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Dynamics of SARS-CoV-2 shedding in the respiratory tract depends on the severity of disease in COVID-19 patients

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Abstract

A fraction of COVID-19 patients progress to a severe disease manifestation with respiratory failure and the necessity of mechanical ventilation. Identifying patients at risk is critical for optimized care and early therapeutic interventions. We investigated the dynamics of SARS-CoV-2 shedding relative to disease severity.

We analyzed nasopharyngeal and tracheal shedding of SARS-CoV-2 in 92 patients with diagnosed COVID-19. Upon admission, standardized nasopharyngeal swabs or sputum were collected. If patients were mechanically ventilated, tracheal aspirates were additionally obtained. Viral shedding was quantified by real-time PCR detection of SARS-CoV-2 RNA.

45% (41 of 92) of COVID-19 had a severe disease course with the need for mechanical ventilation (severe group). At week 1, the initial viral shedding determined from nasopharyngeal swabs showed no significant difference between non-severe and severe cases. At week 2, a difference could be observed as the viral shedding remained elevated in severely ill patients. A time course of C-reactive-Protein (CRP), Interleukin-6 (Il-6), and Procalcitonin (PCT) revealed an even more protracted inflammatory response following the delayed drop of virus shedding load in severely ill patients. A significant proportion (47.8%) of patients showed evidence of prolonged viral shedding (>17 days), which was associated with severe disease courses (73.2%).

We report that viral shedding does not differ significantly between severe and non-severe cases upon admission to the hospital. Elevated SARS-CoV-2 shedding in the second week of hospitalization, a systemic inflammatory reaction peaking between second and third week and prolonged viral shedding are associated with a more severe disease course.

Introduction:

In COVID-19, rapid pulmonary worsening is frequently observed after an initial period of symptom stability. Clinical features of SARS-CoV-2 infections or COVID-19 were previously reported [1-3]. Several reports have described viral shedding to occur for extended periods [4, 5]. Complete assessment of viral shedding can give valuable insight into the underlying immunological mechanisms [6]. Detection of viral RNA by PCR is not necessarily associated with an infectious virus since infectivity was shown to be significantly reduced at later time points despite the presence of SARS-CoV-2 RNA [7-10].

Pneumonia represents the most important clinical manifestation of COVID-19 infection and is the primary determinant of prognosis in severely ill patients. There is a remarkable heterogeneity in the individual course and severity of the disease. Therefore pulmonary clearance of the virus is of particular interest [10]. An exaggerated response or reduced immune-dependent viral clearance in some patients may aggravate the pulmonary manifestation [11]. Individual differences in viral tropism, viral shedding load, duration of viral shedding and viral tissue distribution may play a role therein. Data about the tissue distribution and temporal dynamics of viral shedding are scarce, and further clinical characterization is necessary. Recent investigations shed light on the longitudinal inflammatory response associated to Covid-19 [11], it remains of high interest connecting clinically viable inflammatory parameters to virus shedding.

In our hospital, patients diagnosed with COVID-19 were repeatedly tested for evidence of SARS-CoV-2 RNA in material from the respiratory tract, including repeated endotracheal aspirates (ETA), sputum, and nasopharyngeal swabs (NPS).

Here we report the clinical and virological findings describing the dynamic of viral shedding in the cohort of 92 consecutive patients admitted to our hospital due to COVID-19 between 29th February and 17th May 2020.

Methods:

Study design

This study is a retrospective cohort study of all laboratory-confirmed COVID-19 patients admitted consecutively to the University Hospital of LMU Munich from 29th February 2020 to 17th May 2020.

Patients

All consecutive patients were either referred to or walked into the emergency care unit of our University hospital, a major academic center in southern Germany, with suspected COVID-19. These patients were retrospectively identified as confirmed COVID-19 cases by positive SARS-CoV-2 PCR. Only adults (age≥18 years) were included. We used a simple classification for disease severity: severe cases were defined as patients with the need for mechanical ventilation as it was used before [12]. Moderate disease in our patients was defined by the absence of mechanical ventilation and the need for oxygen insufflation, while the absence of both defined mild disease courses. Non-severe disease includes mild to moderate disease.

Samples

NPS, sputum, or ETA (in 7 patients with intubation at admission) were routinely obtained on admission and were performed according to local guidelines. NPS samples were taken on clinical suspicion of COVID-19. In addition, sputum samples were obtained when CT scanning showed COVID-19 typical infiltrates and NPS were negative or for clinical monitoring purposes. At admission, up to two NPS samples (with at least 12h distance) and one sputum sample (if necessary) were obtained.

Repeated collection of either sample (NPS, sputum, and ETA) was performed for clinical monitoring. When COVID-19 symptoms receded and two consecutive NPS (at least with a day distance) showed a negative result, testing was stopped.

Viral load analysis

Viral loads are expressed as SARS-CoV-2-RNA copy numbers per ml sputum, ETA, or transport medium of the swab sample. The standard swabs used in our hospital contain 1 ml liquid Amies transport medium (eSwab™, COPAN Diagnostics).

The following PCR assays were used for quantification in the accredited routine diagnostics laboratory of the Max von Pettenkofer-Institute: The *nucleocapsid* (N1) reaction of the CDC protocol [13], the *envelope* amplification of the Charité protocol [14, 15], the *nucleocapsid* amplification of the Seegene Allplex 2019-nCoV Assay and the Roche Cobas SARS-CoV-2 *nucleocapsid reaction*.

Standard curves were generated in multiple diluted replicates using either a plasmid containing the *nucleocapsid* gene (2019-nCoV-N-PositiveControl, IDT) or a clinical sample with copy numbers based on digital droplet PCR results as described previously[16]. Different formulas were derived for each PCR assay to convert Ct/Cp values to copy number estimates: 80*1,95^(40,29-Cp) for CDC (N1), 80*1,99^(39,34-Cp) for Charité (E), 80*2,00^(38,63-Ct) for Seegene Allplex 2019-nCoV Assay (N) and 80*1,99^(39,34-Ct) for Roche Cobas SARS-CoV-2 (N). These calculations do not take into account variability between separate PCR runs, different PCR chemicals, or different nucleic acid extraction methods. However, since these variabilities apply to all patient groups, they do not affect the interpretation of the results in this study.

The term "viral shedding" is used synonymously with the detection of SARS-CoV-2 RNA by PCR in respiratory material. However, this parameter, which we quantify, could also include subviral particles or RNA from dying cells and is not equivalent to the excretion of complete virions or even infectivity.

Serum inflammatory parameters

Procalcitonin was measured on a Cobas 8000 platform (Roche Diagnostics, Basel, Switzerland) and Interleukin-6 were measured on a Cobas e801 platform (Roche Diagnostics, Basel, Switzerland). C-reactive protein (CRP) levels were measured on a Cobas c702 platform by using the Tina-quant C-Reactive Protein assay (Roche Diagnostics, Switzerland).

Statistical analysis

Differences in parametric continuous variables such as the viral loads were examined with the Student's T-Test or ANOVA as appropriate. Distribution of clinical characteristics was examined by the usage of the Mann-Whitney U test or χ^2 test, as appropriate. Curve fitting was performed with a smoothing spline with 4 knots. Cox-regression analysis was performed to investigate the association of viral shedding duration with clinical characteristics such as

gender, age, arterial hypertension, diabetes, coronary artery disease, and Charlson Comorbidity score[17]. Patients without repeated negative results were censored on the last day of positivity. Statistical significance was defined as $p<0.05$. Statistical analysis was performed using SPSS 25 or Graphpad Prism 8.0.1.

Ethics Statement

The local ethics board approved this study of the Ludwig Maximilian University of Munich (project no. 20-454).

Results:

Upon admission, all patients had either NPS sampling, ETA sampling, or both. In all 92 cases, SARS-CoV-2 infection was confirmed in respiratory samples by real-time PCR. Patient characteristics are depicted in **Table 1a and b.** Patients were retrospectively identified as confirmed COVID-19 cases admitted from 29th February to 17th May 2020. On admission, the majority of cases (85/92) were breathing spontaneously and had a non-severe disease. 7 patients were transferred to our hospital, already receiving mechanical ventilation. Of the 85 non-severe patients at admission, 34 patients developed a severe disease course during the hospital stay. Of the remaining 51 (non-severe) patients, 20 patients developed a moderate disease course. The median age was 62 years (interquartile range 51-75). A significant proportion of patients had several comorbidities with an average Charlson comorbidity score of 2,5 (interquartile range 1 to 4), with arterial hypertension (49%) and diabetes mellitus (17%) being the most common comorbidities. Additional patient characteristics are shown in **Table S1**. A total of 473 respiratory samples (245 NPS, 228 tracheal aspirates, and 9 sputum samples) were examined. On average, 5.3 samples were collected per patient, and the testing frequency was similar among both groups **Table S2**.

Differences of SARS-CoV-2 viral shedding in ETA and NPS samples

Assessment of sample collection in patients with mechanical ventilation showed that ETA and NPS sample pairs taken at the same time point (n=13 patients) correlated significantly with each other (r=0.499 and p=0.041), but paired ratio T-Test revealed significantly higher viral shedding in ETA versus NPS type (p=0.0041) **Figure 1**. Therefore NPS and ETA were separately analyzed in subsequent tests. Individual sampling of NPS (correlation: r=0.8231, p<0.0001; ratio paired T-test: p=0.2575) **Figure 1b** and ETA (correlation: r=0.7948, p<0.0001, ratio paired T-test: p=0.1436) **Figure 1c** show high reproducibility of each sampling method.

SARS-CoV-2 viral shedding and disease severity

Viral shedding, according to disease severity, as shown in **Figure 2**. Initial virus shedding was not different among the severely diseased or non-severly disease Figure 2a. We excluded an influence of time to first testing to virus shedding load **Figure 2b**. For subsequent tests, we have calculated the average patient viral shedding for each week to reduce the influence of sample timing and sampling bias. According to disease severity, a direct comparison of viral

shedding showed a significantly elevated viral shedding at week 2 in severely ill patients Figure 2c + Table S2.

In nasopharyngeal swabs of patients with non-severe disease, SARS-CoV-2 viral shedding showed a significant drop at week 2 (p=0.0098), week 3 (p=0.0003), and week 4 (p=0.0004) when compared to week 1 Figure 2d. In patients with severe disease, viral shedding was not different at week 2 (p=0.3089) but decreased at week 3 (p=0.0056) and week 4 (p<0.0001), as depicted in Figure 2d.

In ETA of patients with severe disease, viral shedding dropped significantly at week 3 (p=0.0358) and week 4 (p=0.0022) compared to week 1 Figure 2e.

SARS-CoV-2 viral shedding and systemic inflammation

To further characterize the longitudinal inflammatory response to viral shedding and disease severity, we characterized the time course of Interleukin-6, Procalcitonin, and CRP. We calculated the weekly average values of Interleukin-6, Procalcitonin, and CRP to prevent sampling bias. Patients receiving tocilizumab were excluded from the analysis of CRP and Il-6 (n=4). Statistical analysis showed significantly elevated values of Interleukin-6 and CRP in the severe group at early time points and decreased at later time points (week 3-4) except for Procalcitonin. Initial viral shedding load did not correlate with peak PCT, peak Il-6 or peak CRP Figure 3b. Curve fitting revealed Interleukin-6 and Procalcitonin peaking at weeks 2-3, whereas CRP peaked between weeks 1 and 2 and dropped at weeks 2 and 3 Figure 3. Procalcitonin levels directly at admission (PCT measured <48h after admission) were significantly increased in patients with severe disease Figure S2a, further stratification according to coinfection or secondary infection (Table S4) and Figure S2b are attached in supplementary materials.

SARS-CoV-2 viral shedding duration

Viral shedding duration was capable of discriminating the need for mechanical ventilation Figure S2 with a Youden index of 0.467; a cutoff of 17 was determined optimal. Protracted viral shedding (>17days) was observed in 34 % of the 92 patients reported. The duration of viral shedding varied significantly according to disease severity Table 1; this is illustrated in Figure 4.

To correct for influences by other variables, prolonged viral shedding was investigated by uni- and multivariate Cox-Regression analysis. To validate the definition "duration of viral shedding" the Cox-Regression analysis was also performed with "duration of viral shedding" defined as the time from onset of symptoms to the first negative test result. The significance and interpretation of the results are basically unchanged between both definitions (see supplementary Figure S1). Multivariable analysis confirmed the association of prolonged virus shedding with severe disease. Further, no correlations between viral shedding and immunosuppression were found in Table 2.

Discussion:

Our study shows that viral shedding remains elevated the first two weeks in COVID-19 patients with severe disease, whereas it drops earlier in the non-severe patient group in NPS. Furthermore, we show an association of persistent viral shedding with disease severity. In a cohort of European patients, the time course and viral shedding of SARS-CoV-2 have not been investigated. Characterization of viral shedding dynamics is of high interest since it may indicate underlying immunological processes.

Previous investigations of viral shedding of SARS-CoV-2 in respiratory tract samples have shown a higher viral shedding in deeper respiratory tract samples [10, 18]. Huang et al. investigated SARS-CoV-2 viral shedding in different respiratory tract sample types (bronchial and nasopharyngeal) and found that patients with severe courses exhibited elevated viral shedding in deeper respiratory tract samples [19]. These findings are in line with our study, in which ETA and NPS testing showed a high variability when both sample types were compared directly. Therefore, in subsequent tests, they were separately analyzed **Figure 1**. This variability may be explained by differences in the tropism of the SARS-CoV-2 virus, but technical limitations of NPS for nasopharyngeal specimen may add to it [20].

Several studies investigating SARS-CoV-2 viral shedding and disease severity subsumed bronchial and nasopharyngeal tract samples as respiratory tract samples [8, 21, 22]. As discussed before, a separate analysis of lower respiratory tract samples and upper respiratory tract samples may prevent a sampling bias. When analyzed separately, we found that SARS-CoV-2 nasopharyngeal viral shedding remained high at week 2 in the severe patient group, whereas it dropped at week 2 of the non-severe group. When comparing absolute viral shedding at admission, we did not find significant differences according to disease severity. The persistent elevation at week 2 in the severe group indicates a lack of virus clearance as a causative mechanism for pulmonary worsening. Initial viral loads did not differ according to disease severity, which may further suggest a replication ceiling as a consequence of the saturation of ACE-II receptor binding [23]. The recently published study of Zheng et al. showed elevated shedding of SARS-CoV-2 virus in respiratory tract samples of severely diseased patients when compared to patients with mild disease [21]. In this study, respiratory tract samples were not differentiated between sputum or saliva, which may explain the observed differences in viral shedding since elevated levels may also be caused by the inclusion of more sputum samples in the severe group.

When analyzing systemic markers of inflammation, we observed a protracted systemic inflammatory response of Il-6 and Procalcitonin at week 2-3 after an initial elevation and decrease of CRP (week 2). These results support previously published data characterizing the immunological response in severely diseased patients [11, 24, 25]. Interestingly a small but relevant proportion of COVID-19 patients develop hyperinflammatory severe disease courses, while initial viral loads do not differ between severely and non-severely diseased patients but stay elevated in patients with severe disease at week 2. These findings are in line with the reported efficacy of the RECOVERY trial, which showed immune suppression by steroid therapy led to a highly significant reduction of 28-day mortality [26]. Analogies can be drawn to other imbalanced hyperinflammatory syndromes, e.g., only a small fraction of patients after EBV infection develop haemophagocytic lymphohistiocytosis [27]. The absence of efficacy of IL-6 receptor blockade by tocilizumab in moderately ill COVID-19 patients indicates other underlying pathways involved in this inflammatory process [28].

The discordant movement of Il-6 and CRP is suggestive of innate factors dominating the early immune response. It was recently shown that Il-6 does not exclusively correspond to CRP (which is commonly produced by hepatocytes in response to IL-6) despite a certain (low) threshold of Il-6 being necessary for CRP production [29-31]. Two larger studies have shown that serum IL-6 is superior to CRP, ferritin, liver enzymes, and other simple clinical laboratory markers for predicting COVID-19 clinical outcomes, such as respiratory failure and death, with an optimal cutoff of 80 and 86 pg/L, respectively [32, 33].

A procalcitonin value of 0.2-0.5 ng/ml is recognized to be sensitive and specific for bacterial pneumonia in patients with lower respiratory tract symptoms, and pulmonary infiltrates [34, 35]. Interestingly, we observed in the group with and without coinfection or superinfection highly elevated PCT levels, which may indicate the presence of a subclinical bacterial coinfection (Figure S2b). It has to be emphasized that timing of sputum/ETA culture may be preceded by antibacterial therapy, therefore the proportion of patients with positive sputum might be underestimated. Further investigations addressing the importance of bacterial coinfection, subclinical coinfection and colonization in COVID-19 patients are warranted [36-38].

The duration of viral shedding of SARS-CoV-2 has been investigated in several selected patient groups so far. In an early comprehensive study of clinical characteristics of 191 Chinese COVID-19 inpatients, prolonged viral shedding was evident. However, data on absolute copy

numbers or sampling sites (sputum, NPS, or ENTA) are not available [4]. Another study investigated viral shedding and transmissibility, and the temporal pattern of viral shedding was stratified according to patient subgroups [7]. Increased duration of viral shedding was not shown in any of the investigated subgroups. However, in this analysis, only a few patients in the non-severe and severe subgroup were included, and a definition of these subgroups was not available. Our study demonstrates the persistence of viral shedding in our hospitalized patients (n=44; 44.8% patients had viral shedding at least 17 days after onset of symptoms) occurs more frequently in patients with severe disease Table 2.

Persistently elevated SARS-CoV-2 viral shedding in respiratory specimens suggests a decreased immune clearance in patients with severe courses. Whereas in individuals of young age and few comorbidities, viral clearance was swift, but prolonged viral shedding was observed among a few oligosymptomatic patients [11]. Important underlying factors responsible for this phenomenon might be differences in host factors or immune response. Interestingly male gender was associated with prolonged viral shedding in Table 2. The delayed viral clearance of male patients may be explained by immunological and epidemiological gender-specific differences [39, 40].

Further, viral RNA's presence more than 50 days after onset of symptoms may be suggestive for ongoing viral replication, which gives rise to a chronic local inflammatory response. These findings can explain the often difficult and protracted recovery of COVID-19 patients, accompanied by an ongoing local immune reaction with detrimental effects on the respiratory system and other organs [41, 42]. In SARS, similar viral shedding patterns were observed [43]. As in SARS, the slow decrease in SARS-CoV-2 viral shedding despite seroconversion suggests an ongoing cellular clearance with an ineffective antibody-mediated clearance in COVID-19 [10, 43]. Further investigations should focus on the impact of immunological factors on the course and outcome of COVID-19.

Limitations of the study

Our study has several limitations. Firstly, it is a retrospective single-center cohort study with a moderate sample size. This might lead to an unbalanced distribution of confounders in subgroup analyses. The number of patients tested decreases later due to shorter hospital stays in non-severe patients, while other patients were still ventilated when our analysis was performed; the collected samples may be less representative when comparing viral shedding.

Viral shedding measurements by PCR mostly rely on the sample collection and preanalytical factors, influencing the measured viral shedding. Host factors such as increased bronchial susceptibility with an increase of necrotic/apoptotic cells may additionally affect viral shedding measurements.

Conclusion

Our findings show that viral shedding remains elevated in severe disease courses in the first weeks and may persist over longer durations. A protracted and imbalanced inflammatory response may ultimately contribute to disease severity. Further studies should investigate individual host factors associated with these phenomena to elucidate underlying mechanisms.

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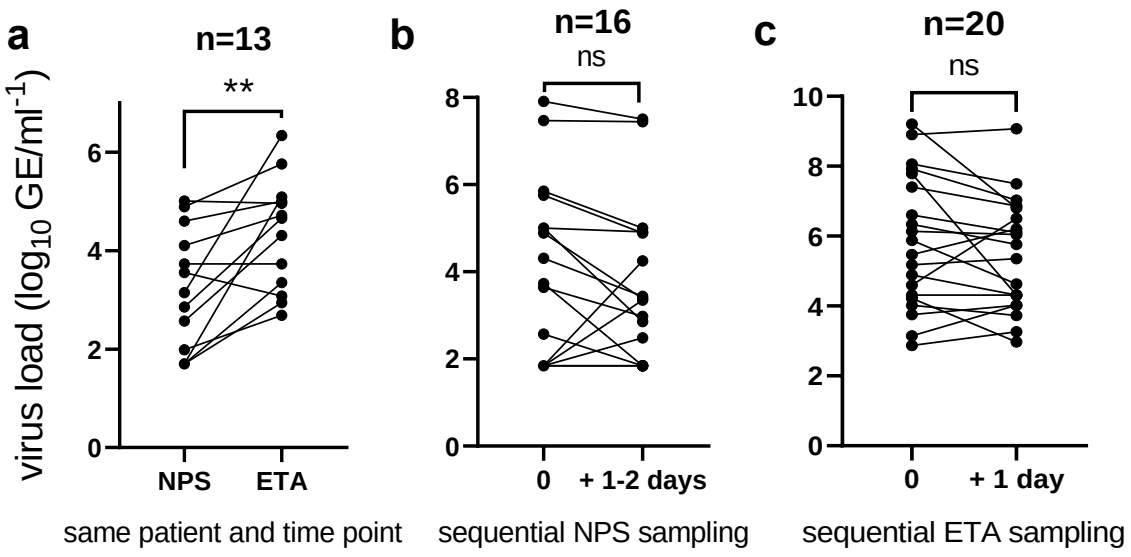


Figure 1: SARS-CoV-2 viral load was investigated in paired ETA and NPS samples collected at the same time point (b) serial samples of NPS of the same patients; (c) serial samples of ETA of the same patients; NPS: nasopharyngeal swabs; ETA endotracheal aspirates

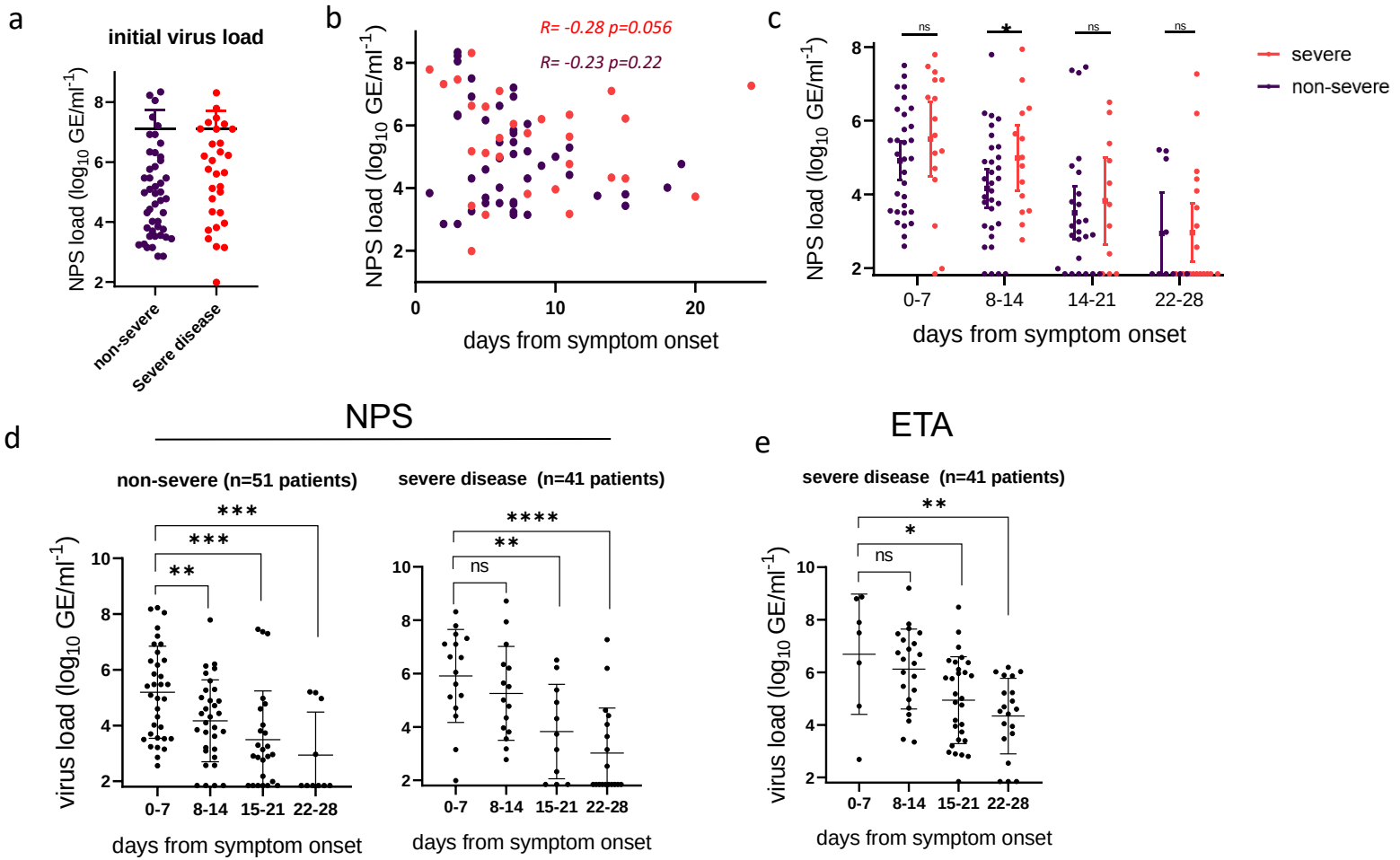


Figure 2: Viral shedding dynamics of severe respiratory syndrome coronavirus 2 (SARS-CoV-2) by disease severity, sample type and time from symptom onset; a. initial SARS-CoV-2 virus shedding load comparison in NPS according disease severity. **b** initial virus load according to days of symptom onset, corresponding Pearson correlation **c** comparison of virus shedding in NPS in patients of non-severe to severe disease patients according to week of symptom onset and **d** dynamics of virus shedding in NPS of non-severe and severely diseased patients. **e** virus shedding dynamics measured exclusively in ETA of severely diseased patients. Non-severe summarizes mild and moderate courses. NPS: nasopharyngeal swabs; ETA: endotracheal aspirate. P-values were calculated with Student's T-Test. Error bars denote mean and standard deviation. **Table S2** shows corresponding statistical data.

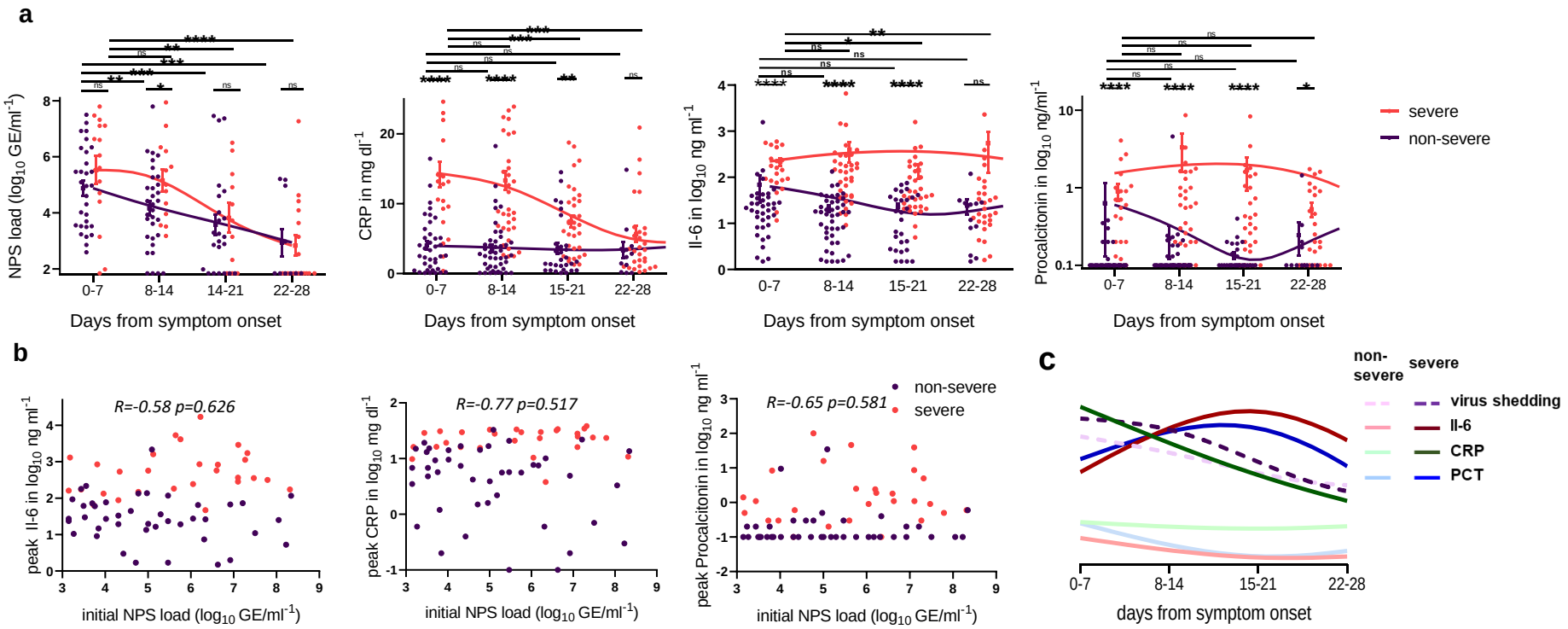


Figure 3: time course of the inflammatory response in non-severe and severe COVID-19 patients. a, Virus shedding in nasophagryngeal swabs, serum measurements of C-reactive protein, Interleukin-6 and Procalcitonin were plotted over time and grouped by disease severity. **b,** initial virus load (only nose swabs of spontaneously breathing patients at admission) were plotted against either peak values of CRP, peak-Interleukin-6 and peak Procalcitonin and a Pearson correlation was calculated. **c,** representative overview of the longitudinal course of inflammatory parameters and virus shedding in NPS. For curve fitting a spline with 4 knots was calculated. Error bars denote the s.e.m.. Student's T-Test determined differences of means (Table S3).

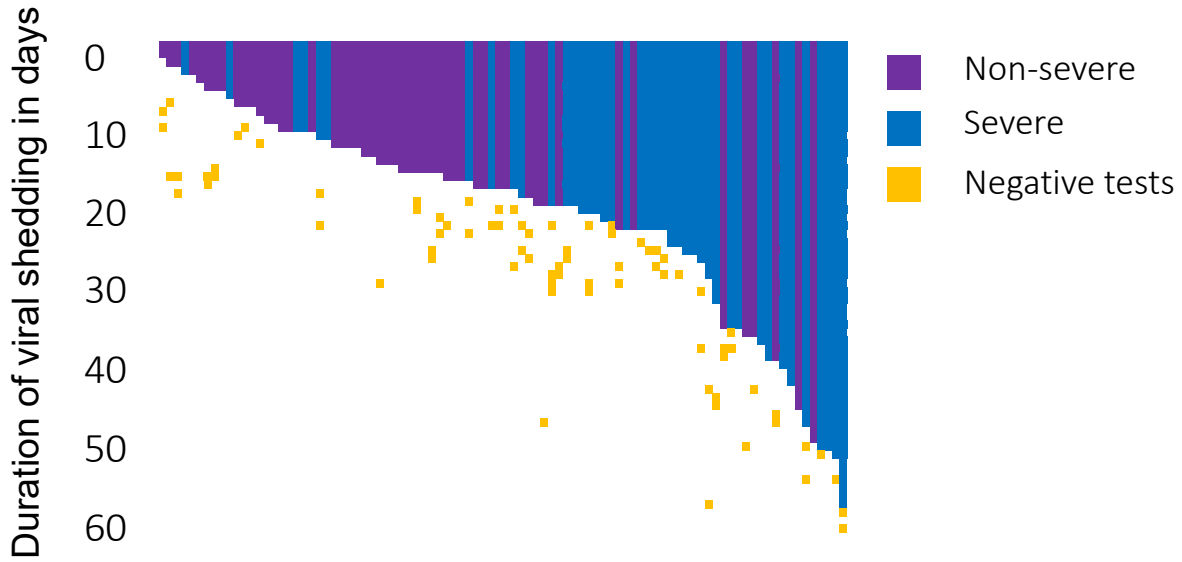


Figure 4: Visualization of the duration of virus shedding according to disease severity. Colored lanes depict each patients' duration of virus shedding from first positive testing until the last positive testing. Yellow boxes represent negative tests.

		Non-Severe disease No mechanical ventilation	Severe disease Mechanical Ventilation necessary	p-Value
	n=92	n=51 (55.4%)	n=41 (44.6%)	
Age, mean ± SD	60.2 ± 15.8	57.9 ± 18.1	63.1 ± 12.7	0.258
Male, n (%)	71 (77.3 %)	36 (70.6 %)	34 (82.9 %)	0.22
Continuous oxygen insufflation, n (%)	61 (66.3 %)	21 (41.2 %)	41 (100 %)	<0.001
Admission to ICU, n (%)	47(51.1 %)	9 (17.6 %)	41 (100 %)	<0.001
Days of mechanical ventilation ± SD		n.a.	22.6 ± 14.1	n.a.
Days of hospitalization ± SD	18.5 ± 13.4	13.1 ± 7.8	25.3 ± 15.6	<0.001
Use of ECMO, n (%)	5 (5.4 %)	n.a.	5 (12.2 %)	n.a.
Days of ECMO use		n.a.	13.6 ± 3.8	n.a.
ECMO mortality, n (%)		n.a.	3 (60 %)	n.a.
Discharge, n (%)	66 (72.5 %)	45 (88.2 %)	21 (47.2 %)	<0.001
Fatal, n (%)	7 (7.6 %)	0 (0.0 %)	7 (17.9 %)	0.003
Presence of COVID-19 typical radiological changes, n (%)	85 (92.4%)	44 (86.3 %)	41 (100 %)	0.013
Initial viral load in nose swabs (copies/ml) ± SD	12.8 x 10^6 ± 41.1 x 10^6	12.6 x 10^6 ± 43.1 x 10^6	13.0 x 10^6 ± 39.9 x 10^6	0.127
Initial viral load in endotracheal aspirate (copies/ml) ± SD		n.a.	67.2 x 10^6 ± 273 x 10^6	n.a.
Duration of viral shedding in days (with twice confirmed negativity) ± SD	18. 7 ± 12.0	13.9 ± 9.5 (n=16)	25.8 ± 11.8 (n=18)	0.025
Persistent viral shedding (≥17days), n (%)	44 (47.8 %)	14 (27.5 %)	30 (73.2 %)	<0.001
Time to first testing in days ± SD	7.4 ± 4.7	6.5 ± 4.0	8.4 ± 5.3	0.12
Comorbidities n (%)				
Arterial hypertension	48 (52.2 %)	24 (47.1 %)	24 (58.5 %)	0.30
Diabetes mellitus Type 2	18 (19.6 %)	8 (15.7 %)	10 (24.4 %)	0.43
Coronary artery disease	15 (16.3 %)	9 (17.6 %)	6 (14.6 %)	0.78
COPD	11 (12.0 %)	4 (7.8 %)	7 (17.1 %)	0.21
Immunosuppression	22 (23.9 %)	13 (25.5%)	9 (22.0%)	0.81
Charlson comorbidity index ± SD	2.5 ± 1.8	2.5 ± 1.9	2.6 ± 1.7	0.62

Table 1a: Baseline characteristics of the study population. Data are mean (SD) or n (%). p values were calculated by Mann-Whitney U test or χ^2 test, as appropriate. Severe disease was defined by the need of mechanical ventilation. COVID-19 typical changes included either ground glass opacities or diffuse bilateral infiltrates. Duration of nasopharyngeal viral shedding was defined by the time between symptom begin and last positivity for viral shedding in standardized nose swabs or endotracheal aspirates.

		Non-Severe disease No mechanical ventilation	**Severe disease** Mechanical Ventilation necessary	**p-Value**
	n=92	n=51 (55.4%)	n=41 (44.6%)	
Inflammation parameters				
Initial CRP (mg/dl)	7.9 ± 9.0	4.7 ± 5.2	12.6 ± 11.3	<0.001
Peak CRP (mg/dl)	15.4 ± 12.1	8.5 ± 7.9	25.6 ± 9.8	<0.001
Initial PCT (ng/ml)	0.41 ± 0.73	0.22 ± 0.33	0.68 ± 1.04	<0.001
Peak PCT (ng/ml)	4.14 ± 13.7	2.95 ± 14.2	5.91 ± 12.9	<0.001
Initial IL-6 (pg/ml)	189.3 ± 737.8	75.3 ± 292.4	359.9 ± 1095.5	<0.001
Peak IL-6 (pg/ml)	841.8 ± 2300.5	118.9 ± 321.7	1916.3 ± 3352.2	<0.001
Initial WBCs (G/l)	10.8 ± 31.6	6.2 ± 3.0	9.5 ± 5.0	<0.001
Peak WBCs (G/l)	18.1 ± 43.2	8.5 ± 4.0	21.5 ± 9.7	<0.001
Specific medication				
Use of broad spectrum antibiotics*	n=58 (63.0 %)	19 (37.3 %)	39 (95.1 %)	0,01
Use of Azithromycin	n=49 (53.3 %)	20 (39.2 %)	29 (70.7 %)	0.14
Use of antiviral agents**	n=9 (9.8 %)	4 (7.8 %)	5 (12.2 %)	0.78
Use of hydroxchloroquin	n=24 (26.1 %)	8 (15.7 %)	16 (39.0 %)	0.09
Use of prednisolone	n=3 (3.3 %)		3 (7.3 %)	
Use of tocilicumab	n=4 (4.4 %)	1 (1.1 %)	3 (3.3%)	0.23

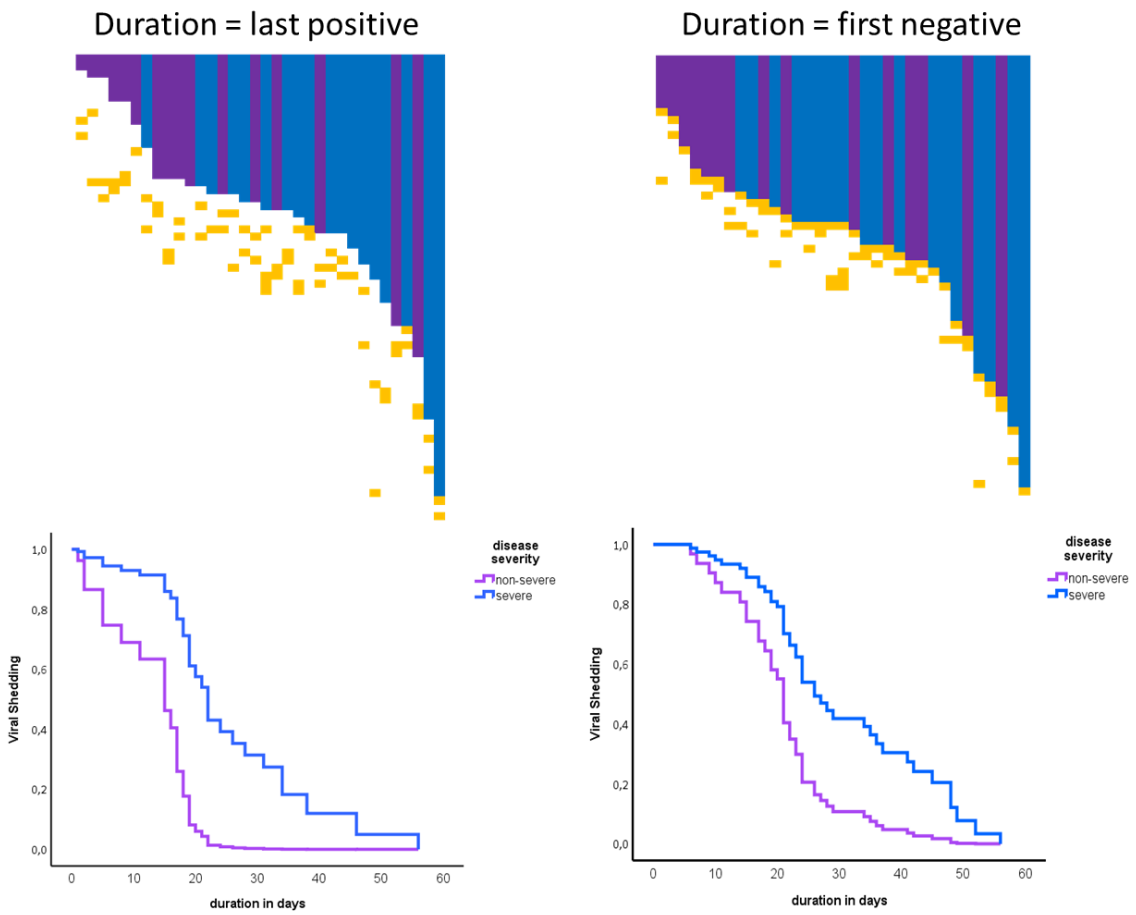
Table 1b: Baseline characteristics of the study population. Inflammation parameters and specific medication of subgroups. CRP = C-reactive protein; PCT = procalcitonin; IL-6 = Interleukin 6; WBC = White blood cell count.

*meropenem or piperacillin and tazobactam

**lopinavir/ritonavir (n=8) or Tamiflu (n=1)

	Univariate analysis				Multivariate analysis			
	Significance	Hazard Ratio	95% confidence interval		Significance	Hazard ratio	95% confidence interval	
			Lower	Higher			Lower	Higher
age	0.335	1.013	0.987	1.041	0.831	0.995	0.954	1.03
sex (m=1, f=2)	0.415	1.395	0.627	3.102	0.077	2.531	0.905	7.073
Disease severity (severe=1, non-severe=0)	0.075	1.894	0.939	3.824	**0.025**	3.260	1.162	9.147
Oxygen insufflation necessary (yes=1, no=0)	0.573	1.321	0.502	3.473	0.057	3.960	0.961	16.319
Hydroxychloroquin therapy (yes=1, no=0)	0.082	0.490	0.219	1.095	0.263	0.597	0.242	1.474
Lopinavir/Ritonavir treatment (yes=1, no=0)	0.796	1.149	0.401	3.296	0.384	1.713	0.509	5.765
Immunsuppressive treatment (Tocilizumab/Prednisolon/others; yes=1, no=0)	0.233	1.723	0.704	4.215	0.110	2.748	0.794	9.511
Diabetes Mellitus (yes=1, no=0)	0.953	0.975	0.422	2.254	0.704	1.243	0.404	3.825
Arterial hypertension (yes=1, no=0)	0.621	1.193	0.593	2.401	0.572	0.765	0.302	1.939
Coronary artery disease (yes=1, no=0)	0.787	1.141	0.440	2.959	0.968	0.978	0.326	2.930
Charlson Score (0-7)	0.425	1.082	0.891	1.314	0.850	1.036	0.719	1.493

Table 2 Cox-Regression analysis of factors associated with prolonged SARS-Cov-2 positivity.



	Significance	Exp(B)	95,0% CI	
			Lower	Upper
Disease Severity (severe=1, non-severe=0)	0,001	5,099	1,998	13,014
Charlson Score (0-7)	0,467	0,897	0,669	1,202
Age	0,104	0,963	0,919	1,008
Gender (m=1)	0,079	0,444	0,18	1,098

	Significance	Exp(B)	95,0% CI	
			Lower	Upper
Disease Severity (severe=1, non-severe=0)	0,008	2,562	1,272	5,161
Charlson Score (0-7)	0,1	0,811	0,632	1,041
Age	0,584	1,009	0,977	1,042
Gender (m=1)	0,252	0,626	0,281	1,396

Figure S1: Cox-Regression analysis was performed with "duration of viral shedding" defined as the time from onset of symptoms to the last positive result (left figure) and until the first negative test result (right curve).

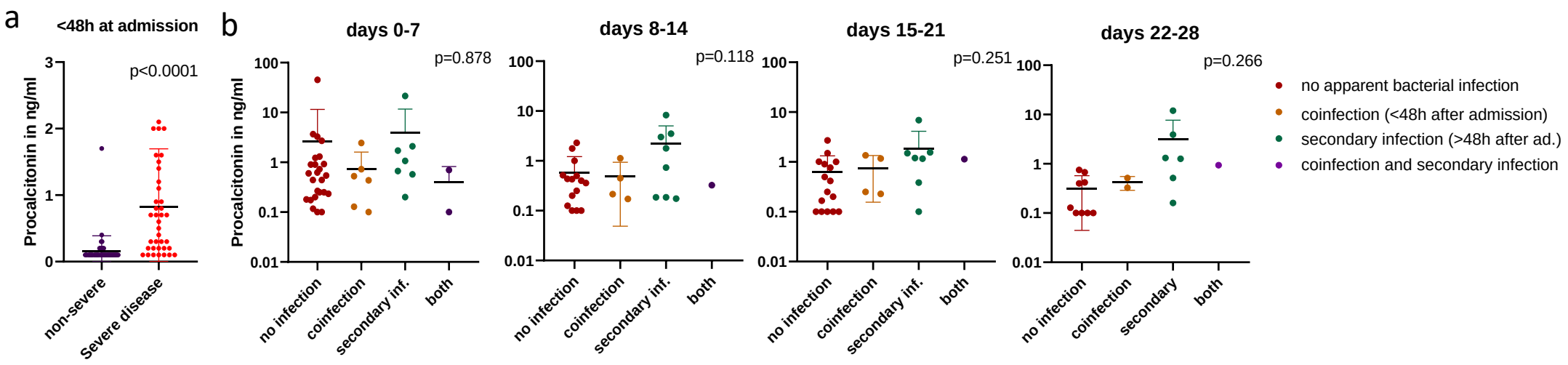


Figure S2a: PCT values at admission (PCT measured <48h after admission) of severe and non-severe disease **b** PCT levels in the subgroups of bacterial coinfection and secondary bacterial infection. ANOVA determined differences of means. Error bars denote mean and standard deviation.

		Non-Severe disease No mechanical ventilation	Severe disease Mechanical Ventilation necessary	p-Value
	n=92	n=51 (55.4%)	n=41 (44.6%)	
Number of tests per patient, ± SD	5.3 ± 4.2	3.3 ± 1.7	7.7 ± 4.9	<0.001
Testing Frequency (number of tests/day)	0.31 ± 0.14	0.29 ± 0.13	0.33 ± 0.15	0.169
Twice negative testing available before discharge n (%)	34 (37.0%)	16 (31.3 %)	18 (43.9 %)	0.154
Comorbidities n (%)				
Asthma bronchiale	4 (4.3 %)	3 (5.5 %)	1 (2.7 %)	0.63
Smoking habit	25 (27.2 %)	15 (27.3%)	10 (27.0%)	0.81
Rheumatic disease	3 (3.3 %)	3 (5.5 %)	0 (0 %)	0.25
History of solid cancer	10 (10.9 %)	4 (7.8 %)	6 (14.6 %)	0.33
Solid organ transplantation	5 (5.4 %)	2 (3.9 %)	3 (7.3%)	0.65

Table S1: Extended patient characteristics: data are mean (SD) or n (%). p values were calculated by Mann-Whitney U test or χ^2 test, as appropriate. Severe disease was defined by the need of mechanical ventilation. Testing frequency was defined as the number of tests divided by the length of the hospital stay.

		week 1	week 2	week 3	week 4
Severe	Number of patients tested	16	15	11	18
	Mean Average viral load (in copies/ml)	$22x10^6$	42×10^6	0.47×10^6	1.1×10^6
Mild to moderate	Number of patients tested	34	32	24	10
	Mean Average viral load (in copies/ml)	15×10^6	2.1×10^6	2.9×10^6	0.04×10^6
T-Test	P value	0.17	0.03	0.60	0.90

Table S2: Comparison of average viral load in NPS according to time point and disease severity. (Student's T-Test). NPS: nasopharyngeal swabs.

CRP					
		week 1	week 2	week 3	week 4
Severe	Number of patients tested	21	37	33	30
	Mean Average concentration in mg dl^{-1}	14.19	13.34	7.50	5.66
Mild to moderate	Number of patients tested	38	44	29	12
	Mean Average concentration in mg dl^{-1}	4.01	3.57	3.60	3.36
T-Test	P value	<0.0001	<0.0001	0.0014	0.25

Il-6					
		week 1	week 2	week 3	week 4
Severe	Number of patients tested	21	35	32	29
	Mean Average concentration in ng ml^{-1}	214.1	400.1	141.4	549.5
Mild to moderate	Number of patients tested	38	43	28	12
	Mean Average concentration in ng ml^{-1}	69.0	21.3	22.3	24.3
T-Test	P value	<0.0001	<0.0001	<0.0001	0.1199

Procalcitonin					
		week 1	week 2	week 3	week 4
Severe	Number of patients tested	21	32	29	24
	Mean Average concentration in ng ml^{-1}	0.90	3.30	1.67	0.53
Mild to moderate	Number of patients tested	36	44	28	12

	Mean Average concentration in ng ml^{-1}	0.632	0.222	0.13	0.24
T-Test	P value	<0.0001	<0.0001	<0.0001	0.90

Table S3: Comparison of CRP, Il-6 and PCT according to time point and disease severity. (Student's T-Test).

	At admission <48h of hospital stay		>48h of hospital stay	
	Clinical evidence of bacterial coinfection	ETA/sputum samples positive	Clinical evidence of secondary infection	ETA/Sputum samples positive
Severe	8 of 41 (19.5%)	6 of 25 (24.0%)	10 of 41 (24.3%)	0 of 9 (0%)
Non-severe	1 of 51 (2.0%)	0 of 5 (0%)	4 of 51 (8.0%)	1 of 5 (20%)
p-Value	0.005	n.a.	0.028	n.a.

Table S4 Distribution of coinfection, positive ETA/sputum samples and secondary infection in severe and non-severe COVID-19 patients.

Bacterial coinfection was defined as evidence for bacterial infection at admission, either by sputum, ETA culture or additional radiological signs of bacterial pneumonia. Secondary bacterial infection was defined as being an infection of any origin and being acquired during the hospital stay, according to patient charts.