobachtungszeitraums höhere Viruslasten beobachtet: Jede 10-fache Erhöhung der Viruslast im LRT in den ersten 30 Tagen war mit einer 33% Erhöhung der Wahrscheinlichkeit der 90-Tage-Gesamtmortalität verbunden (p<0,0001). Im Blut wurden bei versterbenden Patienten höhere Viruslasten bei der Aufnahme und während des Nachbeobachtungszeitraums beobachtet: Jeder 10-fache Anstieg der Viruslast im Blut in den ersten 30 Tagen war mit einer 434% Erhöhung der Wahrscheinlichkeit der 90-Tage-Gesamtmortalität verbunden (p<0,0001). Angesichts der unterschiedlichen Zeitspanne bis zur Virusbeseitigung bei Patienten mit und ohne Exitus könnte eine anhaltend hohe Viruslast, insbesondere im Blut, als Frühindikator für das spätere Versterben dienen. Im zweiten Teil der Arbeit wurden die potenzielle Infektiosität von verstorbenen COVID-19 Patienten und der prädiktive Wert von diagnostischen Verfahren für die fortbestehende Infektiosität untersucht. Die postmortale Virusstabilität wurde durch eine Reihe von Experimenten nachgewiesen: Es wurde kein Zusammenhang zwischen nasopharyngealen Viruslast bei Leichenannahme und postmortalen Intervallen festgestellt. Auch sequenzielle nasopharyngeale Viruslasten von Annahme bis zu 168 Stunden nach Leichenannahme ergaben keine Anzeichen für eine Abnahme viraler RNA. Lebensfähige Viren konnten bei 20% der verstorbenen COVID-19 Patienten bis zu 13 Tage postmortal mittels Zellkultur aus den nasopharyngealen Proben isoliert werden. Eine schwache Evidenz für einen Unterschied in den erwarteten und beobachteten Anteilen erfolgreicher Viruskulturen zwischen Patienten mit und ohne Fäulnisveränderungen deuten darauf hin, dass der postmortale Zerfallsprozess die Infektiosität im postmortalen Umfeld beeinflusst. Es wurde kein Zusammenhang zwischen der Viruslast und dem Ergebnis der Viruskultur beobachtet. Viruskulturergebnisse korrelieren mit der Serokonversion für anti-Spike Antikörper und anti-Spike Antikörperspiegeln. Weiterhin bestand ein schwacher Zusammenhang zwischen dem AgRDT Ergebnis und dem Ergebnis der Viruskultur. Antigen-Schnelltests zeigten eine hohe Sensitivität (80-86 %) und Spezifität (92-100 %) für den Nachweis von SARS-CoV-2 in postmortalen nasopharyngealen Proben im Vergleich zur RT-PCR.

Declaration of own contribution

The MD student conducted the study conception and ethics application primarily. Literature research was largely done by the MD student. The MD student derived the study protocol as well as methods of analysis with the help of a medical statistician at the Institute of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. The MD student predominantly conducted data collection for papers 1 and 2, and entirely for paper 3 from the Institute of Medical Microbiology, Virology, and Hygiene databases. The MD student largely did data analysis and statistical programming for all three papers. The MD student largely discussed the results for all three papers, supported by medical statisticians, medical microbiologists, and forensic and intensive care medicine specialists. The manuscripts for papers 1 and 2 were largely compiled by the MD student, and for paper 3, a medical microbiologist joined the writing process. The MD student also largely did the reasoning in the discussion section.

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Thank you all for making this journey possible.

Curriculum vitae

Fabian Heinrich, DOB: 28th of February 1996

Qualification	
2022 to 2023	MSc Medical Statistics, London School of Hygiene and Tropical Medicine, United Kingdom
2014 to 2021	Medicine, University Medical Center Hamburg-Eppendorf, Germany
Prices and scholarships	
2023 to today	Scholarship from the Germany Research Foundation within the iD Fellows Clinician Scientist Programme
2017 to 2023	Scholarship from the German National Academic Foundation (Studienstiftung des Deutschen Volkes)
2022	Bronze medal of the Dr. Martini Preis
2016 to 2021	Mentoring programme for excellent students funded by the Claussen-Simon Foundation
2017-2018	Scholarship from the German Research Foundation within the Collaborative Research Centre 841

Publications as part of the doctoral thesis in reverse order of publication	Own contribution	IF (2022)
Stanelle-Bertram, S., et al., CYP19A1 mediates severe SARS-CoV-2 disease outcome in males. Cell Rep Med, 2023. 4(9): p. 101152.	C	17.0
Heinrich, F., et al., New Postmortem Perspective on Emerging SARS-CoV-2 Variants of Concern, Germany. Emerg Infect Dis, 2023. 29(3): p. 652-656.	D,L,C,A,W,I	11.8
Heinrich, F., et al., Using autopsies to dissect COVID-19 pathogenesis. Nature Microbiology, 2023. 8(11): p. 1986-1994.	D,L,C,A,W,I	21.4
Bertram, F., et al., The Mental and Physical Health of the Homeless. Dtsch Arztebl International, 2022. 119(50): p. 861-868.	D,L,C,A,W,I	8.3
Heinrich, F., et al., The Prevalence and Determinants of Viral Hepatitis Among Homeless Individuals in Hamburg. Dtsch Arztebl International, 2022. 119(1-2): p. 8-9.	D,L,C,A,W,I	8.3
Heinrich, F., et al., Dying of VOC-202012/01 — multimodal investigations in a death case of the SARS-CoV-2 variant. International Journal of Legal Medicine, 2022. 136(1): p. 193-202.	D,L,C,A,W,I	2.1
Zickler, M., et al., Replication of SARS-CoV-2 in adipose tissue determines organ and systemic lipid metabolism in hamsters and humans. Cell Metabolism, 2022. 34(1): p. 1-2.	C,I	29.0
Fitzek, A., et al., Prospective postmortem evaluation of 735 consecutive SARS-CoV-2-associated death cases. Scientific Reports, 2021. 11(1): p. 19342.	C,A	5.0
Ondruschka, B., et al., Multiorgan tropism of SARS-CoV-2 lineage B.1.1.7. International Journal of Legal Medicine, 2021. 135(6): p. 2347-2349.	D,L,C,A,W,I	2.1
Schröder, A.S., et al., The handling of SARS-CoV-2 associated deaths - infectivity of the body. Forensic Science, Medicine and Pathology, 2021. 17(3): p. 411-418.	A	1.8
Klein, A., et al., [SARS-CoV-2 incidental findings among Hamburg deaths: an epidemiological monitoring during the dynamic infection event in spring 2020]. Rechtsmedizin (Berl), 2021. 31(5): p. 427-433.	D,L,C,A,W,I	0.5
Wichmann, D., et al., Autopsy Findings and Venous Thromboembolism in Patients With COVID-19. Annals of Internal Medicine, 2020. 173(4): p. 268-277.	D,C,A	39.2
Braun, F., et al., SARS-CoV-2 renal tropism associates with acute kidney injury. The Lancet, 2020. 396(10251): p. 597-598.	C	168.9
Heinrich, F., et al., Germany's first COVID-19 deceased: a 59-year-old man presenting with diffuse alveolar damage due to SARS-CoV-2 infection. Virchows Archiv, 2020. 477(3): p. 335-339.	D,L,C,A,W,I	3.5
Klein, A., et al., [The first COVID-19 hotspot in a retirement home in Hamburg]. Rechtsmedizin (Berl), 2020. 30(5): p. 325-331.	D,L,C,A,W,I	0.5
Edler, C., et al., Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in Hamburg, Germany. Int J Legal Med, 2020. 134(4): p. 1275-1284.	C	2.1

Abbreviations: IF, impact factor; D, design; L, literature search; C, data collection; A, analysis; W, writing; I, interpretation.

Supplementary Material

Table of contents:
Supplementary materials are organized based on their occurrence in the main text to make it easier to refer to them.

- **Supplementary Material 4.** Discussion on missing data issues in the generalized linear mixed model approach for live COVID-19 patients.

Supplementary Material 1. Methods.

Methods for live COVID-19 patients

Population

The study enrolled patients diagnosed with clinically suspected or molecular-genetic confirmed COVID-19 admitted to one of 12 intensive care units in the Department of Intensive Care Medicine at the University Medical Center Hamburg-Eppendorf, Hamburg, Germany, between March 2020 and March 2021. All patients aged above 18 were eligible for inclusion; patients with multiple ICU stays were excluded. The study was presented to the Ethics Committee of the Hamburg Chamber of Physicians; due to its retrospective nature, informed consent was waived (WF-094/21). Partial data from a subset of the cohort have been previously analysed and published elsewhere (n=30/170)[45,97].

Primary outcome

All-cause mortality was the primary outcome of this study. The time origin was hospitalisation, and the time scale was days from hospitalisation to death or last follow-up. Administrative right-censoring was employed after 90 days as mortality at these later times was considered likely unrelated to the underlying COVID-19 diagnosis. The median follow-up time was calculated using the reverse Kaplan-Meier-based method[98].

Exposure

Time-updated quantified SARS-CoV-2 RNA loads in the upper respiratory tract, lower respiratory tract, and blood during the first 30 days after ICU admission were obtained from the IMMVH databases. Upper respiratory tract samples comprised nasopharyngeal swabs stored in UT (MANTACC, Shenzhen, China) or Amies (Copan, Brescia, Italy) medium. Lower respiratory tract samples comprised sputum, bronchial fluid, or bronchial lavage fluid. Blood samples comprised EDTA blood samples (Sarstedt, Nümbrecht, Germany). All samples were taken as part of routine clinical practice, collected daily from admission to the ICU until discharge or death.

Clinical characteristics

Baseline sociodemographic and medical characteristics were obtained as described in the main manuscript. The state of being immunocompromised was defined based on pre-existing medical conditions as follows: all patients diagnosed with AIDS, leukaemia, lymphoma, other malignancies, or collagenosis were considered immunocompromised and labelled as immunosuppressed.

Microbiological analysis

Quantitative reverse-transcription quantitative polymerase chain reaction (qRT-PCR)

SARS-CoV-2 RT-PCR was conducted at the IMMVH. SARS-CoV-2 RNA in respiratory specimens (upper respiratory tract [URT] and lower respiratory tract [LRT]) was detected and quantified using the commercially available assays Xpert Xpress SARS-CoV-2 (Cepheid, California, USA), Cobas SARS-CoV-2 (Roche, Mannheim, Germany), and laboratory-developed tests (LDTs) run on the cobas6800 system (Roche, Mannheim, Germany), NeuMoDx system (Qiagen, Maryland, USA), and the Light Cycler 480 II (Roche, Mannheim, Germany) as previously described[99,100]. Also, as described before, SARS-CoV-2 RNA in blood specimens was detected and quantified using the commercially available assay Cobas SARS-CoV-2 (Roche, Mannheim, Germany)[101]. Standard RNA reference material was used for quantification (INSTAND e.V., Düsseldorf, Germany). SARS-CoV-2 RNA copies/ml (y) were calculated from cycle threshold (ct)-values (x) using linear transformation formulae as follows:

Xpert Xpress SARS-CoV-2 (target: E2): $y = -0.290x + 12.83$
Cobas SARS-CoV-2 for URT and LRT (target: T2): $y = -0.308x + 13.81$
Cobas SARS-CoV-2 for blood (target: T2): $y = -0.247x + 12.27$
SARS-CoV-2 UCT (LDT, target: E2): $y = -0.291x + 12.97$
NeuMoDx (LDT, target: E2): $y = -0.425x + 14.80$
LightCycler 480 II (LDT, target: E2): $y = -0.318x + 13.32$

A threshold of 1×10^3 copies/ml was set for quantification to account for the nonlinearity of viral RNA quantification using standard RNA material with extreme values. The lower limit of detection is assay-dependent and can be found in the manufacturer protocol or the respective publications for laboratory-developed tests. Single imputation for measurements below the lower limit of quantification and limit of detection was used, with those values imputed as a SARS-CoV-2 RNA load of $1x10^2$ copies/ml and $1x10^1$ copies/ml, respectively.

Multiplexed typing polymerase chain reaction (PCR)

Nasopharyngeal swab samples were analysed to detect the circulating virus variants of concern (VOCs). Multiplexed typing PCRs were designed to detect variant-specific mutations in the SARS-CoV-2 genome. Multiplexed typing PCR performance was evaluated as previously described[102,103].

Statistical Analysis

Continuous variables were checked for approximate normality using Q-Q plots. Descriptive summary statistics were calculated. Continuous variables were summarised as the mean (SD) or median (IQR), as appropriate. Categorical variables were summarised as numbers (%). Quantified viral loads were $\log_{10}$ transformed to improve approximate normality for positively skewed data. Statistical comparisons between median SARS-CoV-2 RNA loads at admission and median peak SARS-CoV-2 RNA loads were done using a Mann-Whitney U test when the conditional normality assumption seemed violated and when a location shift was a plausible hypothesis. The independence of groups was assumed based on clinical knowledge. Expected and observed proportions of categorical variables were compared using the χ^2-test and Fisher's exact test, as appropriate.

Non-parametric methods were used to investigate the survival patterns between baseline covariates with all-cause mortality. Censoring was assumed to be uninformative about event times. To obtain Kaplan-Meier estimates for continuous variables, they were categorised using three quantiles. The percentiles defined the left-hand ends of grouping intervals and were rounded to the nearest integer values. The survival function was estimated using the Kaplan-Meier estimate, performed separately for groups. 95% confidence intervals were calculated using Greenwood's formula, assuming the normality of survival probabilities. Estimates of survival curves were plotted for visual comparison, and differences in the survival time distributions between different groups were tested using the log-rank test.

Non-parametric methods were used to investigate the virus clearance patterns between patients who were alive at the end of follow-up and those who were not. Censoring was assumed to be uninformative about event times. The competing risk of all-cause mortality was neglected in the analysis in the main manuscript. Patients who died were censored, and cumulative incidence functions of viral clearance were derived through the cause-specific hazard function as Kaplan-Meier estimated survival probabilities for the event of interest are downward biased in the presence of competing risk[104]. A weighted log-rank test was used to compare cause-specific cumulative incidence functions, assuming a joint censoring distribution for survivors and non-survivors[105]. Successful RNA clearance was defined as the absence of SARS-CoV-2 RNA from the respective compartment for at least three days. For missing data, a last observation carried forward approach was used.

The method section in the main manuscript described the use of a generalised linear mixed model with the use of a binomial outcome distribution and canonical link function (identity link) to examine whether upper respiratory tract SARS-CoV-2 RNA loads, lower respiratory tract SARS-CoV-2 RNA loads and blood SARS-CoV-2 RNA loads over time in the first 30 days are predictive of 90-day all-cause mortality in COVID-19 patients admitted to the ICU. The 90-day survival status served as the dependent variable. SARS-CoV-2 RNA loads ($\log_{10}$ copies/ml) in each compartment were used as independent variables. Viral load measurements below the lower limit of quantification and lower limit of detection were excluded. Potential confounding variables for the model included age [continuous, years], sex [binary, make or female], body mass index [continuous, kg/m^2], Charlson comorbidity index [continuous, 1-12], the presence of ARDS [binary, yes or no], the need for mechanical ventilation [binary, yes or no], and the need for extracorporeal membrane oxygenation [binary, yes or no]. These were included on a clinical basis. In the models examining the effect of upper respiratory tract and lower respiratory tract viral load on all-cause mortality, viremia status [binary, yes or no] was additionally included as an independent variable. A linear functional form of all continuous variables in the models on the log odds of 90-day all-cause mortality was assumed. A random effect model with random patient intercept and time slope was used with a first-order autoregressive variance-covariance structure. Backwards variable selection was performed using a stepwise approach based on the Akaike Information Criterion. Model optimisation was done for the lower respiratory tract, and the model was subsequently transferred without further adjustments to the other compartments to ensure comparability. Restricted maximum likelihood estimation was used to fit the model due to the unbalanced nature of the data. Firth's bias correction was used due to collinearity issues between mechanical ventilation and ECMO status[13].

Methods for deceased COVID-19 patients

Population

All corpses admitted to the Institute of Legal Medicine (ILM), University Medical Center Hamburg-Eppendorf, Hamburg, Germany, were swabbed for SARS-CoV-2 RNA testing using UTM swabs (MANTACC, Shenzhen, China). Nasopharyngeal swabs were taken by medically qualified staff according to current standards[106]. Study phase 1 (March 22nd to May 1st, 2020) included a consecutive sample of 79 SARS-CoV-2 RNA-positive corpses to examine the correlation between semiquantitative SARS-CoV-2 RNA loads and the postmortem interval. A consecutive subset of 11/79 corpses with short postmortem intervals (<35 hours) was used to examine semiquantitative SARS-CoV-2 RNA trajectories, and a consecutive subset of 6/79 corpses that underwent conventional autopsy was used to investigate

[13] Indications of divergences from the original statistical analysis plan were noted, but with the trial statistician having changed companies, tracing back the specific actions taken is impossible. Therefore, the subsequent analysis was interpreted as if the original analysis plan had been followed.

surrogates of sustained infectivity. Corpses exhibiting advanced putrefactive changes, defined as mummification or marbling, were excluded from the study. Study phase 2 (November 1st, 2020, to February 28th, 2021) included a consecutive sample of 128 SARS-CoV-2 RNA-positive corpses for virus culture and to evaluate AgRDT sensitivity. In the first month of the study, a consecutive sample of 72 SARS-CoV-2 RNA-negative corpses was included to assess AgRDT specificity. Corpses with postmortem intervals exceeding 14 days from death until admission and/ or advanced putrefaction changes defined as marbling or mummification were excluded from the study. Corpses were stored at 4°C upon admission. Informed consent was obtained from relatives or legal representatives. The Hamburg Chamber of Physicians ethics committee approved the study (reference numbers PV7311 and 2020-10353-BO-ff).

Primary outcome
Time-updated nasopharyngeal and pulmonary SARS-CoV-2 RNA loads were a primary outcome of the first study phase. Nasopharyngeal and pulmonary swabs were repeatedly performed on a subset of SARS-CoV-2 RNA-positive corpses. Nasopharyngeal swabs were taken at fixed time points, as described above, namely 0, 12, 24, 36, 48, 60, 72, 96, and 168 hours after admission to the ILM. Likewise, pulmonary swabs were taken at the same fixed time points before the conventional autopsy using thoracic puncture and after the conventional autopsy by directly swabbing the lungs. From admission until discharge, corpses and ex-situ lungs were stored in the cooling chamber at 4°C. Replicable virus presence at admission was the primary outcome of the second study phase.

Exposure
Sociodemographic characteristics and characteristics related to the decay process of corpses, including the time from death to admission, the time from admission to testing, and minor putrefactive changes. Standard diagnostic test results.

Clinical characteristics
Sociodemographic and medical data were collected from the death certificate and medical records. Data on corpse storage were collected from institutional databases. Antemortem and postmortem virological data were collected from databases from the Institute of Medical Microbiology, Virology, and Hygiene (IMMVH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Forensic pathology analysis
Full autopsies and tissue sample collection
Full autopsies were conducted following the recommendations of the German Society of Legal Medicine with special consideration of the guidelines on handling COVID-19 deaths[107,108]. Native tissue samples of 1 cm^3 were collected using separate scalpels and forceps for each sample, snap-frozen at -127°C, and stored at -80°C until further analysis. Tissue samples were prepared for further analysis as described elsewhere[93].

Microbiological analysis
Quantitative reverse-transcription quantitative polymerase chain reaction (qRT-PCR)
SARS-CoV-2 RT-PCR was conducted at the IMMVH. As described above, SARS-CoV-2 RNA was detected and quantified using commercially available and laboratory-developed tests (LDTs). In study phase 1, the analysis approach described by Pfefferle et al. was used only. SARS-CoV-2 RNA copies/ml were calculated from ct-values using the linear transformation formulae above. The nonlinearity of viral RNA quantification using standard RNA material with extreme values remained unconsidered in all analyses. The lower limit of quantification and lower limit of detection are assay-dependent and can be found in the manufacturer protocol or the respective publications for laboratory-developed tests. Single imputation was used for measurements below the lower limit of detection, with those values imputed as a cycle threshold of 50 in the first study phase. Cycle thresholds inversely correlate with viral loads.

Subgenomic ribonucleic acid polymerase chain reaction (sg-RNA PCR)
Subgenomic RNA loads were determined using previously described methods[9,109]. Briefly, the N-gene subgenomic RNA gene was quantified by combing a 5-UTR region primer (leader sequence, sgLead-SARSCoV2-F; 5-CGATCTCTTGTAGATCTGTTCTC-3′13) with a reverse primer (2019-nCoV_N1-R, 5′-TCT GGT TAC TGC CAG TTG AAT CTG-3′) and probe (2019-nCoV_N1-P, 5′-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3′) binding to the nucleotide gene[109]. Positive and negative controls were included, with SARS-CoV-2 infected and non-infected Vero cells as controls[109].

Virus culture
Vero E6 cells were maintained and cultivated under standard conditions. 500 µL of swab medium was used to infect the cells. After 72 hours of incubation at 37°C, supernatants were harvested, and virus growth was analysed using RT-qPCR as previously described[110,111].

Antigen-detecting rapid diagnostic tests (Ag-RDTs)

Three antigen-detecting rapid diagnostic tests were used according to manufacturer recommendations. Two independent examiners carried out readouts of antigen-detecting rapid diagnostic tests by visual inspection. The following antigen-detecting rapid diagnostic tests were used: Test 1 (Panbio™ COVID-19 Ag Rapid Test Device, Abbott, Chicago, USA), Test 2 (SARS-CoV-2 Rapid Antigen Test, Roche Diagnostics Deutschland GmbH, Mannheim, Germany), and Test 3 (MEDsan® SARS-CoV-2 Antigen Rapid Test, MEDsan GmbH, Hamburg, Germany). Further test specifications are given in the supplementary table of the main manuscript. All assays were listed by the Paul-Ehrlich Institute as meeting the requirements of the common list of coronavirus antigen rapid tests[94,112].

Serological testing using enzyme-linked immunosorbent assay (ELISA)
Blood samples were analysed using ELISA to detect antibodies specific to SARS-CoV-2. The quantitative detection of antibodies against SARS-CoV-2 spike protein was done using Elecsys Anti-SARS-CoV-2-S (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Qualitative detection of antibodies against SARS-CoV-2 nucleocapsid protein was done using Elecsys Anti-SARS-CoV-2 (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). All tests were conducted as recommended by the manufacturer on the Cobas e411 (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). When appropriate, patient sera were manually diluted in 1:100 increments to determine SARS-CoV-2 antibody titres. Cut-offs for antibody positivity were according to manufacturer recommendations.

Statistical analysis
Categorical variables were inspected with tables and summarised in numbers and percentages. Continuous variables were inspected with summary statistics and scatter plots and were summarised with the mean and standard deviation (SD) or the median and interquartile range (IQR) as appropriate. Continuous variables were inspected for approximate normality using histograms and Q-Q plots. All variables were inspected for missing values. Exact binomial 95% confidence intervals were calculated for test sensitivity and specificity. Spearman's correlation coefficient was used to examine the relationship between two continuous variables, where a monotonic and non-linear relationship was a plausible assumption. Moreover, this was done to increase robustness to potentially outlying values. A Wilcoxon signed-rank test was used to test for a median difference between antemortem and postmortem SARS-CoV-2 RNA loads where substantial violations of the normality assumption were observed while accounting for subject blocks relying on a continuous distribution. A Mann-Whitney U test was conducted to compare medians of continuous variables between two independent groups where substantial violations of the normality assumption were observed, and a location shift was plausible. The χ^2 or Fisher's exact test were used to formally assess the null hypothesis of no difference in expected and observed counts between groups, depending on expected cell counts above or below 5 (χ^2 test was conducted where >5).

In the first study phase, a linear mixed model was used to investigate postmortem SARS-CoV-2 RNA load trajectories in a subset of corpses with a short time from death until admission. The dependent variable was semiquantitative SARS-CoV-2 RNA loads as determined from cycle-threshold values from RT-qPCR. Absolute quantified SARS-CoV-2 RNA loads were not available at that time. A clinically saturated model, including all covariables clinically relevant to the exposure-outcome relationship, was used to explore an appropriate random effect structure for the model. Postmortem interval [continuous, hours from death until admission], time from admission [continuous, hours from admission until measurement], sampling location [binary, nasopharynx or lung], and time-varying conventional autopsy status [binary, yes or no] were included as independent covariables in the model. Linear functional forms of continuous independent covariates were assumed with the aim of a parsimonious model. An interaction was fitted between sampling location and time-varying conventional autopsy status. Time-varying confounding was assumed by conventional autopsy status without exposure-confounder feedback. Thus, regression adjustment was deemed sufficient to adjust for confounding. Using likelihood ratio tests, the fit of a linear regression and a random intercept model were compared with the fixed-effect component of the model unchanged. Measurements below the limit of detection, sequential, were and were not excluded from sensitivity analyses. Because the dataset was not balanced, restricted maximum likelihood estimation was used to overcome the potential downward bias in estimating the within-patient variance. Linear combinations of coefficients were obtained using the estimated variance-covariance matrix from the model.

In the second study phase, a generalised linear model was used to investigate factors associated with true AgRDT positivity. A generalised linear model with a binomial response distribution and its canonical link function (logit link) was used to investigate the association between patient baseline characteristics and true positive testing in the cohort of SARS-CoV-2 RNA-positive corpses. Test outcome was used as the dependent variable in the model. Patient baseline clinical covariates were included on a clinical basis, separately for univariable models and altogether for multivariable models. Postmortem interval [continuous; hours], $\log_{10}$ SARS-CoV-2 RNA load [continuous; copies/ml], and putrefactive changes [binary; yes or no] were included as independent covariables in the model. Linear functional forms of continuous independent covariates were assumed, aiming for a parsimonious model. No interaction term was assumed. Quantified viral loads were $\log_{10}$ transformed to improve approximate normality for positively skewed data. The

estimates were transformed to the odds ratio scale. Model-based predicted probabilities of true positive testing were obtained. 95% confidence intervals were obtained on the linear scale and transformed into the probability space.

For all studies, a significance level of 0.05 was defined for all statistical analyses. Statistical analyses were conducted in STATA/MP 18.0 (StataCorp LLC, Texas, USA) and SAS 9.4 (SAS Institute Inc., North Carolina, USA). GraphPad Prism Version 10.2.3 (GraphPad Software, California, USA) and Adobe Illustrator (Adobe, California, USA) were used for data illustration.

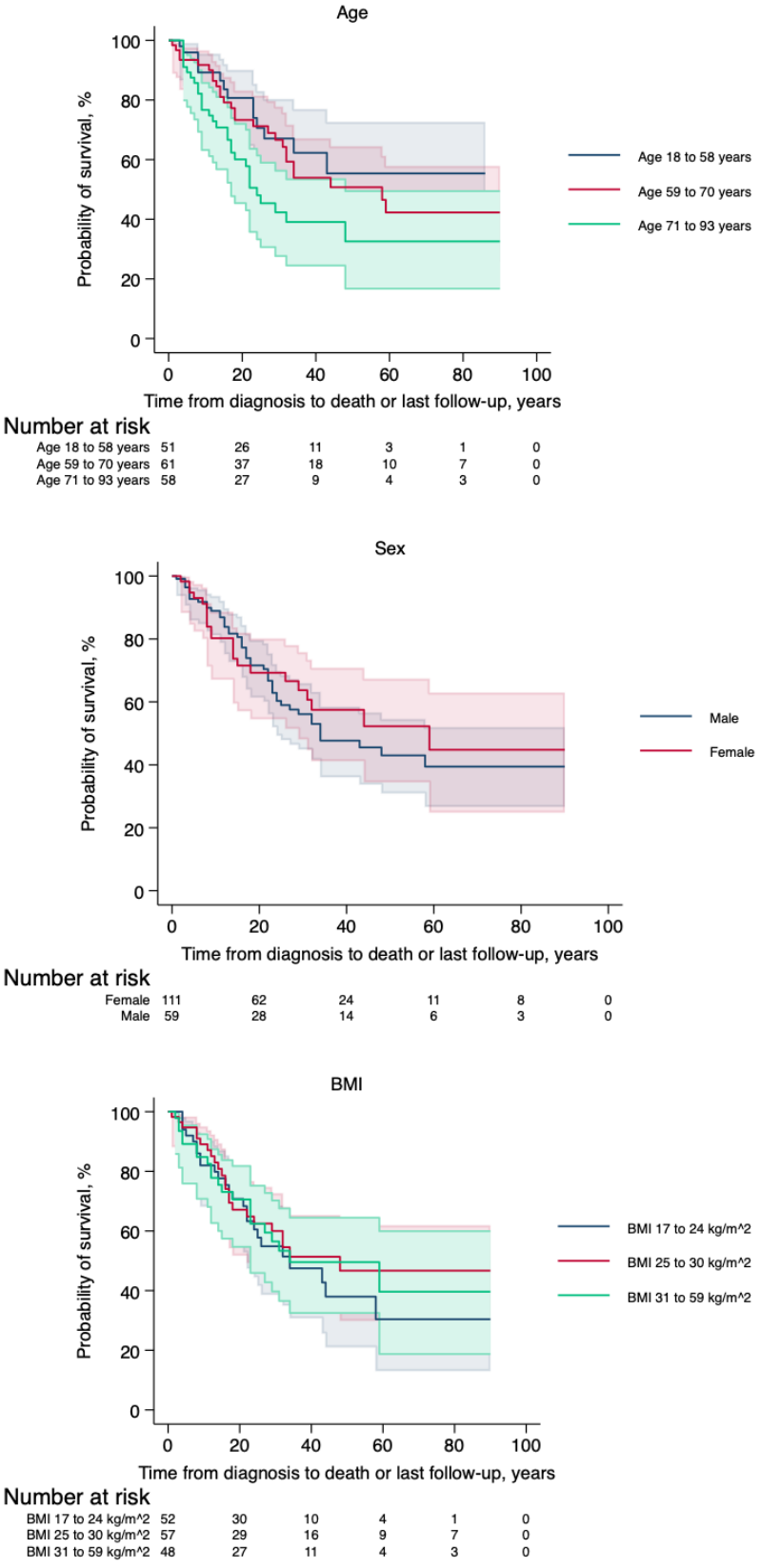
Supplementary Table 1. Baseline characteristics of live COVID-19 patients cohort stratified by 90-day all-cause mortality.

	Survivors Numbers (%) or median (IQR)	**Non-survivors** Numbers (%) or median (IQR)	**Total** Numbers (%) or median (IQR)
	N=100	N=70	N=170
Age, years*	60.0 (51.0-72.0)	66.5 (59.0-76.0)	63.0 (55.0-73.0)
Sex			
Male	63 (63.0%)	48 (68.6%)	111 (65.3%)
Female	37 (37.0%)	22 (31.4%)	59 (34.7%)
BMI, kg/m^{2}*	28.1 (24.8-31.6)	26.3 (24.2-31.9)	27.3 (24.5-31.9)
Comorbidities			
Chronic lung disease	13 (13.0%)	11 (15.7%)	24 (14.1%)
Diabetes mellitus	35 (35.0%)	23 (32.9%)	58 (34.1%)
Arterial hypertension	55 (55.0%)	43 (61.4%)	98 (57.6%)
History of myocardial infarction	14 (14.0%)	17 (24.3%)	31 (18.2%)
History of heart failure	13 (13.0%)	11 (15.7%)	24 (14.1%)
Peripheral arterial disease	2 (2.0%)	6 (8.6%)	8 (4.7%)
Cerebrovascular disease	16 (16.0%)	13 (18.6%)	29 (17.1%)
Dementia	1 (1.0%)	2 (2.9%)	3 (1.8%)
Gastric ulcer	6 (6.0%)	1 (1.4%)	7 (4.1%)
Mild liver disease	0 (0.0%)	1 (1.4%)	1 (0.6%)
Moderate to severe liver disease	2 (2.0%)	0 (0.0%)	2 (1.2%)
Hemiplegia	2 (2.0%)	3 (4.3%)	5 (2.9%)
Moderate to severe kidney disease	17 (17.0%)	10 (14.3%)	27 (15.9%)
Immunosuppression	23 (23.0%)	28 (40.0%)	51 (30.0%)
Charlson comorbidity index at admission*	1.0 (1.0-3.0)	2.0 (1.0-4.0)	2.0 (1.0-3.0)
COVID-19 disease characteristics			
Time from COVID-19 diagnosis until ICU admission*	3.0 (1.0-8.0)	7.0 (1.0-13.0)	4.0 (1.0-10.0)
SAPS II score at admission*	37.0 (30.0-43.0)	42.0 (37.0-52.0)	40.0 (32.0-47.0)
SOFA score at admission*	5.0 (3.0-11.0)	10.0 (5.0-13.0)	7.0 (3.0-12.0)
ARDS at any time	50 (50.0%)	63 (90.0%)	113 (66.5%)
ARDS severity among patients with ARDS			
Mild	2 (4.1%)	1 (1.7%)	3 (2.8%)
Moderate	14 (28.6%)	9 (15.0%)	23 (21.1%)
Severe	33 (67.3%)	50 (83.3%)	83 (76.1%)
COVID-19 treatment characteristics			
High-flow nasal oxygen at any time	36 (36.0%)	25 (35.7%)	61 (35.9%)
Non-invasive ventilation at any time	22 (22.0%)	21 (30.0%)	43 (25.3%)
Mechanical ventilation at any time	57 (57.0%)	65 (92.9%)	122 (71.8%)
Time of ventilation among patients on MV, days*	16.0 (8.0-41.0)	13.0 (6.0-24.0)	14.5 (7.0-29.0)
Tracheotomy at any time	26 (26.0%)	17 (24.3%)	43 (25.3%)
Renal replacement therapy at any time	28 (28.0%)	52 (74.3%)	80 (47.1%)
ECMO at any time	17 (17.0%)	32 (45.7%)	49 (28.8%)

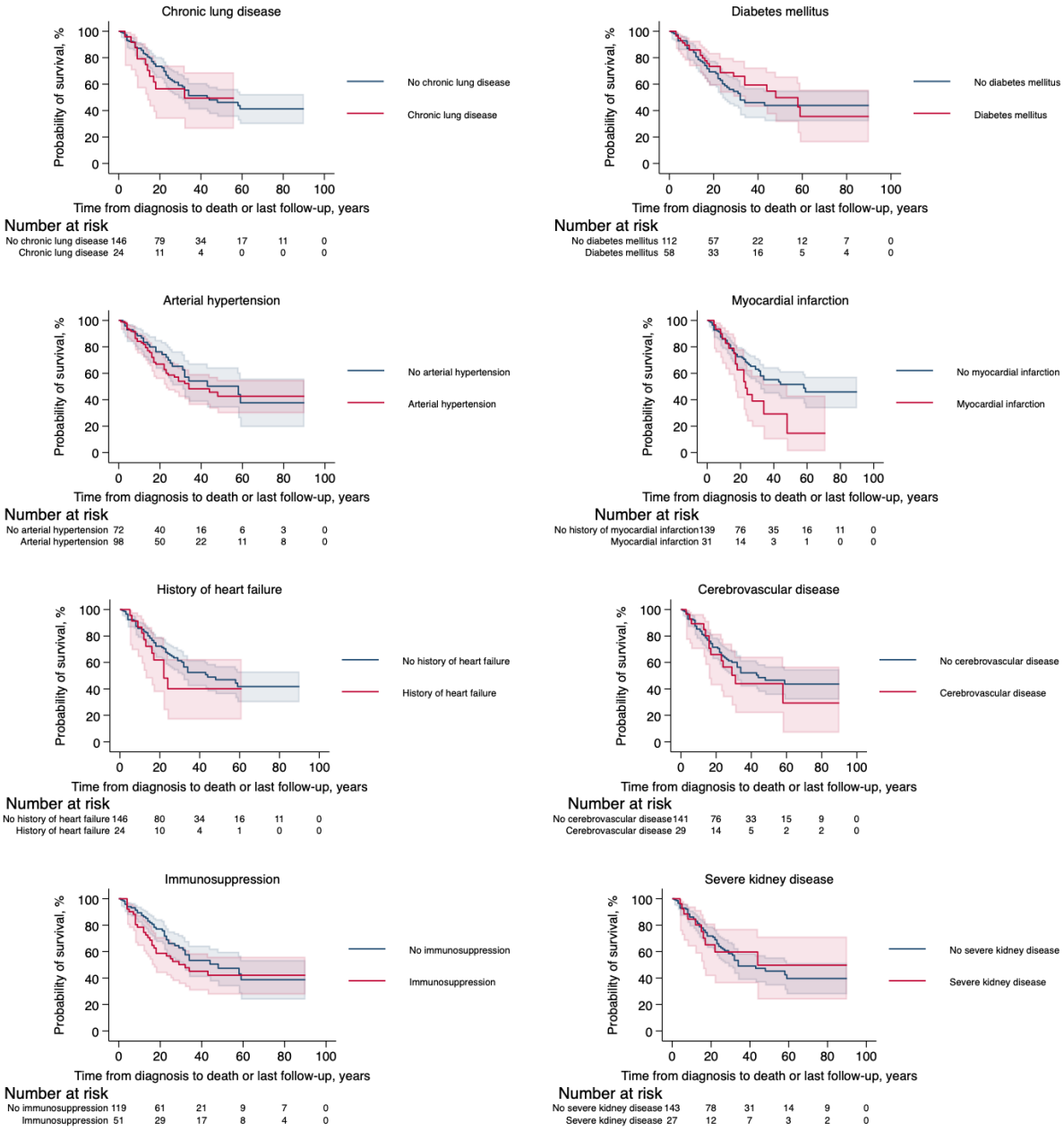
Vasopressors at any time	66 (66.0%)	68 (97.1%)	134 (78.8%)
Dexamethasone at any time	39 (39.0%)	35 (50.0%)	74 (43.5%)
Remdesivir at any time	22 (22.0%)	11 (15.7%)	33 (19.4%)
Antibody therapy at any time	0 (0.0%)	3 (4.3%)	3 (1.8%)
Plasmapheresis at any time	3 (3.0%)	3 (4.3%)	6 (3.5%)

*The median and IQR are provided in cases of high skewness or kurtosis. **Abbreviations:** BMI, body mass index; ICU, intensive care unit; COVID-19, coronavirus disease-2019; SAPS II score, simplified acute physiology score II; SOFA, sequential organ failure assessment; MV, mechanical ventilation; ECMO, extracorporeal membrane oxygenation.

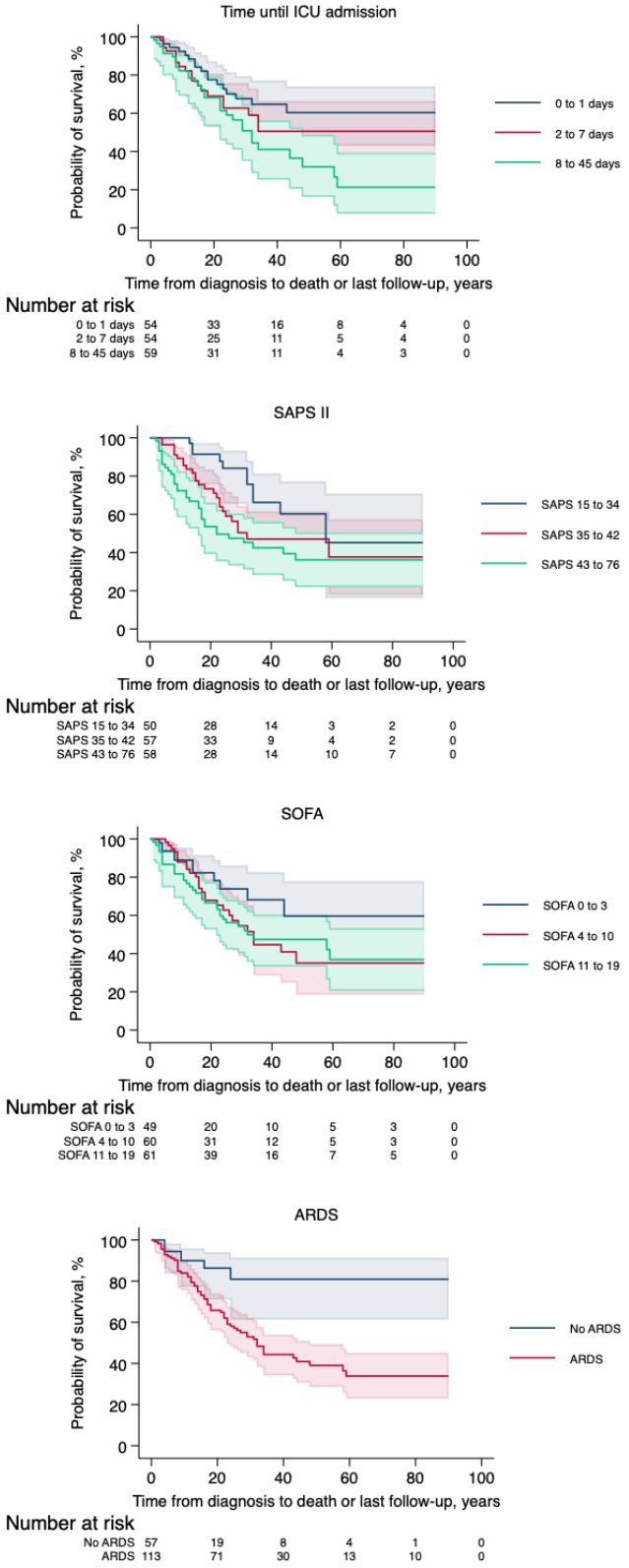
Supplementary Figure 1. Kaplan-Meier estimated survival curves with 95% confidence intervals in live COVID-19 patients. The number of patients in follow-up is illustrated in risk tables below the graph stratified by group.



Supplementary Figure 2. Kaplan-Meier estimated survival curves with 95% confidence intervals in live COVID-19 patients. The number of patients in follow-up is illustrated in risk tables below the graph stratified by group.

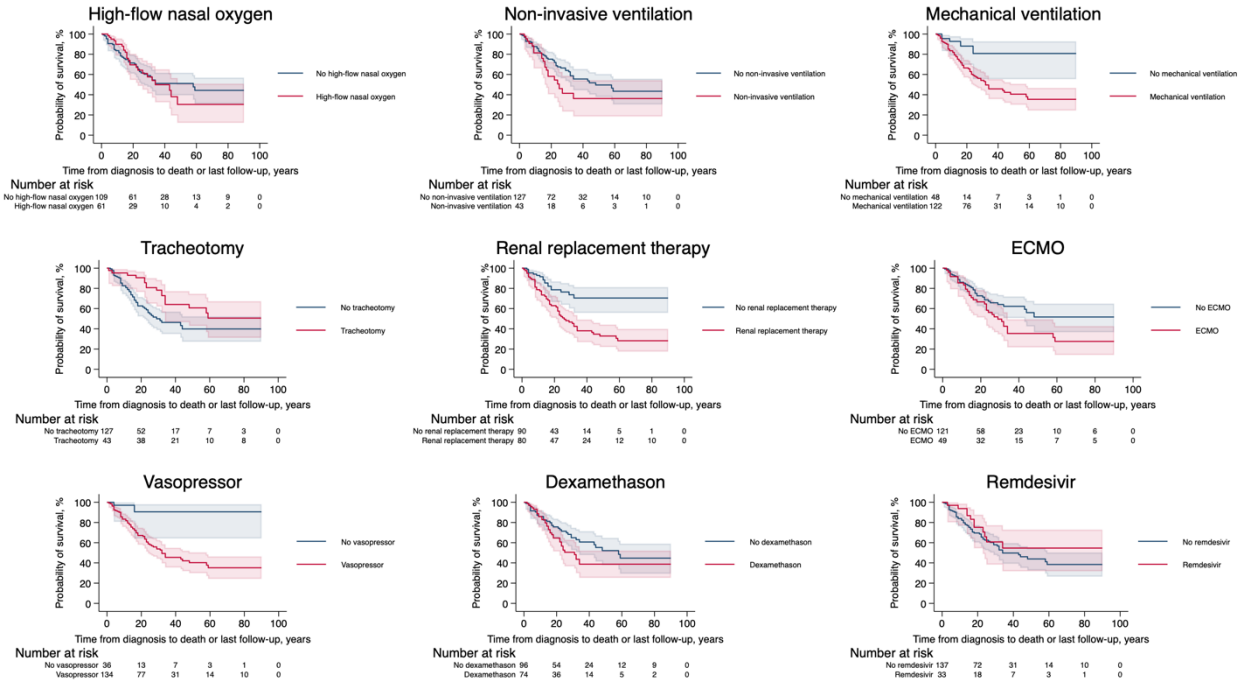


Supplementary Figure 3. Kaplan-Meier estimated survival curves with 95% confidence intervals in live COVID-19 patients. The number of patients in follow-up is illustrated in risk tables below the graph stratified by group.



Abbreviations: ICU, intensive care unit; SAPS II, simplified acute physiology score II; SOFA, sequential organ failure assessment score; ARDS, acute respiratory distress syndrome.

Supplementary Figure 4. Kaplan-Meier estimated survival curves with 95% confidence intervals in live COVID-19 patients. The number of patients in follow-up is illustrated in risk tables below the graph stratified by group.



Abbreviations: ECMO, extracorporeal membrane oxygenation.

Supplementary Material 2. A detailed description of missingness proportions, missingness patterns, and assumed missingness mechanisms in SARS-CoV-2 RNA loads in different compartments in live COVID-19 patients.

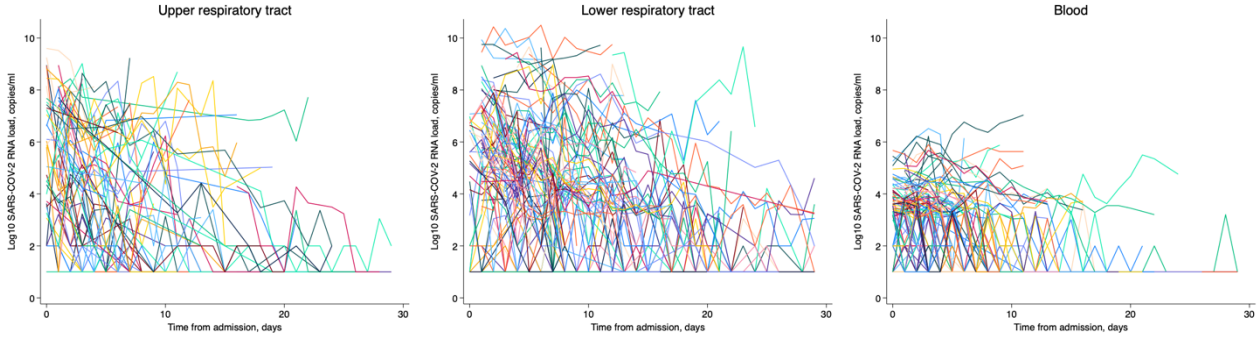
On average, patients had four samples of SARS-CoV-2 RNA loads taken from the upper respiratory tract throughout the observation period (SD: 4.45). In total, 20 patients had equal to or more than ten observations available (12%), 88 patients had equal to or more than 2/30 observations available (52%), and 55 cases had no observations available (32%). When investigating the pattern of missingness for upper respiratory tract data, the pattern was inversely monotone, with most patients having initial but not later observations missing. Missingness was assumed completely at random for the analyses. On average, patients had nine samples of SARS-CoV-2 RNA loads taken from the lower respiratory tract throughout the observation period (SD: 9.18). In total, 64 patients had equal to or more than ten observations available (38%), 108 patients had equal to or more than 2/30 observations available (64%), and 44 cases had no observations available (26%). When investigating the pattern of missingness for lower respiratory tract data, the pattern was inversely monotone, with most patients having initial but not later observations missing. Missingness was assumed completely at random for the analyses. On average, patients had 12 SARS-CoV-2 RNA loads in the blood available throughout the observation period (SD: 9.22). In total, 86 patients had equal to or more than ten observations available (51%), 155 patients had equal to or more than 2/30 observations available (91%), and 10 cases had no observations available (6%). When investigating the pattern of missingness for blood sample data, the pattern was inversely monotone, with most patients having initial but not later observations missing. Missingness was assumed completely at random for the analyses.

Supplementary Table 2. Number and proportion of missing baseline covariates in this cohort of COVID-19 patients admitted to the ICU at the University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

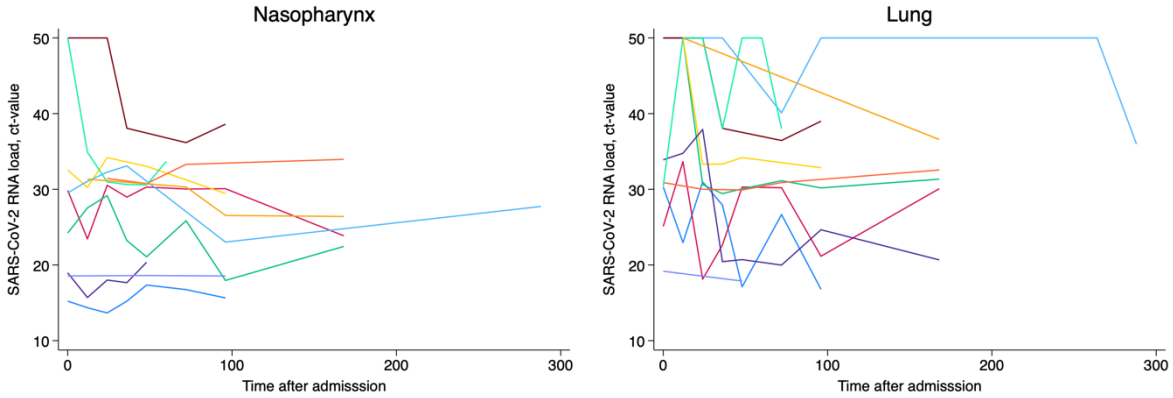
Variable	**Missing**, n	**Total number**	**Missing**, %
Age	0	170	0.00
Sex	0	170	0.00
BMI	13	170	7.65
Chronic lung disease	0	170	0.00
Diabetes mellitus	0	170	0.00
Arterial hypertension	0	170	0.00
History of myocardial infarction	0	170	0.00
History of heart failure	0	170	0.00
Peripheral arterial disease	0	170	0.00
Cerebrovascular disease	0	170	0.00
Dementia	0	170	0.00
Gastric ulcer	0	170	0.00
Mild liver disease	0	170	0.00
Moderate to severe liver disease	0	170	0.00
Hemiplegia	0	170	0.00
Moderate to severe kidney disease	0	170	0.00
Immunosuppression	0	170	0.00
Charlson comorbidity index at admission	0	170	0.00
Time from COVID-19 diagnosis until ICU admission	1	170	0.59
SAPS II score at admission	5	170	2.94
SOFA score at admission	0	170	0.00
ARDS at any time	0	170	0.00
ARDS severity among patients with ARDS	61	170	35.88
High-flow nasal oxygen at any time	0	170	0.00
Non-invasive ventilation at any time	0	170	0.00
Mechanical ventilation at any time	0	170	0.00
Time of ventilation among patients on MV	47	170	27.65
Tracheotomy at any time	0	170	0.00
Renal replacement therapy at any time	0	170	0.00
ECMO at any time	0	170	0.00
Vasopressors at any time	0	170	0.00
Dexamethasone at any time	0	170	0.00
Remdesivir at any time	0	170	0.00
Antibody therapy at any time	0	170	0.00
Plasmapheresis at any time	0	170	0.00

Abbreviations: BMI, body mass index; ICU, intensive care unit; SAPS II, simplified acute physiology score 2; SOFA, sequential organ failure assessment; ARDS, acute respiratory distress syndrome; MV, mechanical ventilation; ECMO, extracorporeal membrane oxygenation.

Supplementary Figure 5. Individual SARS-CoV-2 RNA trajectories from the time of ICU admission in the upper respiratory tract, lower respiratory tract, and blood are shown.



Supplementary Figure 6. Individual semiquantitative nasopharyngeal and pulmonary SARS-CoV-2 RNA load trajectories from admission are illustrated over time in deceased COVID-19 patients.



Supplementary Material 3. Detailed description of linear mixed model results in deceased COVID-19 patients.

With every hour increase from death until admission, a mean difference of 0.05 (95% CI: -0.41 to 0.52) in ct-values was found when adjusting for other covariables in the model, with no evidence for a difference in ct-values with hourly increase when adjusting for other covariables in the model (p=0.83). A mean difference of 7.41 (95% CI: 4.51 to 10.32) in ct-values was found when comparing semiquantitative SARS-CoV-2 RNA loads in the nasopharynx and lung in deceased individuals who had not yet undergone conventional autopsy when adjusting for covariables in the model; there was very strong evidence for this difference in ct-values between anatomical locations when adjusting for covariables in the model, with higher ct-values observed in the lungs (p<0.001). When comparing nasopharyngeal semiquantitative SARS-CoV-2 RNA loads in individuals who did and who had not yet undergone conventional autopsy, a mean difference of 1.48 (95% CI: -1.31 to 4.27) in ct-values was found when adjusting for covariables in the model, but without evidence that this difference in ct-values was statistically significant when adjusting for covariables in the model (p=0.30). When comparing pulmonary semiquantitative SARS-CoV-2 RNA loads in individuals who did and who had not yet undergone conventional autopsy, a mean difference of -3.41 (95% CI: -6.48 to -0.33) in ct-values was found when adjusting for covariables in the model, with moderate evidence for a difference in these ct-values when adjusting for covariables in the model, with lower pulmonary ct-values observed in individuals who had undergone autopsy (p=0.03).

Supplementary Table 3. Linear mixed model with semiquantitative SARS-CoV-2 RNA loads throughout the study included as the dependent variable in the model. SARS-CoV-2 RNA loads below the limit of detection were excluded from the analysis. Measurements below the lower limit of detection were single-imputed as a ct-value of 50.

	Average mean difference	95% CI	P-value
Time after admission	-0.002	-0.03 to 0.02	0.87
Location (Ref: Nasopharynx)	11.16	8.06 to 14.26	<0.001
PMI, hours	0.10	-0.51 to 0.71	0.74
Time-varying conventional autopsy status	-1.28	-4.80 to 2.24	0.48
Location x Time-varying conventional autopsy status	-7.08	-11.11 to -3.05	0.001
Random effects	**Coefficient***	**95% CI**	
Between patient variance			
Patient intercept	71.07	27.02 to 186.95	
Within patient variance			
Residual variance	32.37	24.97 to 41.96	

Abbreviations: 95% CI, 95% confidence interval; PMI, postmortem interval (defined as the time from death until admission). "x" denotes interaction terms. *If not stated otherwise, coefficients refer to the variance.

Supplementary Table 4. Linear mixed model with semiquantitative SARS-CoV-2 RNA loads throughout the study included as the dependent variable in the model. SARS-CoV-2 RNA loads below the limit of detection were excluded from the analysis. Small-sample bias correction was applied as suggested by Kenward and Roger (Kenward MG, Roger JH. Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. Biometrics 1997; 53(3): 983-97).

	Average mean difference	**95% CI**	**P-value**
Time after admission	-0.005	-0.02 to 0.01	0.58
Location (Ref: Nasopharynx)	7.41	4.46 to 10.32	<0.001
PMI, hours	0.05	-0.49 to 0.59	0.83
Time-varying conventional autopsy status	1.48	-1.36 to 4.32	0.30
Location x Time-varying conventional autopsy status	-4.89	-8.39 to -1.38	0.01
Random effects	**Coefficient***	**95% CI**	
Between patient variance			
Patient intercept	40.89	15.38 to 108.73	
Within patient variance			
Residual variance	16.45	12.32 to 21.96	

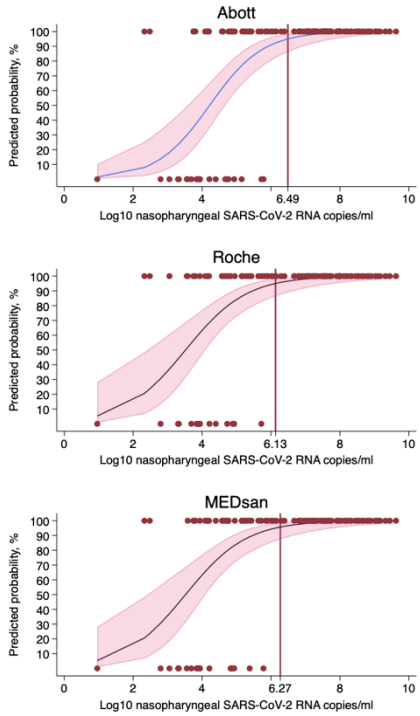
Abbreviations: 95% CI, 95% confidence interval; PMI, postmortem interval (defined as the time from death until admission). "x" denotes interaction terms. *If not stated otherwise, coefficients refer to the variance.

Supplementary Table 5. Linear mixed model with semiquantitative SARS-CoV-2 RNA loads throughout the study included as the dependent variable in the model. SARS-CoV-2 RNA loads below the limit of detection were excluded from the analysis. Measurements below the lower limit of detection were single-imputed as a ct-value of 50. Small-sample bias correction was applied as suggested by Kenward and Roger (Kenward MG, Roger JH. Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. Biometrics 1997; 53(3): 983-97).

	Average mean difference	**95% CI**	**P-value**
Time after admission	-0.002	-0.03 to 0.02	0.87
Location (Ref: Nasopharynx)	11.16	8.02 to 14.29	<0.001
PMI, hours	0.10	-0.61 to 0.81	0.75
Time-varying conventional autopsy status	-1.28	-4.85 to 2.28	0.48
Location x Time-varying conventional autopsy status	-7.08	-11.15 to -3.00	0.001
Random effects	**Coefficient***	**95% CI**	
Between patient variance			
Patient intercept	71.07	27.02 to 186.95	
Within patient variance			
Residual variance	32.37	24.97 to 41.96	

Abbreviations: 95% CI, 95% confidence interval; PMI, postmortem interval (defined as the time from death until admission). "x" denotes interaction terms. *If not stated otherwise, coefficients refer to the variance.

Supplementary Figure 7. The model predicted probabilities and 95% confidence intervals. 95% confidence intervals were obtained on a linear scale and transformed into the probability space. This figure was adapted from the main manuscript.



Abbreviations: SARS-CoV-2 RNA, Severe acute respiratory distress syndrome coronavirus-2 ribonucleic acid.

Supplementary Material 4. Discussion on missing data issues in the generalized linear mixed model approach for live COVID-19 patients.

Generally, a high proportion of missing data was encountered in SARS-CoV-2 RNA loads over time, with, on average, four observations available from the upper respiratory tract, nine from the lower respiratory tract, and 12 from the blood. The missingness pattern was inverse monotonic, with earlier observations often missing and later observations consecutively observed. The generalised linear mixed model with random intercepts and time slopes is an optimal approach to deal with such a scenario. Exclusion of SARS-CoV-2 RNA loads below the lower limit of detection and quantification likely introduces selection bias towards individuals with higher SARS-CoV-2 RNA loads. The intensive care medicine department indicated that they aimed to obtain upper respiratory tract, lower respiratory tract, and blood samples as part of their daily clinical routine for every patient, regardless of COVID-19 severity. Missingness completely at random was assumed for SARS-CoV-2 RNA loads, and consequently, a complete case analysis was employed. However, as sampling from the lower respiratory tract, for instance, involves the need for medical interventions that are likely not done when there is no individual benefit for the patient, the missingness completely at random assumption for missingness in SARS-CoV-2 RNA loads likely is violated. In stepwise logistic regression with robust standard errors and a significance level of 0.05 as a cut-off for stepwise removal[14], missingness in SARS-CoV-2 RNA loads across all compartments was less likely in patients with a range of comorbidities. It was positively associated with SOFA scores for lower respiratory tract and blood viral loads. Patients developing ARDS at any time were more likely to have missing viral loads in all compartments except for the lower respiratory tract, where patients were less likely to have missing viral loads when developing ARDS. Patients with intubation and tracheotomy were less likely to have missing viral loads for all compartments, as were patients on COVID-19-specific therapy. With the majority of the variables above included in the generalised linear mixed model, the model should appropriately handle missing data that occur at random. Earlier in the above-presented analysis, missingness not at random for peak viral loads was assumed, which can be plausible as patients may not undergo interventions to obtain lower respiratory tract samples when clinicians anticipate low viral loads. Missingness not at random in SARS-CoV-2 RNA loads is not appropriately handled using the modelling approach outlined. Sensitivity analyses can be conducted to examine the implication of possible missingness not at random. Less than 5% of the cases had potential confounding variables missing, so they were neglected in the above analyses.

[14] Post-hoc analysis to better understand the missingness mechanism in the dataset.

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