SARS-CoV2 persistent viral shedding in the context of hydroxychloroquine-azithromycin treatment

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Abstract:

SARS-CoV-2 nasopharyngeal shedding contributes to the spread of the COVID epidemic. Among 3,271 COVID-19 patients treated at the Hospital University Institute Méditerranée Infection, Marseille, France from March 3 to April 27, 2020, tested at least twice by RT-PCR, the median SARS-CoV-2 nasopharyngeal shedding was 6 days (range 2-54 days). Compared with short shedders (qRT-PCR positivity < 10 days), 34 (1.04%) persistent shedders (qRT-PCR positivity ≥ 17 days; mean ± SD: 23.3 ± 3.8 days) were significantly older, with associated comorbidities, exhibiting lymphopenia, eosinopenia, increased D-dimer and increased troponin ($p<0.05$), and were hospitalized in intensive care unit in 17.7% vs. 1.1% cases ($p<0.0001$). Viral culture was positive in 6 persistent shedders after day 10, including one patient after day 17, and no viral co-pathogen was detected in 33 tested patients. Persistent shedders received azithromycin plus hydroxychloroquine ≥ 3 days in 26/34 (76.5%) patients, a figure significantly lower than in short shedders (86.6%) ($p=0.042$). Accordingly, mortality was 14.7% vs. 0.5% ($p<0.0001$). Persistent shedding was significantly associated with persistent dyspnea and anosmia/ageusia ($p<0.05$). In the context of COVID-19 treatment, including treatment with azithromycin plus hydroxychloroquine, the persistence of SARS-CoV-2 nasopharyngeal shedding was a rare event, most frequently encountered in elderly patients with comorbidities and lacking azithromycin plus hydroxychloroquine treatment.

Keywords: SARS-CoV-2; COVID-19; viral persistence; culture; RT-PCR; hydroxychloroquine; azithromycin.

1. Introduction

SARS-CoV-2 responsible for COVID-19, is detected in the nasopharynx, which could constitute a major portal of entry for this emerging pathogen [1]. Indeed, not only is SARS-CoV-2 RNA routinely detected in nasopharyngeal swabs of COVID-19 patients [2], but this clinical material yields living virus after it is appropriately cultivated in cell culture systems [3, 4]. These data suggest that SARS-CoV-2 nasopharyngeal shedding is of interest for the natural history of COVID-19 in patients and in populations, as it may relate to the prognosis of the infection and its contagiousness. Two meta-analyses, including respectively 79 and 28 studies, converged to indicate a viral shedding duration of 17 days (mean) and 18.4 days (median), respectively [5, 6].

Monitoring more than 3,200 COVID-19 patients firmly documented by RT-PCR at the Hospital University Institute Méditerranée Infection, Marseille, France in March-April 2020 provided the opportunity to clarify the clinical and virological characteristics of patients with persistent nasopharyngeal shedding of SARS-CoV-2 [2].

2. Materials and Methods

2.1. ***Patients.*** This retrospective study aimed to describe the duration of SARS-CoV-2 viral shedding among 3,737 real-time RT-PCR (qRT-PCR)-confirmed COVID-19 patients followed from March 3 to April 27, 2020 in the Méditerranée Infection Institute, as previously reported [2]. Clinical, radiological and laboratory data were collected, as previously reported [2, 7] and the severity of patient illness was evaluated using the National Early Warning Score version 2 (NEWS-2) and the Charlson score [8]. All patients

without contraindications were offered oral hydroxychloroquine (HCQ) (200 mg, three times a day for ten days) and azithromycin (AZ) (500 mg at day 1, followed by 250 mg per day for 4 days), and ceftriaxone or ertapenem was added for seven days in case of NEWS-2 ≥ 5 [9, 10]. Initiation of medical care (i.e., medical consultation and initiation of treatment) was defined as day 0, outpatient reevaluation was offered at day 2, 6 and 10 (more if needed), while hospitalized patients were evaluated twice daily.

2.2. ***Virology.*** All patients were SARS-CoV-2-diagnosed based on at least one positive RT-PCR test performed by nasopharyngeal swab. SARS-CoV-2 genome sequencing was performed directly from nasopharyngeal swab RNA extract using the Illumina MiSeq sequencer and Nextera XT paired-end technology (Illumina, Evry, France) and genome sequences were compared with the reference SARS-CoV-2 isolate Wuhan Hu-1, genome sequence (NC 045512.2) [11]. In case of qRT-PCR cycle threshold (Ct) value > 18, partial spike gene PCR amplification (nucleotides 21,296-23,424 in reference to NC 045512.2) was performed, as previously described [DOI:https://doi.org/10.35088/4y1e-ec62]. Viral cultures were performed as previously described [3, 4]. Patients for whom the qRT-PCRs became negative within 10 days were qualified as short viral shedders, patients with positive qRT-PCRs between day 10 and 17 as long viral shedders and patients with positive qRT-PCRs after day 17 as persistent viral shedders.

2.3. ***Co-pathogen detection***. Co-pathogens in the nasopharyngeal swabs were screened by using a multiplex test incorporating 21 targeted pathogens, following the instructions of the supplier (FTD Respiratory pathogens 21; Siemens Fast Track Diagnosis, Luxembourg).

2.4. ***Statistical analysis*****.** We used the Student t-test, Mann-Whitney U test, Chi2 test, or Fisher's exact test to compare differences between short viral shedding and persistent viral shedding groups. To explore risk factors associated with persistent viral shedding, we performed multivariable analyses using logistic regression models. All variables significant at $p<0.10$ in univariate analyses were introduced in the initial multivariate model. A stepwise approach was then used to assess the iteration of variables and to control potential confounders (both significance level values for entry and stay were set at 0.05). A primary model was performed over the full sample and a secondary model was performed among patients aged 65 years and older [12]. A secondary analysis using a multivariable logistic regression model was also performed to identify risk factors associated with prolonged COVID-19 symptoms. The same stepwise approach was applied to this secondary outcome. A two-sided α of less than 0.05 was considered statistically significant. All analyses were carried out using SAS 9.4 statistical software (SAS Institute, Cary, NC).

2.5. ***Ethics statement*****.** Data were collected retrospectively from the routine care setting. This non-interventional retrospective study was approved by our institutional review board committee (Méditerranée Infection No.:

2020–021). In compliance with European General Data Protection Regulation No. 2016/679, patients were informed of the potential use of their medical data and that they could refuse the use of their data. The analysis of collected data followed the reference methodology MR-004 registered on No. MR 5010010520 in the AP-HM register, in compliance with European General Data Protection

3. Results

3.1. ***Population description*****.** Among 3,737 COVID-19 patients followed at the Hospital University Institute Méditerranée Infection between March 3 and April 27 [2], 3,271 patients had at least 2 positive SARS-CoV-2 RT-PCRs performed within less than 10 days, had a median duration of shedding of 6 days (range, 2-54 days): in detail, 2,800 (85.6%) patients were short shedders, with a 4.8 ± 3.1-day (mean ± SD) shedding; 437 (13.3%) patients were long shedders, with a 15.2 ± 3.8-day shedding; and 34 (1.04%) patients were persistent shedders, with a 23.3 ± 3.8-day shedding (Table 1). Compared to short shedders, persistent shedders were significantly ($p < 0.01$) older (≥ 65 years old), although the age range was 21-93 years, and 13/34 patients were < 45 years old, with age-related comorbidities including chronic heart disease, hypertension, and a NEWS-2 ≥ 5, and a Charlson score ≥ 5, indicative of an 85% probability of death in 13/34 (38.2%) patients and subsequent hospitalization (Table 1). Furthermore, the initial laboratory check-up of persistent shedders indicated a higher neutrophil/lymphocyte ratio, eosinopenia and higher D-dimer, troponin and C-reactive protein levels compared to short shedders

(Table 2). Multivariate logistic regression indicated that persistent viral shedding was associated with hospitalization, chronic heart disease and eosinopenia <0.04 G/L.

3.2. ***Care and treatment*****.** A higher percentage of persistent shedders was hospitalized in intensive care unit compared to short shedders, 17s.7% vs. 1.1% ($p < 0.0001$). In this cohort, 26/34 (76.5%) persistent shedders had HCQ-AZ therapy ≥ 3 days versus 2,426/2,800 (86.6%) short shedders ($p=0.042$, Mantel-Haenszel Chi-2 test). Indeed, 8/34 persistent shedders had HCQ-AZ therapy < 3 days: more precisely, one patient refused HCQ and AZ treatment, and seven other patients received AZ only because HCQ was contraindicated due to cardiac contraindications (n=6) and drug interaction (n=1) (Table 1). Persistent viral shedding was also associated with persistent dyspnea 30 days after the onset of follow up. Mortality was 14.7% among persistent shedders vs. 0.5% among short shedders ($p<0.0001$). At ~ 9-10-month follow-up in January 2021, 5/34 persistent shedders had died and 7 had been seen in consultation: three patients had persistent post-COVID-19 dyspnea, including one patient who had persistent ageusia. Kinetics analysis showed that eosinophil and lymphocyte counts were significantly lower in patients with persistent viral shedding than in those with short viral shedding, whereas neutrophils were higher in persistent viral shedders. D-dimers and troponin were significantly and persistently higher in persistent viral shedders.

3.3. ***Viral genotype analysis*****.** A total of 21 complete (n=11) or partial (approximately the first half of the spike-encoding gene, n=10) genome sequences were obtained for the 34 persistent shedders, comprising 14 classified in Nextstrain clade 20A, 3 in clade 20B and 4 in clade 20C [13]. This distribution into viral clades did not differ from that observed in non-persistent viral shedders during the same period (310 in clade 20A, 55 in clade 20B and 94 in clade 20C, for a total of 466 genome sequences). All spike sequences harbored the D614G substitution that was present in almost all SARS-CoV-2 genomes in Europe since the epidemic onset. No additional mutations were present within the spike.

3.4. ***Viral culture*****.** A total of 177 nasopharyngeal swabs were cultured in the 34 patients, of which 69 swabs collected in 20 patients were positive in culture. More precisely, eight swabs collected in six patients had a positive culture > 10 days after the onset of follow-up, at day 11, 13, 14, 15, 17 and 33 (Figure 1).

4. Discussion

In a large series of RT-PCR-documented COVID-19 cases followed at the Méditerranée Infection Institute in March and April 2020, the median SARS-CoV-2 nasopharyngeal shedding was six days, shorter than that reported in the literature [14]. Also, less than 1% of cases (34/3,737) exhibited shedding ≥ 17 days, and persistent positivity of qRT-PCR in nasopharyngeal swabs correlated with culture positivity. We observed positive cultures more than 10 days after the onset of the follow-up in 8 of 34 patients, a situation rarely reported, as nasopharyngeal shedding is usually reported on the sole basis of qRT-PCR positivity [6]: one case of 4-month

viral shedding was reported in the absence of culture and contagiousness [15], whereas anecdotal persistent viral shedding with up to 60-days positive culture has been reported in cancer patients receiving chemotherapy [16].

Previous case reports and a small series of patients experienced prolonged shedding with SARS-CoV-2 (Table 3)[17-35], mostly involving immunocompromised individuals, including patients diagnosed with hematological malignancies in most cases. This was not our situation, where only one patient had lymphoma and another immunosuppression following kidney transplantation. In addition, several cases of viral shedding beyond 90 days occurred in patients who received treatments with convalescent plasma and/or remdesivir [17, 18]. These situations were not encountered in this series, as no patient received remdesivir or convalescent plasma and the figures reported here were observed in the context of standardized care, including the prescription of hydroxychloroquine and azithromycin treatment. It is noteworthy that almost one quarter of the 34 persistent shedders reported here did not receive the combination of hydroxychloroquine and azithromycin, a prevalence significantly higher than that in short shedders. This standardized care notably differed in other series reporting a higher proportion of persistent shedders, and this observation suggests that the combination of hydroxychloroquine plus azithromycin at the dosage prescribed may participate in reducing the time of SARS-CoV-2 nasopharyngeal shedding.

This reported series mirrors the first COVID-19 epidemic in our region, which was caused by SARS-CoV-2 of clades 20A, 20B and 20C. Accordingly, population next-generation sequencing, we did not observe any genotype pattern in any of the 21 patients whose viral genome could be explored. Further ultradeep sequencing of paired early and late nasopharyngeal swabs in the 34 patients here reported may

nevertheless reveal quasi-species and minority genotypes that may have escaped the standard genotyping methods used in this report.

Whether the data here reported on the genetic context of SARS-CoV-2 circulating in our region one year ago would apply to that of SARS-CoV-2 variants responsible for current epidemics [36] remains to be explored.

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ACKNOWLEDGEMENTS.

None.

COMPETING INTERESTS.

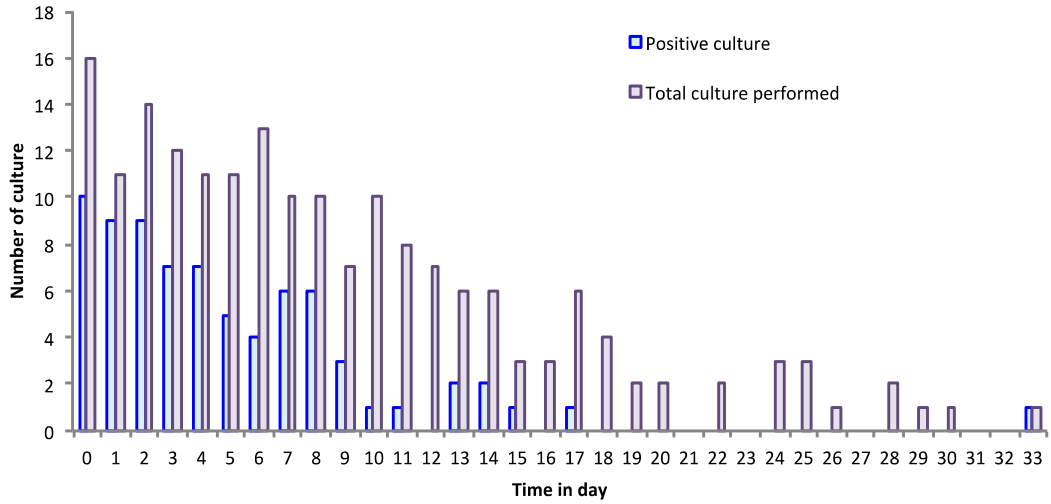
None to declare.

FUNDING.

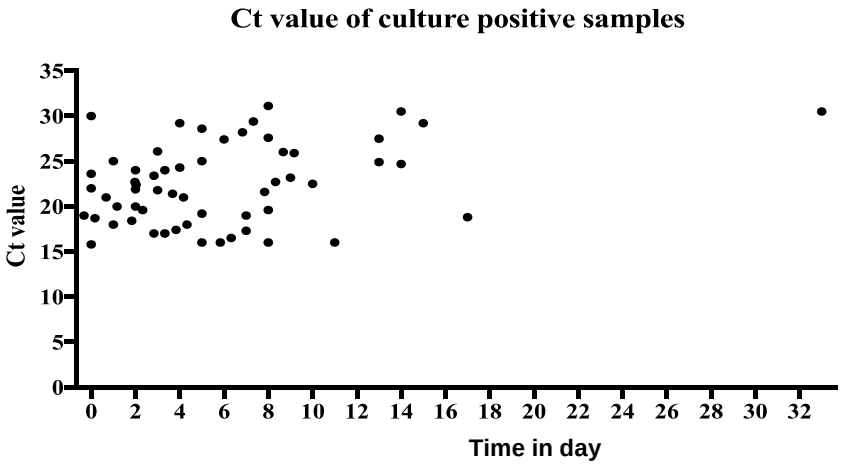
This work was funded by ANR “Investissements d’avenir”, Méditerranée infection 10-IAHU-03, and was also supported by the Région Provence-Alpes-Côte d’Azur. This work received financial support from the Mediterranean Infection Foundation.

Figure 1. Culture results on 34 patients with persistent viral shedding (>17 days)

A. Number of positive culture for SARS-CoV-2 among the number of culture performed



B. SARS-CoV-2 cycle threshold values of samples with positive culture



Ct, cycle threshold value; 11 among the 69 samples had positive SARS-CoV-2 culture without Ct value

415 **Table 1.** Summary of 34 COVID-19 patients with prolonged SARS-CoV-2 viral shedding ≥ 17 days, Marseille, France, March-April

416 2020.

Age (years), gender	Duration of viral shedding (days)	Cause of immunodepression	Serology	NEWS-2 score	Charlson (probability of death in the following year)	Treatment	Death (days), COVID-19-imputable	Genotype (amino acid substitutions or deletions in the spike)
60,M	24	-	+	5	2 (26%)	HCQ-AZ	-	N.a.
41, M	23	-	+	6	2 (26%)	HCQ-AZ	-	20A
21, M	20	-	N.a.	2	0 (12%)	HCQ-AZ	-	20C
41, M	20	-	+	0	1 (26%)	HCQ-AZ	-	20A
30, F	18	-	-	3	0 (12%)	HCQ-AZ	-	N.a.
43, M	19	-	N.a.	4	1 (26%)	HCQ	-	N.a.
37, M	21	-	+	0	0 (12%)	HCQ-AZ	-	20B
37, F	17	-	+	2	0 (12%)	HCQ-AZ	-	20A/25563T/2416T/8371T
74, M	25	-	N.a.	9	8 (85%)	AZ	-	20A
63, M	18	-	-	4	4 (52%)	HCQ-AZ	-	20A
61, F	40	-	+	4	3 (52%)	HCQ-AZ	-	20A
81, M	25	-	-	6	7 (85%)	AZ	-	N.a.
47, F	21	-	+	2	1 (26%)	HCQ-AZ	-	20B
93, F	17	-	N.a.	4	7 (85%)	AZ	-	N.a.
89, F	20	-	+	8	5 (85%)	HCQ-AZ	22, Yes	20A
28, F	26	-	+	2	0 (12%)	HCQ-AZ	-	20A
56, F	54	-	+	2	2 (26%)	HCQ-AZ	-	N.a.
69, M	31	-	N.a.	5	3 (52%)	HCQ-AZ	-	20C
71, M	18	-	+	7	5 (85%)	No HCQ, No AZ	-	N.a.
30, F	21	-	N.a.	5	0 (12%)	HCQ-AZ	-	20A/25563T/2416T/8371T
52, F	20	-	+	4	2 (26%)	HCQ-AZ	-	20A
88, F	17	-	+	7	7 (85%)	AZ	-	20A
70, F	17	-	+	8	6 (85%)	HCQ	289, No	20B
82, M	18	-	N.a.	4	7 (85%)	HCQ-AZ	-	N.a.
43, F	17	-	+	0	1 (26%)	HCQ	-	20C
69, M	19	-	N.a.	6	3 (52%)	HCQ-AZ	-	20A
90, F	25	-	N.a.	6	6 (85%)	HCQ-AZ	51, Yes	20A
76, M	23	-	+	11	5 (85%)	HCQ-AZ	-	N.a.
89, M	21	-	N.a.	12	7 (85%)	HCQ-AZ	-	20C
43, M	19	Lymphoma	+	4	3 (52%)	HCQ-AZ	-	N.a.
73, M	19	-	+	8	7 (85%)	HCQ-AZ	20, Yes	N.a.
64, F	22	-	+	2	3 (52%)	HCQ-AZ	-	N.a.
34, F	23	-	-	2	0 (12%)	HCQ-AZ	-	N.a.
69, F	25	Kidney transplantation	-	3	7 (85%)	HCQ-AZ	-	20A

417 N.a., not available; . HCQ-AZ stands for treatment combining hydroxychloroquine plus azithromycin ≥ 3 days (refer to text for posology).

418 **Table 2.** Comparison biological data for 2,800 short shedders (nasopharyngeal
419 SARS-CoV-2 RT-PCR positivity < 10 days) and 34 persistent shedders
420 (nasopharyngeal SARS-CoV-2 RT-PCR positivity ≥ 17 days).
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	Short viral shedders n=2,800		Persistent viral shedders n=34	
Biological data	N	Mean (std)	N	Mean (std)
Lymphocytes (G/L)	2,358	1.44 (0.64)	31	1.05 (0.47)*
Neutrophils (G/L)	2,285	3.37 (1.8)	27	4.42 (2.6)
Neutrophils/lymphocytes	2,285	2.84 (3.19)	27	4.71 (3.44)*
Eosinophils (G/L)	2,347	0.08 (0.09)	31	0.04 (0.06)*
D dimers (µg/mL)	457	0.99 (2.19)	19	1.25 (1.01)*
Troponin (ng/L)	246	9.99 (12.18)	16	18.71 (18.79)*
CRP (mg/L)	2,13	16.31(34.6)	31	31 (48.09)*

422 CRP, C-reactive protein; * denotes statistical significance using the Chi-square, Fisher's
423 exact or Wilcoxon-Mann-Whitney test where appropriate and a 0.05 P value.
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Table 3. Epidemiological, virological, and clinical features of cases of prolonged SARS-CoV-2 infections in immunocompromised patients.

Reference	Gender, age (years)	Immunodepression cause	Duration of viral shedding	Remdesivir	Convalescent plasma or anti-spike antibodies	Other therapies(s)	Number of amino acid substitutions/deletions in the genome and/or in the spike protein	Outcome
17	Male, 45 y.-o.	Severe antiphospholipid syndrome	151 days	Days 0-4, 72-81, 105-109, 151-155	ASA: day 143	Glucocorticoids, cyclophosphamide, intermittent eculizumab and rituximab, ruxolitinib	24 substitutions, 3 deletions (spike: 12 substitutions, 1 deletion) among which substitution N501Y present in variants 20I/501Y.V1, 20H/501Y.V2 and 20J/501Y.V3[b], and E484K present in variants 20H/501Y.V2 and 20J/501Y.V3[b]	Death on day 154
18	Not reported	Marginal B cell lymphoma (received B cell depletion therapy; hypogammaglobulinemia)	101 days	Days 41-, 54-, 93-	CP: days 63, 65, 95	-	Spike: 5 substitutions among which N501Y and deletion H69/V70 both present in 20I/501Y.V1[b]	Not reported
19	Male, 60 y.-o.	Mantle cell lymphoma	156 days	Days 30-, 122-	CP: days 33, 122	CD20 bispecific antibody, second B-cell directed antibody, cyclophosphamide, doxorubicine, prednisone	6 substitutions	Pursued home hospice care
20	Male, 75 y.-o.	Multiple myeloma	71 days	Days 5-9	CP: days 2, 58	Dexamathasone (days 63-74)	Spike: 9 substitutions between days 4 and 67, including D215G present in 20H/501Y.V2[b], Y144 deletion present in 20I/501Y.V1[c], and N501T at a position mutated in variants 20I/501Y.V1, 20H/501Y.V2 and 20J/501Y.V3[b]	Death on day 74
21	Male, 60-70 y.-o.	Non-Hodgkin lymphoma	268 days	Days 47-51, 77-86, 178-182, 205-209	CP: day 88	Darunavir/ritonavir, hydroxychlorquine, IV methylprednisolone, tocilizumbab, ceftaroline	26 substitutions; spike: 7 including H69Y/P and V70G at positions mutated in variant 20I/501Y.V1[b]	Death on day 271
22	Female, 53 y.-o.	Follicular lymphoma	85 days	Days 63-72, 80-84	CP: day 85[c]	-	No genome sequencing reported	

Table 3 - *Continued*

Reference	Gender, age (years)	Immunodepression cause	Duration of viral shedding	Remdesivir	Convalescent plasma or anti-spike antibodies	Other therapies(s)	Number of amino acid substitutions/deletions in the genome and/or in the spike protein	Outcome
23	Female, 17 y.-o.	Pre-B-cell acute lymphoblastic leukemia	100 days	Days 13-22, days 60-69	CP: day 61	Hydroxychloroquine for two days; methylprednisolone	No genome sequencing reported	qPCR-positive on day 100; no supplemental oxygen
24	Male, 50-60 y.-o.	Chronic lymphocytic leukemia	63 days	Days 23-33, days 45-55	CP: day 58	-	No genome sequencing reported	
25	Female, 41 y.-o.	Severe hypogammaglobulinemia	75 days	No	CP: days 71, 72	Prednisone	No genome sequencing reported	Discharge
25	Male, 65 y.-o.	Common variable immunodeficiency	40 days	No	No	Lopinavir/ritonavir, broad-spectrum antibiotics	No genome sequencing reported	Death on day 40
26	Female, 70-79 y.-o.	Follicular lymphoma	>134 days	No	CP: ≈days 45, 65, 95, 110, and 115	Steroids	24 substitutions, 2 deletions; spike: 3 substitutions including E484K present in variants 20H/501Y.V2 and 20J/501Y.V3[b]; one deletion Y144 present in variant 20I/501Y.V1[c]	Death on day 156
26	Not reported	B-cell depleted lymphoma	91 days	No	No	N.a.	At day 19 post-diagnosis: 2 substitutions	Recovery
27	Female, 71 y.-o.	Chronic lymphocytic leukemia, hypogammaglobulinemia	105 days	No	CP: days 70, 80	-	6 substitutions and 1 deletion on day 49; spike: 2 substitutions; 3 additional substitutions (2 at day 70, 1 at day 85) and one additional deletion on day 70 in the spike	N.a.
28	Female, 47 y.-o.	Follicular lymphoma	59 days	No	No	Obinutuzumab bimonthly, acyclovir, atovaquone, favipiravir, ciclesonide, lopinavir/ritonavir	No genome sequencing reported	Discharge on day 69

Table 3 - *Continued*

Reference	Gender, age (years)	Immunodepression cause	Duration of viral shedding	Remdesivir	Convalescent plasma or anti-spike antibodies	Other therapies(s)	Number of amino acid substitutions/deletions in the genome and/or in the spike protein	Outcome
29	Not reported, median (range), 58 y.-o. (35-77)	Hematological malignancies (n= 15); multiple sclerosis (1); common variable immune deficiency (1)	17 patients (median duration= 56 days; max.= 83 days)	N= 3	No	Anti-CD20 monoclonal antibodies (n= 15); steroids (8); hydroxychloroquine (n= 5); tocilizumab (n= 4); lopinavir/ritonavir (n= 2)	No genome sequencing reported	One death
30	Male, 66 y.-o.	HIV infection (CD4 cell count= 0/mm^3)	123 days	No	No	Multi-antiretroviral therapy	1 substitution, in the spike	Neurological degradation
30	Male, 71 y.-o.	Heart transplantation, diabetes mellitus	121 days	No	No	Prednisone, mycophenolic acid, belatacept	No occurrence of substitutions	N.a.
30	Male, 35 y.-o.	Rheumatoid arthritis	84 days	No	No	Rituximab	Occurrence of 6 substitutions, 1 the spike	Improvement
31	Female, 5 y.-o.	Dermatomyositis	35 days	No	No	Prednisolone	No genome sequencing reported	Resolution
32	Female, 60 y.-o.	Rheumatoid arthritis	>35 days	Day ≈30	CP: Week 5	Rituximab	No genome sequencing reported	Discharge
33	Female, 17 y.-o.	Previously healthy	97 days	No	No	Hydroxychloroquine for 5 days	Coinfection with two SARS-CoV-2 lineages (20A, 20B)	N.a.
34	Male, 61 y.-o.	Liver transplant	Negative on days 35 and 39, then positive again on days 41 and 48	No	No	Tacrolimus, lopinavir/ritonavir, amoxicillin, piperacillin sulbactam, Lianhua Qingwen	No genome sequencing reported	Discharge on day 55
35	Male, 31 y.-o.	X-linked agamma-globulinaemia	62 days (in sputum; 36 days in nasopharyngeal samples)	Days 34-43, 61-70	CP: days 69, 70	Hydroxychloroquine/ azithromycine, meropenem, ceftriaxone, clarithromycin	5 substitutions; spike: 1 substitution	Discharge on day 73

[a] As assessed by qPCR; [b] 20I/501Y.V1= “UK” variant, 20H/501Y.V2= “South African” variant, and 20J/501Y.V3= “Brazilian” variant; [c] at the end of second cure of remdesivir

ASA : anti-spike antibodies; CP: convalescent plasma; PML, progressive multifocal leukoencephalopathy