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# The interplay of viral loads, clinical presentation, and serological responses in SARS-CoV-2 – Results from a prospective cohort of outpatient COVID-19 cases

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ABSTRACT

Risk factors for disease progression and severity of SARS-CoV-2 infections require an understanding of acute and long-term virological and immunological dynamics. Fifty-one RT-PCR positive COVID-19 outpatients were recruited between May and December 2020 in Munich, Germany, and followed up at multiple defined timepoints for up to one year. RT-PCR and viral culture were performed and seroresponses measured. Participants were classified applying the WHO clinical progression scale. Short symptom to test time (median 5.0 days; $p = 0.0016$) and high viral loads (VL; median maximum VL: $3{\cdot}10^8$ copies/mL; $p = 0.0015$) were indicative for viral culture positivity. Participants with WHO grade 3 at baseline had significantly higher VLs compared to those with WHO 1 and 2 ($p = 0.01$). VLs dropped fast within 1 week of symptom onset. Maximum VLs were positively correlated with the magnitude of Ro-N-Ig seroresponse ($p = 0.022$). Our results describe the dynamics of VLs and antibodies to SARS-CoV-2 in mild to moderate cases that can support public health measures during the ongoing global pandemic.

## 1. Introduction

At the end of 2019, cases of pneumonia of unknown origin were registered in Wuhan, China, and a novel coronavirus was subsequently identified as the causative agent (Sun et al., 2020; Zhou et al., 2020; Carvalho et al., 2021). On January 27th, 2020, the first SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) infection was confirmed in Germany at the Division of Infectious Diseases and Tropical Medicine in Munich (Rothe et al., 2020). Shortly after, on January 30th, the WHO declared the outbreak as a public health emergency of

*Abbreviations:* VL, Viral load; STT, Symptom to Test Time; KoCo19, Munich COVID-19 cohort; RT-PCR, Reverse Transcription Polymerase Chain Reaction; RNA, Ribonucleic Acid; ELISA, Enzyme-linked Immunoassay; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

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international concern.

The current reference standard to diagnose acute SARS-CoV-2 infection is reverse transcriptase-polymerase chain reaction (RT-PCR), from nasopharyngeal swabs or other respiratory samples (van Kampen et al., 2021). Active, replicating SARS-CoV-2 virus can also be detected by viral culture in Biosafety Level 3 laboratories and correlates with infectivity. In patients with positive viral culture results, viral loads (VLs) tend to be significantly higher than in patients with negative culture results. In addition, patients with high VLs are described to have more active virus replication and thus being more infectious than those with low VLs (van Kampen et al., 2021; Jefferson et al., 2020; Wölfel et al., 2020).

Dynamics of viral shedding as well as their association with demographic and clinical characteristics during the acute phase of SARS-CoV-2 infection have been described. These indicate that VL decrease gradually after symptom onset and serological responses mostly develop within the first two weeks after infection (Bullard et al., 2020; Sui et al., 2021; Mahallawi et al., 2021; Wellinghausen et al., 2020; Wang et al., 2020a). Studies also suggest that in some cases, RT-PCR positivity can persist more than 30 days from symptom onset, which is described as prolonged viral shedding (Wang et al., 2020b; Jin et al., 2020). The probability of positive culture decreases 10–14 days after symptom onset jointly with declining VLs (Bullard et al., 2020; Kim et al., 2021). Most published studies were conducted in inpatient settings, and data on the interplay of viral, clinical, and serological characteristics in an outpatient cohort are limited.

In the here-presented study, we aimed to assess acute phase VL and shedding dynamics, clinical information, and the SARS-CoV-2-specific antibody response within a long-term prospective cohort in Munich, Germany. We investigated a group of 51 individuals with acute SARS-CoV-2 infection who underwent an in-depth analysis during multiple early time points and for up to week 52 after symptom onset.

## 2. Materials and methods

### 2.1. Study design, setting and population

Individuals with a documented positive SARS-CoV-2 RT-PCR result were recruited in a prospective longitudinal cohort (n = 51) from May to December 2020, under the umbrella of the KoCo19 studies (Pritsch et al., 2021; Radon et al., 2021; Olbrich et al., 2021). Participants were consented and recruited as fast as possible upon RT-PCR confirmation of SARS-CoV-2 infection and followed during weekly visits up to week 4. Additional visits were performed at week 8, 26, and 52. Nasopharyngeal swabs were collected for RT-PCR analyses and viral culture. Semi-quantitative and quantitative antibody responses against nucleocapsid as well as spike/RBD were determined at all timepoints respectively. During time of recruitment, only the SARS-CoV-2 Wuhan strain (lineage A) was circulating in Germany, hence no further viral sequencing was performed within the study.

Questionnaires on clinical and demographic information were collected as previously published (Pritsch et al., 2021; Radon et al., 2021). Symptom to Test Time (STT) was defined as the number of days from the onset of symptoms to documented positive SARS-CoV-2 RT-PCR to reflect the onset of disease more accurately. The clinical presentation of the participants was classified using the WHO clinical progression scale (WHO clinical scale), a scoring system for disease severity during SARS-CoV-2 infection including the following codes: (0) uninfected, (1) asymptomatic cases, (2) mild symptomatic, (3) moderate symptomatic cases who needed assistance, but hospitalization was not necessary, (4) hospitalised but no oxygen therapy, (5) hospitalised and oxygen by mask or nasal prongs, (6) hospitalised and oxygen by NIV or high flow, (7) intubation and mechanical ventilation, (8) mechanical ventilation or vasopressors, (9) mechanical ventilation and vasopressors, dialysis or ECMO, (10) dead (Marshall et al., 2020). The participants of the study were all outpatients, therefore all patients fell within WHO category 1–3.

The study was approved by the Ethics Committee of the Faculty of Medicine at LMU Munich (20–371). Informed consent was obtained prior to any study procedure.

### 2.2. Viral testing methods

Per protocol, two respiratory swabs per sampling time point were obtained per patient, one of which was incubated for viral culture at the Bundeswehr Institute of Microbiology as described elsewhere (Wölfel et al., 2020). Quantitative RT-PCR was performed following RNA extraction using the TANBead Maelstrom™ 9600 (Taiwan Advanced Nanotech) Instrument with the OptiPure Viral Auto Plate (TANBead, Taiwan) kit. Quantification and detection of SARS-CoV-2 RNA was done using the Allplex 209-nCoV assay on a SeeGene Starlet IVD (SeeGene, Germany) automated platform. VL were then calculated back based on the SARS-CoV-2 standard dilution series of INSTAND (Germany) standards, taking Cycle threshold (CT) values of the two amplified targets into consideration. Viral cultures were attempted mostly for samples with a VL above the RKI (Robert-Koch-Institute) defined threshold of $1 \cdot 10^6$ RNA copies/ml, as chance for culture positivity is minimal in samples below this threshold (Robert-Koch-Institut, 2021a). However, for some samples a viral culture was not possible due to limited lab-capacities. For some samples below the threshold the viral culture was attempted to verify negativity.

### 2.3. Serologic testing methods/laboratory assays

Serological assays were performed as previously published (Pritsch et al., 2021; Radon et al., 2021). Commercially available assays were conducted following the manufacturer's instructions. For all sample time-points, the following assays were performed: Euroimmun Anti-SARS-CoV-2-ELISA anti-S1 IgG (hereafter called EI-S1-IgG; Euroimmun, Lübeck, Germany), Roche anti-N and Elecsys Anti-SARS-CoV-2 S anti-S1 (hereafter called Ro-N-Ig and Ro-RBD-Ig-quant, respectively; Roche, Mannheim, Germany). Elecsys Anti-SARS-CoV-2 is an immunoassay for the *in vitro* qualitative detection of antibodies using a double-antigen sandwich format. In this format, the capture as well as detection is performed by using respectively labelled antigens. Thus, the assay is antibody subclass agnostic by design. In addition, the SARS-CoV-2 surrogate virus neutralisation test (GS-cPass; GenScript®, Piscataway, New Jersey, USA) was performed. We chose serological assays based on the following criteria: availability in large quantities, enabled for at least semi-automated workup, acceptable pricing, licenced for the use in Europe, and well-described performance (Olbrich et al., 2021).

## 3. Data analysis

Prior to analysis, data was cleaned and locked. Statistical analysis and visualisation were performed using the software R, version 4.0.5 (https://cloud.r-project.org/). For operational replicates, the first measurement of EI-S1-IgG was used. In the case of Ro-N-Ig and GS-cPass the latest measurement was included, while for Ro-RBD-Ig-quant the most diluted was selected. We report Pearson's correlation coefficient *R* for association among continuous variables. For multiple group comparisons, Kruskal-Wallis tests, followed by post-hoc Dunn tests using the Benjamini Yekutieli adjustment for pairwise comparisons were applied (Yoav and Daniel, 2001).

## 4. Results

We recruited a total of 51 participants and analysed virological, serological, and clinical data. Table 1 provides an overview of the main demographic and clinical cohort characteristics. 57% (29/51) of the cohort were female with a median age of 32 years (IQR 27–47), while

**Table 1**
**Overview: Cohort characteristics.** Response rate of questionnaires: 86% (44/51).

| Total number of participants | 51 |
|---|---|
| **Median age in years (IQR)** | 32 (26–49) |
| Age distribution in years (%) | |
| <18 | 7 (13.7) |
| 18-29 | 10 (19.6) |
| 30-39 | 16 (31.4) |
| 40-50 | 7 (13.7) |
| >50 | 11 (21.6) |
| **WHO Grading** | |
| WHO 1 (%) | 6 (11.8) |
| WHO 2 (%) | 20 (39.2) |
| WHO 3 (%) | 25 (49.0) |
| **Most common symptoms** | |
| Headache (%) | 27 (61.4) |
| Cough (%) | 21 (47.7) |
| Fatigue (%) | 21 (47.7) |
| Loss of Smell (%) | 18 (40.9) |
| Loss of Taste (%) | 18 (40.9) |

IQR = Inter Quartile Range; WHO Grading = Grading by WHO Clinical Progression Scale.

43% (22/51) were male with median age of 35 years (IQR 26–51).

### 4.1. Viral dynamics

Out of 51 participants with an initially documented positive RT-PCR, 82% (42/51) tested RT-PCR positive after recruitment during at least one subsequent visit. Overall, a total of 78 (43%, 78/182) positive samples from different time-points were available for VL assessment. The highest measured VL was $1{\bullet}10^{11}$ copies per mL. Fig. 1 presents the VL dynamics, demonstrating that in our cohort, peak SARS-CoV-2 RNA levels declined rapidly in all but one patient in the first two weeks after symptom onset. One participant was classified as false-positive, as all follow-up RT-PCR tests remained negative and no seroresponse was detected. Five days after symptom onset, 71% (36/51) of the participants were RT-PCR positive and after 10 days, 59% (30/51) were still above the detection limit (~10 copies per mL). The mean VL decreased three orders of magnitude between the first and the second week and four orders of magnitude between the first and third week after symptom onset. After three weeks of STT, 22% (11/51) of the participants were tested positive in the RT-PCR while only 6% (3/38) tested positive after 45 days of STT. All participants tested negative in the RT-PCR after day 61, except one who was still tested positive on day 252. During the whole period, this specific participant had varying positive as well as negative RT-PCRs; and viral sequencing revealed the same strain for the whole follow-up period.

Viral culture was attempted mostly on samples with a VL > $10^6$ RNA copies and could be obtained for 33 samples of 27 patients. Overall, 52% (17/33) of viral culture attempts were successful. 76% (13/17) of these were from samples collected within the first week after symptom onset. Samples with positive and negative viral culture results presented significantly different STT distributions (Fig. 2A, p = 0.0016, STT available for 32 samples). Positive viral cultures were obtained with a median time of 5.0 days after symptom onset. In contrast, swabs resulting in negative cultures were taken at 13.0 days median time after symptom onset. No culture was successful beyond day 22 after symptom onset. Positive samples had significantly higher maximum VLs compared to negative and not attempted cultures (Fig. 2B; KW p = 0.0002; Dunn's post-test: positive-negative p = 0.0084, positive-not attempted p = 0.0003, negative-not attempted P = 0.98; median maximum VL with positive viral culture: $9{\bullet}10^8$ copies/mL; one sample per patient and for three patients the VL was unavailable).

### 4.2. Demographic and clinical data in correlation with VLs

Data on symptoms at baseline (week 1 of study participation) was available for 86% (44/51) of the participants. Out of 23 different symptoms reported (Supplemental Fig. 1), headache was the most frequent (61.4%, 27/44, range of symptom duration 1–11 days), followed by cough (47.7%, 21/44, 3–11 days), fatigue (47.7%, 21/44, 2–12 days), loss of taste (40.9%, 18/44, 3–120 days), and loss of smell (40.9%, 18/44, 3–120 days) (see also Table 1). Following the WHO scale for disease severity (Table 1), most participants were classified as WHO 2 (20/51, 39%) or 3 (25/51, 49%), with mild or moderate symptoms not requiring hospitalization (Characterisation, 2020). To assess the association between individual participants' characteristics and the course of disease, we correlated VLs with sex (p = 0.27) and age (R = 0.24, p = 0.101), none of which were statistically significant (Fig. 3A and B). Multiple linear regression model showed no significant correlation also in the interaction term between sex and age (F-statistics p = 0.4658; p = 0.266; p = 0.121; p = 0.230, respectively).

Participants graded as WHO 3 had significantly higher VLs compared to those graded as WHO 1/2 (median VLs: 7.45 and 3.78, respectively), demonstrating strong evidence of a significant association between VLs and disease severity (p = 0.01, Fig. 4).

### 4.3. Serological analysis

Alongside clinical and virological information, the seroresponse at all sample time points for all participants was examined. As participants were included up to four days after their first RT-PCR result, a number of participants were reactive in one or more of the assays at time of study recruitment: 26% for Ro-N-Ig (13/51), 35% for Ro-RBD-Ig-quant (18/51), 18% for El-S1-IgG (9/51), and 59% (30/51) using GS-CPass.

Fig. 5 visualises the association of VL and antibody responses as

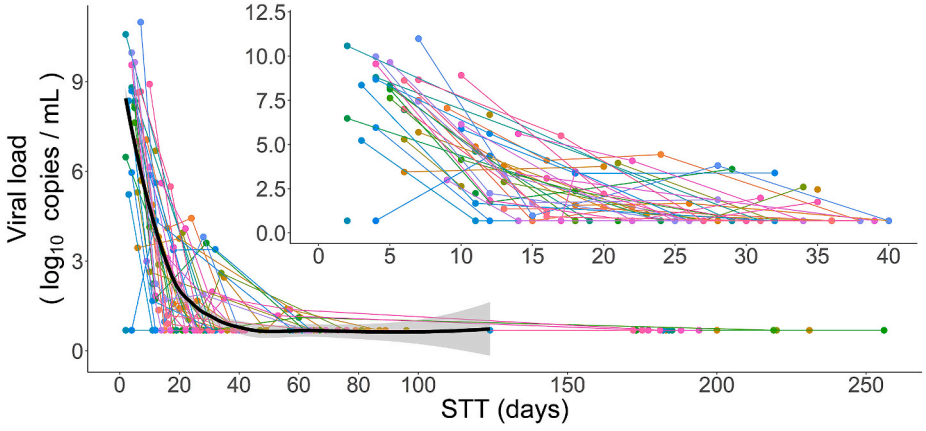


**Fig. 1. Viral load ($log_{10}$ copies per millilitre) over time.** Each colour presents one participant, each dot is one sample. The thick black line shows the LOESS estimation (locally estimated scatterplot smoothing or local regression), modelling the VL drop over Symptom to Test Time (STT) in days. The grey region represents the 95% confidence band of the LOESS. In the first week since symptom onset, the median VL was $7.3{\bullet}10^7$ copies per mL and decreased subsequently. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

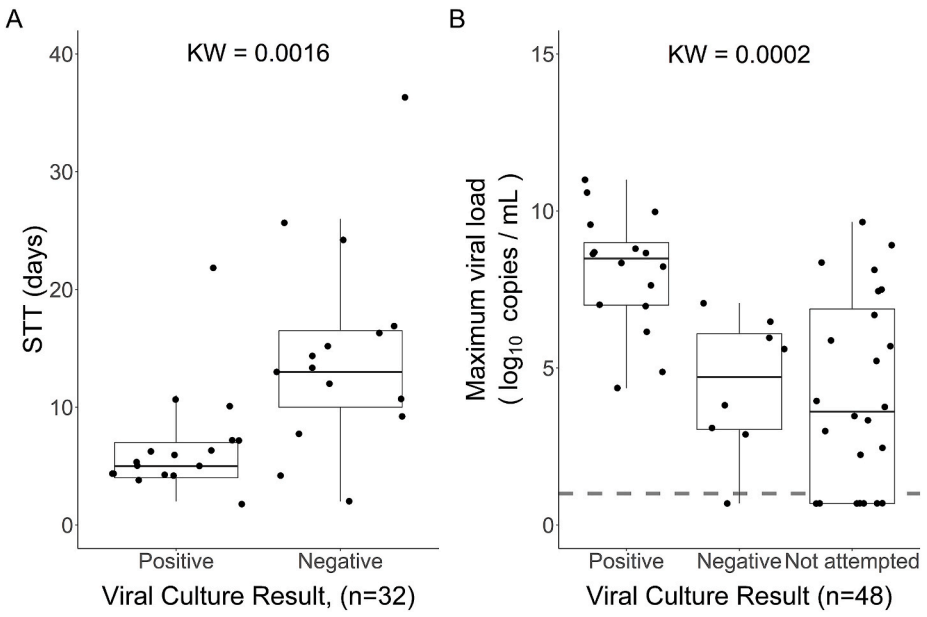


**Fig. 2. Viral culture result analysis. A**: Viral Culture Result and Symptom to Test Time (STT) in days (Kruskal Wallis test = 0.0016, N = 32) for all samples where the viral culture was attempted. Each black dot represents one measurement (n = 32) of 27 patients. A positive viral culture result was characterised by a short STT, and the culture result tended to be negative when STT was high; B: SARS-CoV-2 maximum viral load of each participant and positivity of viral culture (Kruskal-Wallis test = 0.0002). Each black dot represents one patient (n = 48, for three patients no viral load was measured). The grey dashed line represents the detection limit of the VL. Viral culture results were positive when the maximum VLs were high and negative when maximum VLs were low. Positive viral culture results revealed to have significantly different maximum VL distributions compared to negative and not attempted viral cultures (Dunn's post-test = 0.0084 and 0.0003, respectively), while the distributions of negative results and not attempted viral cultures were not significantly different (Dunn's post-test = 0.98).

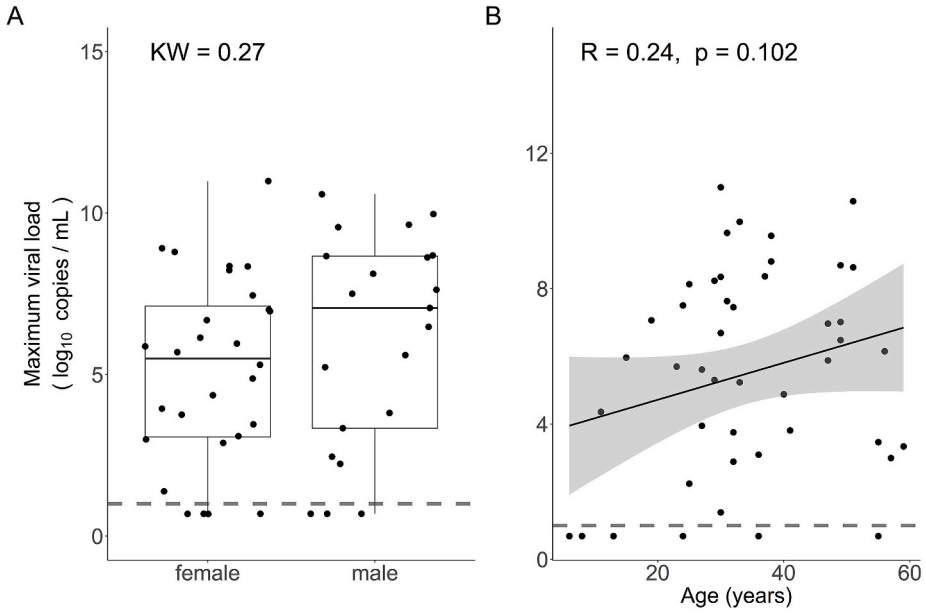


**Fig. 3. SARS-CoV-2 maximum viral load and baseline characteristic analysis.** Each black dot represents one participant and the grey dashed line represents the detection limit of the VL. Maximum VL and A: Sex (Kruskal-Wallis test = 0.27), without significant difference between females and males. B: Age in years (rho = 0.22, P = 0.14). No significant correlation between age and VLs were detected. The grey area is the 95% confidence band on the linear model (black solid line).

detected in the different assays. The highest antibody titre obtained for each participant correlated positively with VLs (p = 0.022) for Ro-N-Ig. Inhibition in GS-cPass showed a trend towards positive association with VL, without reaching statistical significance (p = 0.15). The other assays did not indicate any associations with maximum VLs (Ro-RBD-Ig-quant p = 0.55; El-S1-IgG p = 0.97). Correlation of seroresponse and disease severity were also assessed, however, as shown in Supplemental Fig. 2, no significant association was observed.

## 5. Discussion

In this study, we performed in-depth analyses of association between viral, clinical, and serological data over a long-term follow-up in a prospective cohort of SARS-CoV-2 RT-PCR positive individuals. To our knowledge, this is one of the few studies elucidating the interplay of those factors over an extensive time period in an outpatient setting.

VL decreased fast after the first week of symptoms, suggesting lower infectivity one to two weeks after symptom onset, as described in previous studies (Sui et al., 2021; He et al., 2020). While RT-PCR remains the gold standard for detection of SARS-CoV-2 due to the high sensitivity and specificity, it does not necessarily inform on infectivity as it also detects non-viable virus (Bullard et al., 2020). Understanding how long an individual remains infectious is of major public health interest, informing infection prevention and quarantine durations (Bullard et al., 2020). Detection of culturable virus can be understood as a proxy for infectivity. Consistent with the published literature, probability of a positive viral culture was significantly higher in participants with high VL and short STT in our cohort. No viral culture was successful beyond

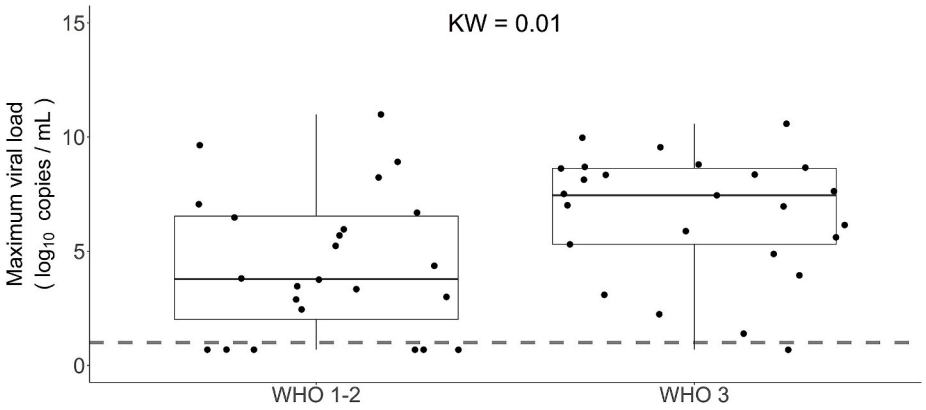


**Fig. 4. Viral load and WHO Grading.** Maximum VL and WHO Scale 1–2 and 3; WHO 1–2 includes asymptomatic and mildly ill participants (left boxplot) and WHO 3 represents moderate cases without the need of hospitalization (right boxplot). Each black dot represents one participant. The grey dashed line represents the detection limit of the VL. Participants graded as WHO 1 and 2 showed lower VLs than those graded as WHO 3 (Kruskal Wallis test = 0.01).

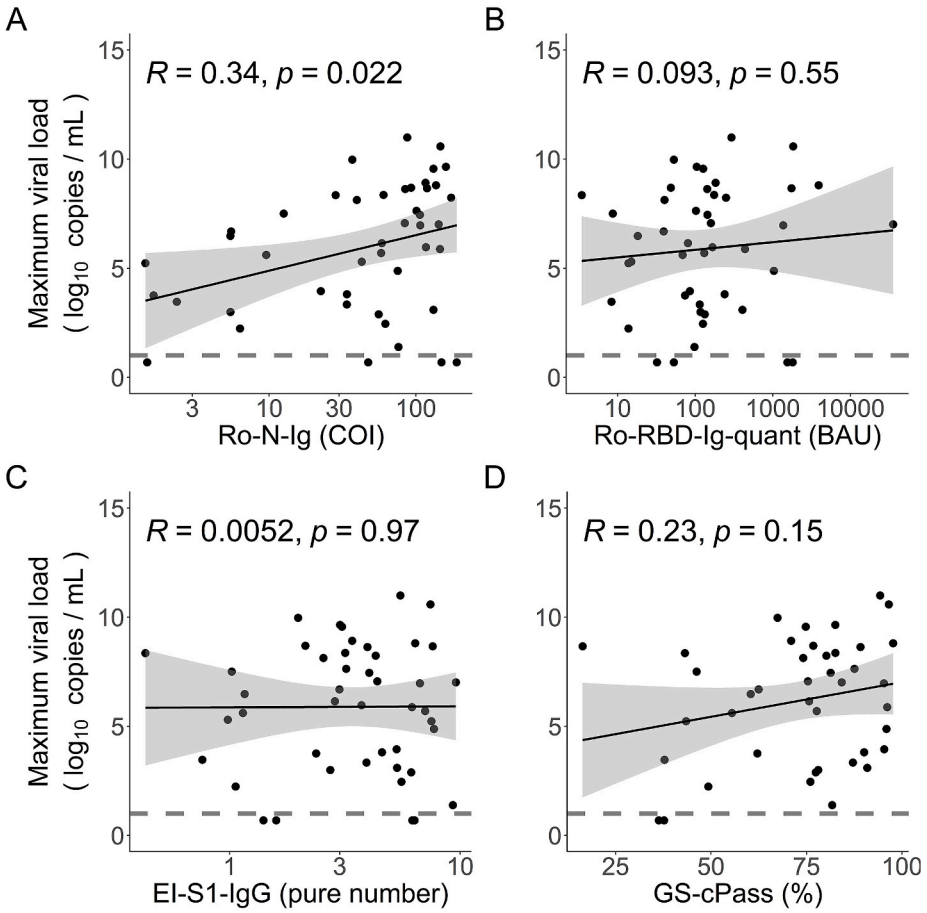


**Fig. 5. Maximum viral loads and antibody response.** The grey area shows the 95% confidence band of the linear model (black solid line) and the grey dash line represents the detection limit of the VL. Each black dot represents one participant. Correlation of maximum VL with A: Ro-N-Ig (R = 0.34, p = 0.0022): Showing significant positive correlation between maximum VL and highest measured Ro-N-Ig value; B: Ro-RBD-Ig-quant (R = 0.093, p = 0.55): Showing no significant correlation between maximum VL and highest measured Ro-RBD-Ig-quant value; C: El-S1-IgG (R = 0.0052, p = 0.97): Showing no positive correlation between maximum VL and highest measured El-S1-IgG value; D: GS-cPass (R = 0.23, p = 0.15): Showing a trend of positive correlation between maximum VL and highest measured GS-cPass value, although not significant.

week 3 since symptom onset which is in line with published literature (Bullard et al., 2020; Kim et al., 2021).

The adaptive immune response of patients infected with SARS-CoV-2, especially antibody responses and their long-term dynamics, is a topic of great relevance, as antibody levels correlate with protection from (re) infection and are used diagnostically to define previous SARS-CoV-2 exposure. Beside factors such as sex, age, or extent of lung infiltration, VL seems likely to have impact on the time to seropositivity and antibody titers (Masia et al., 2021a). For example, in one study which measured the antibodies IgG, IgA, and IgM, non-seroconverters tended to have lower median VLs than seroconverters (Masia et al., 2021b). The four serological tests chosen here had different target structures: Elecsys Ro-N-Ig and Ro-RBD-Ig-quant detect nucleocapsid or a shortened spike, respectively, and all binding immunoglobulin isotypes, while El-S1-IgG targets specifically IgG binding to spike S1. In contrast, GS-cPass is a neutralisation surrogate test and assesses the ability to block the interaction between ACE2 and the RBD of spike, regardless of antibody subclass. In our study, higher VLs correlated significantly with the highest signal detected in the Ro-N-Ig assay, however, this was not the case for the other tests evaluated. This might be due to the abundance of the nucleocapsid antigen in the early phases of viral replication, while the appearance of the anti-S response leads to quick viral control. Here,

the functional neutralisation seems to be more relevant than the titre. This is in line with GS-cPass as functional assay showing a trend to correlation with VL as well. Significance might be reached with better statistical power (larger sample size). Our cohort also includes only outpatients with mild disease severity.

The clinical presentation of SARS-CoV-2 has been described extensively, and it is assumed that infection mostly causes symptoms of common cold such as cough and fever, while severe cases of pneumonia occur in 1% of cases (Robert-Koch-Institut, 2021b; Menni et al., 2020). In this cohort, most participants were asymptomatic- or oligosymptomatic, reporting cough, loss of taste, and loss of smell as the predominant symptoms. This is in accordance with the reports by the Robert Koch Institute (RKI) (Robert-Koch-Institut, 2021b) where most clinical manifestations resolve in the first two weeks after symptom onset. In other studies, loss of smell and loss of taste persisted for up to 4 months and patients with anosmia are likely to recover within 12 months (Robert-Koch-Institut, 2021b; Renaud et al., 2021). This was also observed in two participants in this study.

In our cohort, there was a non-significant trend of higher VLs in male compared to female participants, a finding also described in other studies (Mahallawi et al., 2021; He et al., 2020). Age is reportedly another demographic factor potentially impacting VL, with higher VLs found in older patients and respectively lower VLs in younger individuals (Masia et al., 2021b; Westblade et al., 2020; Pradhan and Olsson, 2020). In our cohort, younger participants tended to have lower VLs, although this observation was not significant. This could be explained by the composition of the cohort, as the median age in years was 32 and only 6% (3/51) of the participants were above 60 years of age. There are ambiguous descriptions regarding an association between VL and severity of disease in published studies. Some studies report a strong association between higher SARS-CoV-2 VL and increasing disease severity (Wang et al., 2020a; Fajnzylber et al., 2020), while others do not find this association (He et al., 2020; Jacot et al., 2020). This might be explained by the time point chosen for VL-assessment, Munker et al. described no difference at admission but saw a significantly higher VL in severely ill patients two weeks following admission (Munker et al., 2021). In our analyses, we used the highest measured VL for each participant and observed a significant correlation with disease severity classified by WHO grading. Munker et al. demonstrated that the site of sampling may influence the magnitude of VLs and disease severity. In their study, samples collected from the lower respiratory tract, especially in severe cases, exhibited higher VL (Munker et al., 2021). However, we focused on upper respiratory samples and bronchial tract samples were not analysed in this study.

Our study has important limitations: Firstly, we have a relatively small sample size of 51 individuals in our cohort. Secondly, due to the study design, participants were recruited at different time points after infection, likely missing the acute phase with highest VLs in some. Thirdly, we did no radiological analysis and have no information about VLs in the lungs.

This study demonstrates that positive viral culture results have high VLs and low STT suggesting increased infectivity in that time range, an essential information for the containment of the SARS-CoV-2 pandemic. Furthermore, lower peak VLs suggest a lower magnitude of seropositivity mainly for anti-N. This data is consistent with published literature and confirms the guidelines for isolation, testing strategies and contact tracing. While the analyses presented here contribute to a deeper understanding of viral, serological, and clinical features of SARS-CoV-2-infection during the acute phase and the first year following infection, further studies with larger cohorts are needed.

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## CRediT authorship contribution statement

**Kerstin Puchinger:** Formal analysis, Conceptualization, Writing – original draft, Resources. **Noemi Castelletti:** Data curation, Visualization, Formal analysis, Software, Writing – original draft, Validation, Conceptualization. **Raquel Rubio-Acero:** Resources. **Christof Geldmacher:** Writing – review & editing, Conceptualization. **Tabea M. Eser:** Resources. **Flora Deák:** Resources. **Ivana Paunovic:** Resources. **Abhishek Bakuli:** Software. **Elmar Saathoff:** Resources. **Alexander von Meyer:** Resources. **Alisa Markgraf:** Resources. **Philine Falk:** Resources. **Jakob Reich:** Resources. **Friedrich Riess:** Resources. **Philipp Girl:** Resources. **Katharina Müller:** Resources. **Katja Radon:** Resources. **Jessica Michelle Guggenbuehl Noller:** Resources. **Roman Wölfel:** Resources. **Michael Hoelscher:** Investigation, Funding acquisition. **Inge Kroidl:** Conceptualization, Supervision, Project administration, Methodology, Data curation, Funding acquisition, Writing – review & editing, Investigation. **Andreas Wieser:** Conceptualization, Methodology, Writing – review & editing, Investigation, Funding acquisition. **Laura Olbrich:** Conceptualization, Supervision, Project administration, Methodology, Data curation, Funding acquisition, Writing – review & editing, Investigation.

## Declaration of competing interest

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

AW and MH (on the behalf of the institute) report personal fees and non-financial support from Roche Diagnostics, LO reports non-financial support from Roche Diagnostics. AW, MH and LO report non-financial support from Euroimmun, non-financial support from Viramed, non-financial support from Mikrogen. AW, MH, LO report grants, non-financial support and other from German Center for Infection Research (DZIF), grants and non-financial support from Government of Bavaria, non-financial support from BMW, non-financial support from Munich Police, non-financial support and other from Accenture. MH and AW report non-financial support from Dr. Becker MVZ during the conduct of the study. In addition, MH and AW have a patent on a sample system for sputum diagnostics of SARS-CoV-2 pending.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2022.02.002.

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