

Accelerated Article Preview

Virological assessment of hospitalized patients with COVID-2019

Received: 1 March 2020

Accepted: 24 March 2020

Accelerated Article Preview

Published online 1 April 2020

Cite this article as: Wölfel, R. et al.
Virological assessment of hospitalized
patients with COVID-2019.
Nature <https://doi.org/10.1038/s41586-020-2196-x> (2020).

Roman Wölfel, Victor M. Corman, Wolfgang Guggemos, Michael Seilmaier, Sabine Zange, Marcel A. Müller, Daniela Niemeyer, Terry C. Jones, Patrick Vollmar, Camilla Rothe, Michael Hoelscher, Tobias Bleicker, Sebastian Brünink, Julia Schneider, Rosina Ehmann, Katrin Zwirgmaier, Christian Drosten & Clemens Wendtner

This is a PDF file of a peer-reviewed paper that has been accepted for publication. Although unedited, the content has been subjected to preliminary formatting. Nature is providing this early version of the typeset paper as a service to our authors and readers. The text and figures will undergo copyediting and a proof review before the paper is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers apply.

Virological assessment of hospitalized patients with COVID-2019

<https://doi.org/10.1038/s41586-020-2196-x>

Received: 1 March 2020

Accepted: 24 March 2020

Published online: 1 April 2020

Roman Wölfel^{1,6}, Victor M. Corman^{2,6}, Wolfgang Guggemos^{3,6}, Michael Seilmaier³, Sabine Zange¹, Marcel A. Müller², Daniela Niemeyer², Terry C. Jones^{2,4}, Patrick Vollmar¹, Camilla Rothe⁵, Michael Hoelscher⁵, Tobias Bleicker², Sebastian Brünink², Julia Schneider², Rosina Ehmann¹, Katrin Zwirgmaier¹, Christian Drosten^{2,7} & Clemens Wendtner^{3,7}✉

Coronavirus disease 2019 (COVID-19) is an acute respiratory tract infection that emerged in late 2019^{1,2}. Initial outbreaks in China involved 13.8% cases with severe, and 6.1% with critical courses³. This severe presentation corresponds to the usage of a virus receptor that is expressed predominantly in the lung^{2,4}. By causing an early onset of severe symptoms, this same receptor tropism is thought to have determined pathogenicity, but also aided the control, of severe acute respiratory syndrome (SARS) in 2003⁵. However, there are reports of COVID-19 cases with mild upper respiratory tract symptoms, suggesting the potential for pre- or oligosymptomatic transmission^{6–8}. There is an urgent need for information on body site-specific virus replication, immunity, and infectivity. Here we provide a detailed virological analysis of nine cases, providing proof of active virus replication in upper respiratory tract tissues. Pharyngeal virus shedding was very high during the first week of symptoms (peak at 7.11×10^8 RNA copies per throat swab, day 4). Infectious virus was readily isolated from throat- and lung-derived samples, but not from stool samples, in spite of high virus RNA concentration. Blood and urine never yielded virus. Active replication in the throat was confirmed by viral replicative RNA intermediates in throat samples. Sequence-distinct virus populations were consistently detected in throat and lung samples from the same patient, proving independent replication. Shedding of viral RNA from sputum outlasted the end of symptoms. Seroconversion occurred after 7 days in 50% of patients (14 days in all), but was not followed by a rapid decline in viral load. COVID-19 can present as a mild upper respiratory tract illness. Active virus replication in the upper respiratory tract puts the prospects of COVID-19 containment in perspective.

There is a close genetic relatedness between severe acute respiratory syndrome coronavirus (SARS-CoV) and the causative agent of COVID-19, SARS-CoV-2. The predominant expression of ACE2 in the lower respiratory tract is believed to have determined the natural history of SARS as a lower respiratory tract infection. Whereas positive SARS-CoV-2 detection in clinical specimens from the upper respiratory tract has been described^{9,10} these observations do not address principal differences between SARS and COVID-19 in terms of clinical pathology. The here-studied patients were enrolled because they acquired their infections upon known close contact to an index case, thereby avoiding representational biases due to symptom-based case definitions. All patients were treated in a single hospital in Munich, Germany. Virological testing was done by two closely-collaborating laboratories using the same standards of technology for RT-PCR and virus isolation, confirming each other's results based on almost all individual samples. Due to extremely high congruence of results, all data are presented together.

Only the serological data are based on results from one laboratory. The patients are part of a larger cluster of epidemiologically-linked cases that occurred after January 23rd, 2020 in Munich, Germany, as discovered on January 27th¹¹. The present study uses samples taken during the clinical course in the hospital, as well as from initial diagnostic testing before admission. In cases when this initial diagnostic testing was done by other laboratories, the original samples were retrieved and re-tested under the rigorous quality standards of the present study.

RT-PCR sensitivity, sites of replication, and correlates of infectivity based on aggregated data

To first understand whether the described clinical presentations are solely caused by SARS-CoV-2 infection, samples from all patients were tested against a panel of typical agents of respiratory viral infection, including HCoV-HKU1, -OC43, -NL63, -229E; Influenza virus A and B,

¹Bundeswehr Institute of Microbiology, Munich, Germany. ²Charité Universitätsmedizin Berlin, Berlin, Germany. ³Klinikum München-Schwabing, Munich, Germany. ⁴Center for Pathogen Evolution, Department of Zoology, University of Cambridge, Cambridge, UK. ⁵University Hospital LMU Munich, Munich, Germany. ⁶These authors contributed equally: Roman Wölfel, Victor M. Corman, Wolfgang Guggemos. ⁷These authors jointly supervised this work: Christian Drosten, Clemens Wendtner. ✉e-mail: christian.drosten@charite.de; clemens.wendtner@muenchen-klinik.de

Rhinovirus, Enterovirus, Respiratory syncytial virus, Human Parainfluenza virus 1-4, Human metapneumovirus, Adenovirus, and Human bocavirus. Interestingly, no co-infection was detected in any patient.

All patients were initially diagnosed by RT-PCR from oro- or nasopharyngeal swab specimens¹². Both specimen types were collected over the whole clinical course in all patients. There were no discernible differences in viral loads or detection rates when comparing naso- vs. oropharyngeal swabs (Figure 1B). The earliest swabs were taken on day 1 of symptoms, with symptoms often being very mild or prodromal. All swabs from all patients taken between days 1 and 5 tested positive. The average virus RNA load was 6.76×10^5 copies per whole swab until day 5 (maximum, 7.11×10^8 copies/swab). Swab samples taken after day 5 had an average viral load of 3.44×10^5 copies per swab and a detection rate of 39.93%. The last positive-testing swab sample was taken on day 28 post-onset. Average viral load in sputum was 7.00×10^6 copies per mL (maximum, 2.35×10^9 copies per mL).

Because swab samples had limited sensitivity for initial diagnosis of cases of SARS^{13,14}, we analyzed the first paired swab and sputum samples taken on the same occasion from seven patients. All samples were taken between 2 and 4 days post-onset. In two cases, swab samples had clearly higher virus concentrations than sputum samples, as indicated by a difference greater than 3 in threshold cycle (Ct) value. The opposite was true in two other cases, while the remaining 5 cases had similar concentrations in both sample types.

None of 27 urine samples and none of 31 serum samples were tested positive for SARS-CoV2 RNA.

To understand infectivity, live virus isolation was attempted on multiple occasions from clinical samples (Figure 1D). Whereas virus was readily isolated during the first week of symptoms from a considerable fraction of samples (16.66% in swabs, 83.33% in sputum samples), no isolates were obtained from samples taken after day 8 in spite of ongoing high viral loads.

Virus isolation from stool samples was never successful, irrespective of viral RNA concentration, based on a total of 13 samples taken between days six to twelve from four patients. Virus isolation success also depended on viral load: samples containing $<10^6$ copies/mL (or copies per sample) never yielded an isolate. For swab and sputum, interpolation based on a probit model was done to obtain laboratory-based infectivity criteria for discharge of patients (Figures 1E, F).

High viral loads and successful isolation from early throat swabs suggested potential virus replication in upper respiratory tract tissues. To obtain proof of active virus replication in absence of histopathology, we conducted RT-PCR tests to identify viral subgenomic messenger RNAs (sgRNA) directly in clinical samples (extended data Figure S1). Viral sgRNA is only transcribed in infected cells and is not packaged into virions, therefore indicating the presence of actively-infected cells in samples. Viral sgRNA was compared against viral genomic RNA in the same sample. In sputum samples taken on days 4/5, 6/7, and 8/9, a time in which active replication in sputum was obvious in all patients as per longitudinal viral load courses (see below), mean normalized sgRNA per genome ratios were ~0.4% (Figure 1G). A decline occurred over days 10/11. In throat swabs, samples taken up to day 5 were in the same range, while no sgRNA was detectable in swabs thereafter. Together, these data indicate active replication of SARS-CoV-2 in the throat during the first 5 days after symptoms onset. No, or only minimal, indication of replication in stool was obtained by the same method (Figure 1G).

During our study we sequenced full virus genomes from all patients. A G6446A exchange was first detected in one patient and later transmitted to other patients in the cluster.¹¹ In the first patient, this mutation was found in a throat swab while a sputum sample from the same day still showed the original allele, 6446G. The SNP was analyzed by RT-PCR and Sanger sequencing in all sequential samples available from that patient (Table 1). The presence of separate genotypes in throat swabs and sputum strongly supported our suspicion of independent virus replication in the throat, rather than passive shedding there from the lung.

Virus shedding, antibody response, and clinical correlation in individual courses

Daily measurements of viral load in sputum, pharyngeal swabs, and stool are summarized in Figure 2. In general, viral RNA concentrations were very high in initial samples. In all patients except one, throat swab RNA concentrations seemed to be already on the decline at the time of first presentation. Sputum RNA concentrations declined more slowly, with a peak during the first week of symptoms in three of eight patients. Stool RNA concentrations were also high. Courses of viral RNA concentration in stool seemed to reflect courses in sputum in many cases (e.g., Figure 2A, B, C). In only one case, independent replication in the intestinal tract seemed obvious from the course of stool RNA excretion (Figure 2D). Whereas symptoms mostly waned until the end of the first week (Table 2), viral RNA remained detectable in throat swabs well into the second week. Stool and sputum samples remained RNA-positive over three weeks in six of the nine patients, in spite of full resolution of symptoms.

All cases had comparatively mild courses (Table 2). The two patients who showed some signs of lung infection were the only cases where sputum viral loads showed a late and high peak around day 10/11, whereas sputum viral loads were on the decline by this time in all other patients (Figure 2F, G). Of note, four of nine patients showed loss of taste and olfactory sensation, and described this loss to be stronger and more long-lasting than in common cold diseases.

Seroconversion was detected by IgG and IgM immunofluorescence using cells expressing the spike protein of SARS-CoV-2 and a virus neutralization assay using SARS-CoV-2 (extended data Figure S2 and Table 3). Seroconversion in 50% of patients occurred by day 7, and in all by day 14 (Figure 1D). No viruses were isolated after day 7. All patients showed detectable neutralizing antibodies, the titers of which did not suggest close correlation with clinical courses. Of note, case #4, with the lowest virus neutralization titer at end of week 2, seemed to shed virus from stool over prolonged time (Figure 2D). Results on differential recombinant immunofluorescence assay indicated cross-reactivity or cross-stimulation against the four endemic human coronaviruses in several patients (Table S1).

Conclusions

The clinical courses in subjects under study were mild, all being young-to middle-aged professionals without significant underlying disease. Apart from one patient, all cases were first tested when symptoms were still mild or in the prodromal stage, a period in which most patients would present once there is general awareness of a circulating pandemic disease⁵. Diagnostic testing suggests that simple throat swabs will provide sufficient sensitivity at this stage of infection. This is in stark contrast to SARS. For instance, only 38 of 98 nasal or nasopharyngeal swab samples tested positive by RT-PCR in SARS patients in Hong Kong¹⁵. Also, viral load differed considerably. In SARS, it took 7 to 10 days after onset until peak RNA concentrations (of up to 5×10^5 copies per swab) were reached^{13,14}. In the present study, peak concentrations were reached before day 5, and were more than 1000 times higher. Successful live virus isolation from throat swabs is another striking difference from SARS, for which such isolation was rarely successful^{16–18}. Altogether, this suggests active virus replication in upper respiratory tract tissues, where SARS-CoV is not thought to replicate in spite of detectable ACE-2 expression^{19,20}. At the same time, the concurrent use of ACE-2 as a receptor by SARS-CoV and SARS-CoV-2 corresponds to a highly similar excretion kinetic in sputum, with active replication in the lung. SARS-CoV was found in sputum at mean concentrations of $1.2 \cdot 2.8 \times 10^6$ copies per mL, which corresponds to observations made here¹³.

Whereas proof of replication by histopathology is awaited, extended tissue tropism of SARS-CoV-2 with replication in the throat is strongly supported by our studies of sgRNA-transcribing cells in throat swab

samples, particularly during the first 5 days of symptoms. Striking additional evidence for independent replication in the throat is provided by sequence findings in one patient who consistently showed a distinct virus in her throat as opposed to the lung. In addition, the disturbance of gustatory and olfactory sense points at upper respiratory tract tissue infection.

Critically, the majority of patients in the present study seemed to be already beyond their shedding peak in upper respiratory tract samples when first tested, while shedding of infectious virus in sputum continued through the first week of symptoms. Together, these findings suggest a more efficient transmission of SARS-CoV-2 than SARS-CoV through active pharyngeal viral shedding at a time when symptoms are still mild and typical of upper respiratory tract infection. Later in the disease, COVID-19 then resembles SARS in terms of replication in the lower respiratory tract. Of note, the two patients who showed some symptoms of lung affection showed a prolonged viral load in sputum. Our study is limited in that no severe cases were observed. Future studies including severe cases should look at the prognostic value of an increase of viral load beyond the end of week 1, potentially indicating aggravation of symptoms.

One of the most interesting hypotheses to explain a potential extension of tropism to the throat is the presence of a polybasic furin-type cleavage site at the S1-S2 junction in the SARS-CoV-2 spike protein that is not present in SARS-CoV¹⁷. Insertion of a polybasic cleavage site in the S1-S2 region in SARS-CoV was shown to lead to a moderate but discernible gain of fusion activity that might result in increased viral entry in tissues with low density of ACE2 expression²¹.

The combination of very high virus RNA concentrations and occasional detection of sgRNA-containing cells in stool indicate active replication in the gastrointestinal tract. Active replication is also suggested by a much higher detection rate as compared to MERS-coronavirus, for which we found stool-associated RNA in only 14.6% samples in 37 patients hospitalized in Riyadh, Saudi Arabia^{22,23}. If virus was only passively present in stool, such as after swallowing respiratory secretions, similar detection rates as for MERS-CoV would be expected. Replication in the gastrointestinal tract is also supported by analogy with SARS-CoV, which was regularly excreted in stool, from which it could be isolated in cell culture²⁴. Our failure to isolate live SARS-CoV-2 from stool may be due to the mild courses of cases, with only one case showing intermittent diarrhea. In China, diarrhea has been seen in only 2 of 99 cases²⁵. Further studies should therefore address whether SARS-CoV-2 shed in stool is rendered non-infectious through contact with the gut environment. Our initial results suggest that measures to contain viral spread should aim at droplet-, rather than fomite-based transmission.

The prolonged viral shedding in sputum is relevant not only for hospital infection control, but also for discharge management. In a situation characterized by limited capacity of hospital beds in infectious diseases wards, there is pressure for early discharge following treatment. Based on the present findings, early discharge with ensuing home isolation could be chosen for patients who are beyond day 10 of symptoms with less than 100,000 viral RNA copies per ml of sputum. Both criteria predict that there is little residual risk of infectivity, based on cell culture.

The serological courses of all patients suggest a timing of seroconversion similar to or slightly earlier than in SARS-CoV infection¹⁸. Seroconversion in most cases of SARS occurred during the second week of symptoms. As in SARS and MERS, IgM was not detected significantly earlier than IgG in immunofluorescence, which might in part be due to technical reasons as the higher avidity of IgG antibodies outcompetes IgM for viral epitopes in the assay. IgG depletion can only partially alleviate this effect. Because IFA is a labor-intensive method, ELISA tests should be developed as a screening test. Neutralization testing is necessary to rule out cross-reactive antibodies directed against endemic human coronaviruses. Based on frequently low neutralizing antibody titers observed in coronavirus infection^{26,27}, we have here developed a particularly sensitive plaque reduction neutralization

assay. Considering the titers observed, a simpler microneutralization test format is likely to provide sufficient sensitivity in routine application and population studies.

When aligned to viral load courses, it seems there is no abrupt virus elimination at the time of seroconversion. Rather, seroconversion early in week 2 coincides with a slow but steady decline of sputum viral load. Whether certain properties such as glycosylation pattern at critical sites of the glycoprotein play a role in the attenuation of neutralizing antibody response needs further clarification. In any case, vaccine approaches targeting mainly the induction of antibody responses should aim to induce particularly strong antibody responses in order to be effective.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-020-2196-x>

1. Zhu, N. et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*, <https://doi.org/10.1056/NEJMoa2001017> (2020).
2. Gorbalenya, A. E. et al. Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. *bioRxiv*, 2020.2002.2007.937862, <https://doi.org/10.1101/2020.02.07.937862> (2020).
3. WHO. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf (2020).
4. Hoffmann, M. et al. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *bioRxiv*, 2020.2001.2031.929042, <https://doi.org/10.1101/2020.01.31.929042> (2020).
5. Leung, G. M. et al. The epidemiology of severe acute respiratory syndrome in the 2003 Hong Kong epidemic: an analysis of all 1755 patients. *Ann Intern Med* **141**, 662–673, <https://doi.org/10.7326/0003-4819-141-9-200411020-00006> (2004).
6. Rothe, C. et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. *N Engl J Med*, <https://doi.org/10.1056/NEJMc2001468> (2020).
7. Holshue, M. L. et al. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med*, <https://doi.org/10.1056/NEJMoa2001191> (2020).
8. Hoehl, S. et al. Evidence of SARS-CoV-2 Infection in Returning Travelers from Wuhan, China. *New England Journal of Medicine*, <https://doi.org/10.1056/NEJMc2001899> (2020).
9. Zou, L. et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *New England Journal of Medicine*, <https://doi.org/10.1056/NEJMc2001737> (2020).
10. Young, B. E. et al. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *Jama*, <https://doi.org/10.1001/jama.2020.3204> (2020).
11. Böhmner, M. et al. Outbreak of COVID-19 in Germany Resulting from a Single Travel-Associated Primary Case, https://ssrn.com/abstract=3551335 (2020).
12. Corman, V. M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* **25**, <https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045> (2020).
13. Drosten, C. et al. Evaluation of advanced reverse transcription-PCR assays and an alternative PCR target region for detection of severe acute respiratory syndrome-associated coronavirus. *J Clin Microbiol* **42**, 2043–2047, <https://doi.org/10.1128/jcm.42.5.2043-2047.2004> (2004).
14. Peiris, J. S. et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* **361**, 1767–1772, [https://doi.org/10.1016/S0140-6736\(03\)13412-5](https://doi.org/10.1016/S0140-6736(03)13412-5) (2003).
15. Poon, L. L. et al. Detection of SARS coronavirus in patients with severe acute respiratory syndrome by conventional and real-time quantitative reverse transcription-PCR assays. *Clin Chem* **50**, 67–72, <https://doi.org/10.1373/clinchem.2003.023663> (2004).
16. Ksiazek, T. G. et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* **348**, 1953–1966, <https://doi.org/10.1056/NEJMoa030781> (2003).
17. Drosten, C. et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* **348**, 1967–1976, <https://doi.org/10.1056/NEJMoa030747> (2003).
18. Peiris, J. S. et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* **361**, 1319–1325, [https://doi.org/10.1016/S0140-6736\(03\)13077-2](https://doi.org/10.1016/S0140-6736(03)13077-2) (2003).
19. Bertram, S. et al. Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts. *PLoS one* **7**, e35876, <https://doi.org/10.1371/journal.pone.0035876> (2012).
20. Xu, H. et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *International Journal of Oral Science* **12**, 8, <https://doi.org/10.1038/s41368-020-0074-x> (2020).
21. Belouzard, S., Chu, V. C. & Whittaker, G. R. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 5871–5876, <https://doi.org/10.1073/pnas.0809524106> (2009).
22. Corman, V. M. et al. Viral Shedding and Antibody Response in 37 Patients With Middle East Respiratory Syndrome Coronavirus Infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **62**, 477–483, <https://doi.org/10.1093/cid/civ951> (2016).

23. Zhou, J. *et al.* Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus. *Sci Adv* **3**, eaao4966, <https://doi.org/10.1126/sciadv.aao4966> (2017).
24. Leung, W. K. *et al.* Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* **125**, 1011-1017, [https://doi.org/10.1016/S0016-5085\(03\)01215-0](https://doi.org/10.1016/S0016-5085(03)01215-0) (2003).
25. Chen, N. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet* **395**, 507-513, [https://doi.org/10.1016/S0140-6736\(20\)30211-7](https://doi.org/10.1016/S0140-6736(20)30211-7) (2020).
26. Drosten, C. *et al.* Transmission of MERS-coronavirus in household contacts. *N Engl J Med* **371**, 828-835, <https://doi.org/10.1056/NEJMoa1405858> (2014).
27. Muller, M. A. *et al.* Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study. *Lancet Infect Dis* **15**, 559-564, [https://doi.org/10.1016/S1473-3099\(15\)70090-3](https://doi.org/10.1016/S1473-3099(15)70090-3) (2015).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2020

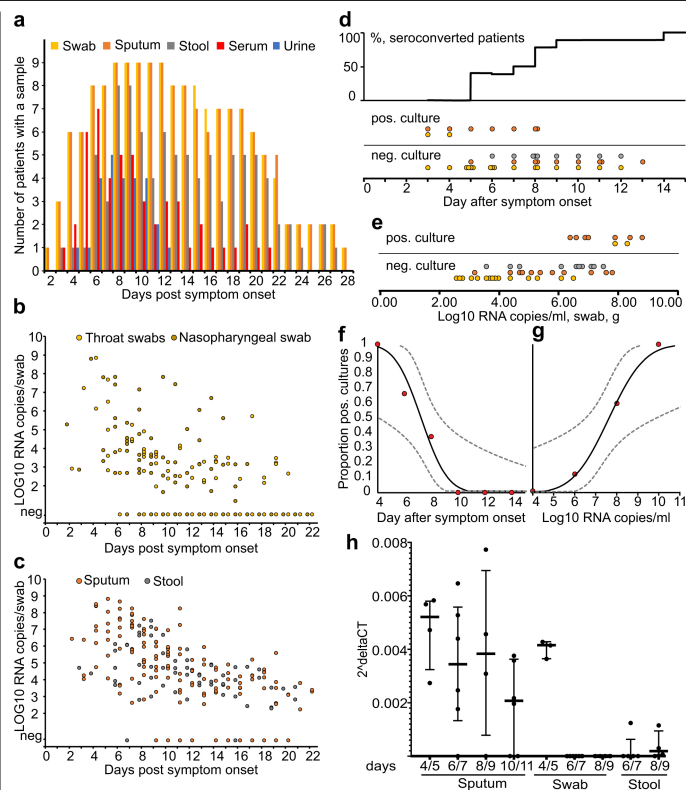


Fig. 1 | Hallmarks of viral shedding in aggregated samples. A, samples and sample types per day. B, viral RNA concentrations in upper respiratory tract samples. C, viral RNA concentrations in sputum and stool samples. D, seroconversion and virus isolation success dependent on day post onset of symptoms. Top panel shows fraction of seroconverted patients, bottom shows aggregated results of virus isolation trials. E, virus isolation success dependent on viral load. F and G, projected virus isolation success based on probit distributions. The inner lines are probit curves (dose-response rule). The outer dotted lines are 95% CI. For less than 5% isolation success, the estimated day was 9.78 (95% CI: 8.45-21.78) days post-onset and the estimated RNA concentration for less than 5% isolation success was estimate to be 6.51 Log₁₀ RNA/ml (95% CI: 4.11-5.40). H, Subgenomic viral RNA transcripts in relation to viral genomic RNA. Dots represent mean values of RT-PCR data obtained from at least two independent experiments on samples from individual patients. Plots show median values with interquartile ranges.

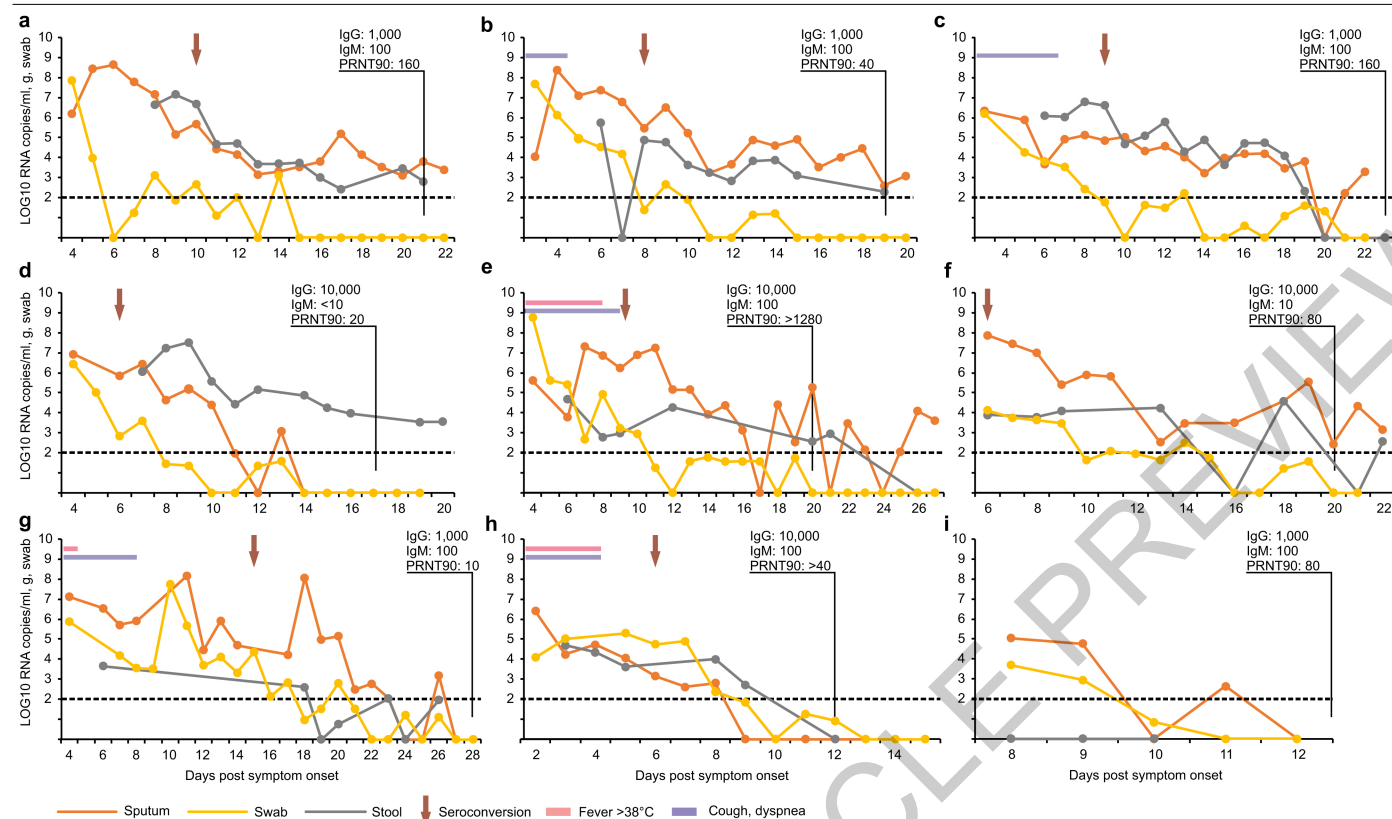


Fig. 2 | Viral load kinetics, seroconversion and clinical observations in individual cases. Panels A to I correspond to cases #1, #2, #3, #4, #7, #8, #10, #14, and #16 in Böhmer et al.¹¹ Dotted lines, limit of quantification. Experiments

were performed in duplicate and the data presented are means of results obtained by two laboratories independently.

Table 1 | Single nucleotide polymorphism at genome position 6446 in clinical samples from case #4

Day p.o.	5	6	7	8	9	10	11
Swab	A		A				
Sputum		G	G	G	G>A		
Stool			G>A	A=G	A=G	G>A	A

Table 2 | Clinical characteristics of all patients

PATIENT ID	COMORBIDITY	INITIAL SYMPTOMS	LATER SYMPTOMS	ANC/ μ l	ALC/ μ l	CRP (mg/l)	LDH (U/l)
#1	hypothyroidism	cough, fever, diarrhea	diarrhoea	4870	1900	46	197
#2	none	sinusitis, cephalgia, cough,	hyposmia, ageusia	3040	1200	4.9	182
#3	COPD	arthralgia, sinusitis, cough,	dysosmia, dgeusia	5040	2600	1.3	191
#4	none	otitis, rhinitis,	hyposmia, hypogeusia	2420	2220	5.9	149
#7	hyper-cholesterinemia	rhinitis, cough,	fever, dyspnea, hyposmia, hypogeusia	4690	900	4.9	209
#8	none	sinusitis, cough		2500	1600	1.7	203
#10	none	sinusitis, cough,	fever, cough	2350	700	7.8	220
#14	none	fever, cough, diarrhea		5040	1500	9.8	220
#16	none	none		4620	900	0.5	201

Abbreviations: ANC = absolute neutrophil count, ALC = absolute lymphocyte count, CRP = C-reactive protein, LDH = lactate dehydrogenase, M = male, F= female

Table 3 | IgG and IgM immunofluorescence titers against SARS-CoV-2 of all patients

PATIENT ID	INITIAL SERUM		FINAL SERUM				
	Day p.o.	IgG	Day p.o.	IgG	IgM	PRNT ₉₀	PRNT ₅₀
#1	5	<10	21	1,000	100	160	>640
#2	4	<10	19	1,000	100	40	320
#3	3	<10	23	1,000	100	160	>640
#4	5	<10	17	10,000	<10	20	160
#7	6	<10	20	10,000	100	>1280	>1280
#8	6	10	20	10,000	10	80	>320
#10	6	<10	28	1,000	10	10	>40
#14	na	na	12	10,000	100	>40	>40
#16	na	na	13	1,000	100	80	>320

Article

Methods

Clinical samples and viral load conversion

Sputum- and stool samples were taken and shipped in native condition. Oro- and nasopharyngeal throat swabs were preserved in 3 mL of viral transport medium. Viral loads in sputum samples were projected to RNA copies per mL, in stool to copies per g, and in throat swabs to copies per 3 mL, assuming that all sample components were suspended in the 3 mL viral transport medium. For swab samples suspended in less than 3 mL viral transport medium, this conversion was adapted to represent copies per whole swab. An aggregated overview of samples received per day post onset of disease from all patients is shown in Figure 1A.

RT-PCR for SARS-CoV-2 and other respiratory viruses

RT-PCR used targets in the E- and RdRp genes as described¹². Both laboratories used a pre-formulated oligonucleotide mixture (Tib-Molbiol, Berlin, Germany) to make laboratory procedures more reproducible. All patients were also tested for other respiratory viruses, including human coronaviruses (HCoV) -HKU1, -OC43, -NL63, -229E; Influenza virus A and B, Rhinovirus, Enterovirus, Respiratory syncytial virus, Human Parainfluenza virus 1-4, Human metapneumovirus, Adenovirus, and Human bocavirus using LightMix-Modular Assays (Roche, Penzberg, Germany). Additional technical details are provided in Section 1 in the Supplementary methods.

Virus isolation

Virus isolation was done in two laboratories on Vero E6 cells. 100 µl of suspended, cleared, and filtered clinical sample was mixed with an equal volume of cell culture medium. Supernatant was harvested after 0, 1, 3, and 5 days and used in RT-PCR analysis. Additional technical details are provided in Section 2a in the Supplementary methods.

Serology

We performed recombinant immunofluorescence assays to determine the specific reactivity against recombinant spike proteins in VeroB4 cells, as described^{26,28}. This assay used cloned CoV spike protein from HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, and SARS-CoV-2. The screening dilution was 1:10. Plaque reduction neutralization tests were done essentially as previously described for MERS-CoV²⁶. Serum dilutions causing plaque reductions of 90% (PRNT90) and 50% (PRNT50) were recorded as titers. Additional technical details are provided in Section 2b and 2c in the Supplementary methods.

Statistical Analyses

Statistical analyses were done using SPSS software (Version 25) or GraphPad Prism (Version 8).

Ethical approval statement

All patients provided informed consent to the use of their data and clinical samples for the purposes of the present study. Institutional review board clearance for the scientific use of patient data has been granted to the treating institution by the ethic committee at the Medical Faculty of the Ludwig Maximilians Universität Munich (vote 20-225 KB).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequence data are available in GISAID under accession number EPI_ISL_406862. All other data are available from the corresponding author upon reasonable request.

28. Corman, V. M. *et al.* Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. *Euro Surveill* **17**, <https://doi.org/10.2807/ese.17.49.20334-en> (2012).

Acknowledgements This work was funded by grants from the German Ministry of Research (01KI1723A) and the European Union (602525) to C. D. as well as by the German Bundeswehr Medical Service Biodefense Research Program. The funders had no role in study design, data collection and analysis or decision to publish. We thank Petra Mackeldanz, Elisabeth Möncke-Buchner, Anja Richter, Marie Schmidt, and Jörn Beheim-Schwarzbach for technical assistance.

Author contributions Roman Wölfel: Planned and supervised laboratory testing and evaluated data. Victor M. Corman: Planned and supervised laboratory testing and evaluated data. Wolfgang Guggemos: Managed patients and evaluated clinical data. Michael Seilmaier: Managed patients and evaluated clinical data. Sabine Zange: Performed laboratory testing. Marcel A. Müller: Managed serological laboratory testing. Daniela Niemeyer: Managed and performed virus isolation studies. Patrick Vollmar: Managed laboratory testing. Camilla Rothe: Managed initial patient contacts. Michael Hoelscher: Managed initial patient contacts and evaluated clinical data. Tobias Bleicker: Performed laboratory testing. Sebastian Brünink: Performed laboratory testing. Julia Schneider: Performed laboratory testing. Rosina Ehmann: Performed laboratory testing. Katrin Zwirgmaier: Performed laboratory testing. Christian Drosten: Designed and supervised laboratory studies, wrote the manuscript. Clemens Wendtner: Designed and supervised clinical management and clinical data.

Competing interests The authors declare no competing interests.

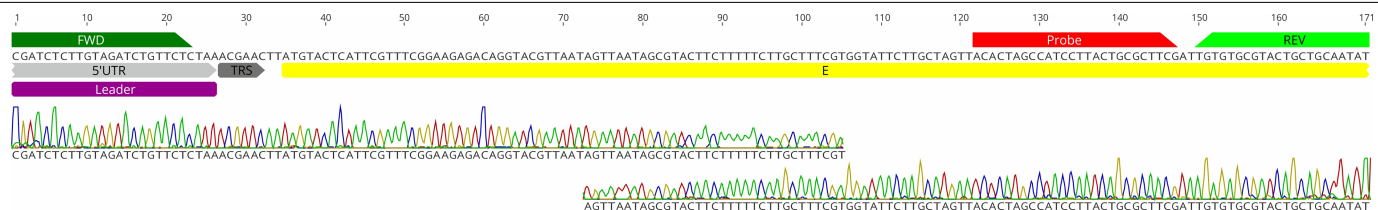
Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41586-020-2196-x>.

Correspondence and requests for materials should be addressed to C.D. or C.M.W.

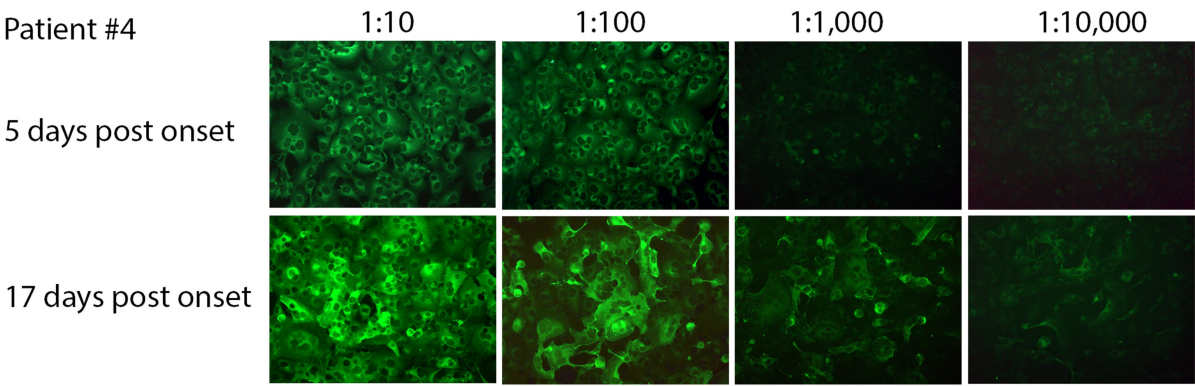
Peer review information Nature thanks Peter Openshaw and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at <http://www.nature.com/reprints>.



Extended Data Fig. 1 | Sequence analysis of E gene sgRNA. The leader sequence (in purple), putative transcription regulatory sequences (TRS, in gray), and nucleotides coding for the 5'-proximal part of the E gene (yellow box)

are shown. PCR primer binding sites used for amplification and RT-PCR detection are shown as green arrows, 5'-nuclease PCR probe as red arrow.



Extended Data Fig. 2 | Recombinant SARS-CoV-2 Spike-based immunofluorescence test shows seroconversion of patient #4.
Representative outcome of a recombinant immunofluorescence test using serum dilutions 1:10, 1:100, 1:1000 and 1:10,000 of patient #4 at 5 and 17 days

post onset of symptoms. Secondary detection was done by using a goat-anti human immunoglobulin labeled with Alexa488 (shown in green). Experiment was performed in duplicate.

Extended Data Table 1 | IgG immunofluorescence titers against endemic human CoVs

Patient ID	Primary serum					Final serum				
	Day p.o.	OC43	NL63	HKU1	229E	Day p.o.	OC43	NL63	HKU1	229E
#1	5	1,000	1,000	1,000	100	15	1,000	1,000	1,000	100
#2	4	1,000	1,000	100	100	13	10,000	100	1,000	10
#3	3	10,000	100	1,000	1000	16	10,000	1,000	10,000	1,000
#4	5	1,000	100	100	100	17	10,000	10	1,000	100
#7	6	1,000	100	1,000	1000	13	10,000	1,000	10,000	10,000
#8	6	1,000	100	1,000	1000	10	10,000	1,000	10,000	100
#10	6	1,000	100	100	1000	11	10,000	1,000	100	1,000
#14	na	na	na	na	na	5	100	100	100	100
#16	na	na	na	na	na	13	10,000	1,000	1,000	100

p.o. = post onset; na = not available; Increases of titer through the final serum are indicated by reciprocal titers in bold face

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

- | | |
|-----------------|--------------------------------|
| Data collection | Microsoft Excel, |
| Data analysis | SPSS, GraphPad Prism, Geneious |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data upon which figures and conclusions are based can be obtained from the corresponding author upon reasonable request. These data cannot be published in an open source because their interpretation may affect aspects of patient privacy.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- ☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Nine Patients.
Data exclusions	None.
Replication	Testing by two different laboratories.
Randomization	None.
Blinding	None.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems		Methods	
n/a	Involved in the study	n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies	<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines	<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology	<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms		
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants		
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data		

Antibodies

Antibodies used	Secondary anti-human IgG reagents (Euroimmun).
Validation	Immunofluorescence.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	In-house collection with reference to ATCC or DSZM.
Authentication	Functional testing for IFN locus inactivation.
Mycoplasma contamination	Regular testing.
Commonly misidentified lines (See ICLAC register)	N/A

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	N.A.
Recruitment	Clinical admission due to symptoms, contact history, and positive initial test.
Ethics oversight	Research ethics board of Ludwig Maximillians University Munich; informed consent to scientific use and publication of anonymized data by each patient.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)
All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	N.A.
Study protocol	No study protocol emergency admissions of patients with new disease.
Data collection	Data collection at treating hospital, and two laboratories as identified in affiliations list.
Outcomes	Laboratory and clinical status. No explicit outcome measure.