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SARS-CoV-2 in an immunocompromised host: convalescent plasma therapy and viral evolution elucidated by whole genome sequencing

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bcr-2023-255255).

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Accepted 18 November 2023

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To cite: Seth-Smith H, Vesenbeckh S, Egli A, *et al*. *BMJ Case Rep* 2023;**16**:e255255. doi:10.1136/bcr-2023-255255

SUMMARY

The evolution of SARS-CoV-2 within immunocompromised hosts who fail to clear the virus over many months has been proposed as a route to the development of Variants of Concern (VoCs). We present a case of an immunocompromised male patient with a prolonged SARS-CoV-2 infection. During hospitalisation, 7 weeks after first diagnosis, his condition worsened to require continuous ventilation support. Resolution of symptoms was observed after convalescent plasma therapy. Whole genome sequencing of the virus showed Pango lineage B.1.221. Between the first sample and the second from bronchoalveolar lavage fluid 7 weeks later, we identified eight mutations, including minor variants, which could be used to estimate the chronology of mutations. This suggests an elevated mutation rate, in-host accumulation of mutations and further evidence for sources of VoCs. Prolonged SARS-CoV-2 infections in immunocompromised hosts increase the likelihood of hospital stays and morbidity, and also pose an increased risk to global public health.

BACKGROUND

In 2019 the new coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2) started spreading around the globe causing a new disease called COVID-19 (coronavirus disease 2019). Over the intervening years, the virus has evolved and is commonly described in categories of Pango lineages.[1] SARS-CoV-2 commonly causes respiratory infections that are usually self-limiting and often oligosymptomatic or even asymptomatic; if symptoms occur, they typically last for no longer than 2 weeks.[2] Several risk factors, including immunosuppressive conditions, increase the risk of more severe COVID-19 with pneumonia and respiratory failure.[3 4]

The virus is typically cleared from the host within 14 days.[5–7] In cases where the virus is detected again in the same patient, whole genome sequencing (WGS) can be used to distinguish between the scenarios of reinfection from a different source, or ongoing infection from the original virus.[8] The latter has been seen in immunocompromised hosts, where infections are often described as lasting longer, with virus detected in some cases over several months.[9–21] The prolonged infections, sometimes in conjunction with convalescent plasma (CCP) therapy, may lead to specific selective pressures and even increased mutation of the virus.[15 22] It has been suggested that such patients increase the risk of the development of immune escape mutants, particularly affecting the S gene encoding the surface spike protein. It has been proposed that such patients may be the source of variants of concern and all descending lineages, such as Alpha (B.1.1.7), Beta (B.1.351), Gamma, (P.1), Delta (B.1.617.2 and AY descendants) and Omicron (B.1.1.529 and BA descendants).[8 13 23 24] Due to the potential emergence of new variants, monitoring of patients infected for several months is important.

We present the case of an immunocompromised host with a long-term SARS-CoV-2 infection, treated with dexamethasone, CCP and remdesivir. The genomic dynamics over 7 weeks were elucidated by WGS from bronchoalveolar lavage fluid (BALF).

CASE PRESENTATION

A Caucasian man in his 70s was admitted to our specialised COVID-19 treatment unit in early 2021 with general weakness, elevated temperature and shivering for 4 weeks, complicated by a dry cough and dyspnoea for 8 days. He had been diagnosed with oligosymptomatic SARS-CoV-2 infection 50 days earlier (=d −50), based on SARS-CoV-2 specific nucleic acid testing (NAT) on a nasopharyngeal swab which gave a Ct value of 21.7 (sample 301120, d −50; Roche-Cobas-SARS-CoV-2 target 1).[7] His only symptom at that time was a light taste and smell disorder and he remained well during 10 days of self-isolation at home without any specific therapy. He had not received SARS-CoV-2 vaccination, as the vaccine was first authorised in Switzerland in December 2020 and roll-out progressed from January 2021.

At the presentation he was tachypnoeic with crackles on auscultation. Initial leucocyte count was normal (5.8 G/L, normal value range 3.2–10), procalcitonin low (0.13 ng/mL, ref. <0.1) and C-reactive protein elevated to 82 mg/L (ref. <5 mg/L). The chest CT scan showed multiple bilateral infiltrates suggestive of COVID-19 pneumonia (figure 1A).

On admission, two nasopharyngeal swabs tested negative for SARS-CoV-2 on NAT (Cepheid XPRSARS-CoV2-10, target E, N2) and no SARS-CoV-2 antibodies were detected, using a qualitative ElectroChemiLuminescence ImmunoAssay (ECLIA) based on nucleocapsid protein (Roche, Elecsys anti-SARS-CoV-2). A bronchoalveolar lavage (BAL) obtained on the same day yielded a positive SARS-CoV-2 NAT result with a Ct value of 17.3 (sample

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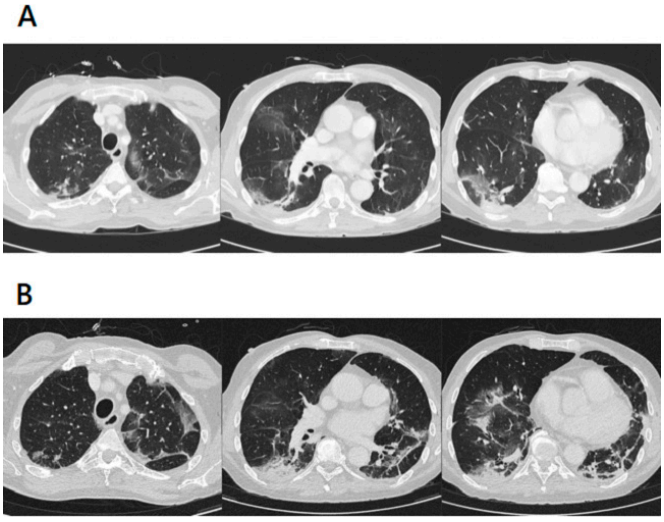

Figure 1 Thoracic CT scans. (A) First CT scan at initial presentation day 1 showing typical COVID-19 findings: multifocal, bilateral ground-glass opacities with superimposed interlobular septal thickening and consolidations in lung regions close to visceral pleural surfaces. (B) Repeat CT scan on day 13 showing worsening of the bilateral ground glass infiltrates and progressive consolidation of the right lower lobe, suggesting bacterial superinfection. Figure 1 by Silvan M Vesenbeckh.

180121, d1, Roche Cobas LIAT SARS-CoV-2 and Influenza A/B, target ORF1ab/N1).

The patient was admitted to the isolation ward and treated with oral dexamethasone 6 mg daily and nasal oxygen. On the following day, a procalcitonin of 49 ng/mL developed with neutrophilic leucocytosis, so that a bacterial superinfection was suspected and antibiotic treatment with intravenous amoxicillin/clavulanic acid 3×2.2 g was initiated for 5 days. On day 3 of admission, he required non-invasive mechanical ventilation and was transferred to our intensive care unit. Over 14 days the fever and laboratory signs of inflammation persisted and the patient remained hypoxic when left without intermittent ventilation support. A repeat chest CT scan on day 13 after admission showed worsening of the bilateral ground glass infiltrates and slightly progressive consolidation of the right lower lobe (figure 1B).

On day 18 after admission the patient required intubation, and a repeat bronchoscopy with BAL on the following day showed persistently high viral levels of replication (Ct 25.2, Roche Cobas LIAT SARS-CoV-2 and Influenza A/B, target ORF1ab/N1) and a viraemia of 1550 copies/mL was found (in-house validated quantitative QNAT based on S gene target). SARS-CoV-2 specific antibodies were still below the level of detection, suggesting that the patient was unable to mount a specific humoral response to SARS-CoV-2.

No concomitant respiratory infection was detected from neither of the two BALs that were performed on day 1 and day 18 after admission. The BIOFIRE Respiratory 2.1 panel (Biomérieux) did not show any evidence of additional viral (adenovirus, coronaviruses 229E/HKU1/NL63/OC43, human metapneumovirus, rhinovirus/enterovirus, influenza A/B virus, parainfluenza virus 1–4, respiratory syncytial virus) or bacterial (*Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*) pathogens. The bacterial, mycobacterial, fungal (including dimorphic fungi) as well as nocardia cultures did not show any growth. *Pneumocystis jirovecii* was evaluated using immunofluorescence and toluidine blue staining, and remained negative. A cystitis was suspected and treated with Ceftriaxone 1×2 g initially but then escalated to piperacillin/tazobactam 3×4.5 g to cover for potential late onset hospital acquired pneumonia and aspiration. Later on, *Pseudomonas aeruginosa* was detected in urine and treated with a cycle of high dose piperacillin/tazobactam 4×4.5 g until treatment failure occurred, and meropenem 3×2 g was started (figure 2).

One day after intubation, the medical team decided to administer CCP as an individual healing attempt according to SAMW (Swiss Academy of Medical Sciences) guidelines.[25] We adjusted a study protocol suggested by Hueso *et al*,[26] and administered 200 mL CCP on two consecutive days (days 19 and 20 after admission). Each plasma was pathogen inactivated using the INTERCEPT blood system (Cerus Corporation, Concord, California, USA).[27] Titres of 2.77 (plasma 1) and 4.21 (plasma 2) were detected using the Euroimmun IgG ELISA (>3.5 considered a high titre according to Food and Drug Administration at that time[28]). Acceptable titres of 5.47 of SARS-CoV-2 neutralising antibodies were additionally confirmed in the first plasma using an ACE dependent NAT quantitative assay.[29] After 5 days the patient was successfully extubated on day 23 after

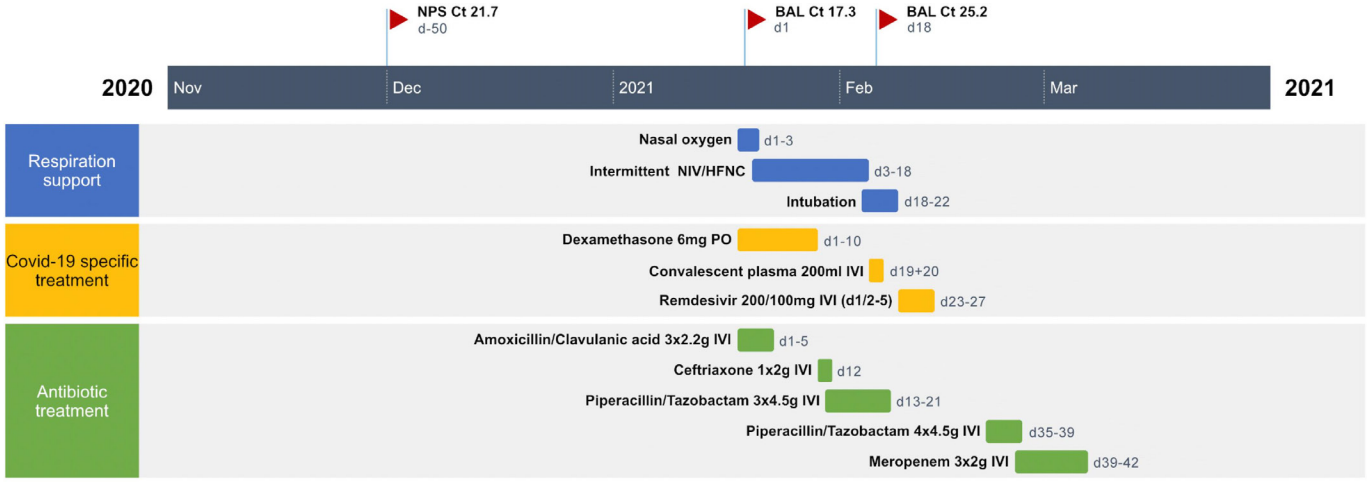

Figure 2 Timeline depicting time points of respiratory samples and time periods of applied therapies. BAL, bronchoalveolar lavage; Ct, cycle threshold; d1, day of presentation to the hospital; HFNC, high flow nasal cannula; IVI, intravenous infusion; NPS, nasopharyngeal swab; PO, per oral. Figure 2 by Silvan M Vesenbeckh.

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admission and gradually weaned from the ventilator. Remdesivir (200 mg loading dose, then 100 mg daily) was administered for 5 days after extubation. On days 21, 23 and 25 after admission persistent clearance of the virus from blood was demonstrated (<1000 copies/mL) and the patient recovered. After 42 days in hospital the patient was discharged in a stable condition. Five weeks after discharge SARS-CoV-2 specific antibodies were detected using the qualitative ECLIA nucleocapsid protein assay mentioned before (Roche, Elecsys anti-SARS-CoV-2) (figure 2).

The patient had been followed by our haematologists since 2019 when he was first diagnosed with chronic non-Hodgkin's lymphoma (NHL) (chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma, Rai I, CLL-prognostic score: intermediate risk). His initial CT scan had shown thoracic and abdominal lymphadenopathy with no involvement of spleen or bone marrow. The patient suffered from recurring infections (viral respiratory infection in late 2019 and community-acquired pneumonia in early 2020, no organisms identified) and progressive lethargy. When progressive pharyngeal lymphadenopathy with difficulty to swallow developed, chemotherapy was indicated and six cycles of bendamustine and rituximab were administered over 6 months in 2020 resulting in complete remission. Rituximab was given in intervals of 4 weeks. The last dose was administered 5 months prior to the first diagnosis of COVID-19. There was no evidence of reactivation of his lymphoma when he was diagnosed with COVID-19 and the patient is still in complete remission in 2022.

The patient suffered from severe hypogammaglobulinaemia (gammaglobulins between 3.2 and 3.8 g/L on four measurements in 2020, normal value range 7.2–14.4) since he was first diagnosed with lymphoma, but was not treated with intravenous immunoglobulins (IVIG) because he had no severe infections prior to COVID-19. A first dose of IVIG was given 3 days prior to discharge from the current hospitalisation episode and from then onwards monthly immunoglobulin substitutions were given. B-cell depletion was evident on lymphocyte subpopulation differentiation performed during the hospitalisation in early 2021: B cells CD19 0/µl (normal value range 80–616), CD3/CD4 80/µl (normal value range 404–1612) and CD3/CD8 136/µl (normal value range 220–1129), which may explain the lack of humoral immunity against SARS-CoV-2.

INVESTIGATIONS

Genome analysis

Sequencing of the samples 301120 (d −50) and 180121 (d 1; the sample 050221 d18 was unfortunately unavailable) used the Artic V3 primers in a weighted mixture (https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019) and 30 or 40 amplification cycles and was performed on an Illumina NextSeq 500 platform following Illumina DNA Prep library preparation. Mapping of resulting reads to the Wuhan-Hu-1 reference sequence (NC_045512) and quality control was performed using the COVGAP pipeline (https://github.com/appliedmicrobiologyresearch/covgap).[30] One hundred per cent of the reference genome was covered with mean coverage in excess of 2000×. Pango lineages[1] were assigned on 7 December 2021 using https://cov-lineages.org/resources/pangolin.html. Mapped reads (bam files) were viewed and subsequently analysed in CLC Genomics Workbench V20.0.2. Single nucleotide variants (SNVs) were called when present in over 20% of the reads at any position. Minor variants were considered as those in <70% of reads at any position. All B.1.221 sequences from Basel-Stadt available on GISAID (https://gisaid.org/) on 7 December 2021[31] (epicov.org, only sequences with <2000 bp missing data, online supplement table S1), the Wuhan-Hu-1 reference genome and the patient samples were aligned using MAFFT V7.467[32] and a phylogenetic tree was generated with IQ-TREE V2.[33 34] Mapped data and consensus sequences are available on GISAID under the ID EPI_ISL_1389121, strain_name hCoV-19/Switzerland/BS-UHB-42565379/2020 and ENA accession ERS6466100 for sample 301120, and EPI_ISL_1388920 strain name hCoV-19/Switzerland/BS-UHB-2103035433/2021 and ENA accession ERS6465694 for sample 180121.

The first sample (301120) sequenced from this patient was found to contain virus belonging to Pango lineage B.1.221. This lineage made up 13.5% of the circulating lineages in Basel-Stadt over the week around the sampling date. Sample 300120 contained 18 mutations relative to the Wuhan-1 reference sequence, each mutation present in over 99% of the sequencing reads (table 1). No minor variants were called from this viral sample.

The second sample (180121, taken at d 1 on admission, 50 days after the first sample) also contained virus belonging to Pango lineage B.1.221, which had decreased to 9.5% of the circulating lineages in the week around this sampling date. A phylogeny of viral samples in lineage B.1.221 from Basel-Stadt shows the close relatedness of the samples (figure 3). There are three additional related sequences from a similar time period (sequences 1–3 in online supplemental table S1) which share all mutations present in 301120 and have acquired additional mutations. Sample 180121 had eight additional mutations in comparison to 301120, each present in 23–100% of the sequencing reads (table 1; figure 4). Of these eight mutations, seven result in amino acid changes, two of which are in the spike protein (codons 76 and 97, not within the receptor binding domain (RBD)). None of the observed mutations have highly deleterious fitness consequences (table 1; [35]). No indels were observed.

OUTCOME AND FOLLOW-UP

The patient was last seen by our team in early 2022. By then he had received three doses of SARS-CoV-2 vaccination (Moderna/Spikevax). His antibody titres using a quantitative ECLIA detecting antibodies against the RBD of the SARS-CoV2 S-protein (Elecsys Anti-SARS-CoV-2) were 429 U/mL and 179 U/mL at two measurements after the first two doses, and >2500 U/mL after the third dose in early 2022. His B-cells have partially recovered 18 months after the last dose of rituximab (CD19 162/µl, CD3/CD4 280/µl, CD3/CD8 872/µl).

DISCUSSION

The case reported here is an example of severe and most likely prolonged SARS-CoV-2 infection in an immunocompromised patient with NHL who had received B-cell depleting therapies until 6 months previous to the infection. Most SARS-CoV-2 infections are oligosymptomatic, but immunocompromised hosts seem to be more likely to develop more severe symptoms including respiratory failure.[3 4] Patients with impaired immune system such as patients with NHL often have prolonged or persistent infections, especially when treated with B-cell depleting therapies such as rituximab.[36 37]

The viral load in upper respiratory tract specimens typically decreases after a peak of 3 days in symptomatic and asymptomatic patients and is not detectable after 14 days.[5] Once pneumonia develops in COVID-19 (IQR 2–7 days after initial symptoms[38]), lower respiratory tract specimens sometimes yield a positive result while nasopharyngeal swabs remain negative.

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Table 1 Single nucleotide variants (SNVs) identified in both samples in comparison to the Wuhan-Hu-1 reference sequence (NC_045512.2)

SNV position relative to NC_045512.2	Ref	SNV	Read coverage	Frequency in 301120 (%)	Frequency in 180121 (%)	Forward/ reverse balance	Gene affected	Mutation caused	Fitness of mutation	Possible order of mutations
241	C	T	1536	99.37	99.9	0.4	Intergenic			
3037	C	T	2633	99.68	99.8	0.4	ORF1ab			
3602	C	T	3948	99.82	100	0.3	ORF1ab			
6662	**G**	**A**	**1975**		**64.4**	**0.5**	**ORF1ab**	**V2133I**	**−0.1287**	**7**
6941	C	T	4009	99.75	100	0.4	ORF1ab			
8983	T	G	1558	100	99.9	0.1	ORF1ab			
9474	**C**	**T**	**3043**		**66.8**	**0.1**	**ORF1ab**	**A3070V**	**0.86129**	**6**
9608	T	C	2743	100	99.9	0.2	ORF1ab			
11083	**G**	**T**	**1907**		**86.6**	**0.1**	**ORF1ab**	**L3606F**	**no data**	**3**
12161	C	A	2154	99.9	99.9	0.5	ORF1ab			
14408	C	T	3122	99.63	99.8	0.5	ORF1ab			
15324	C	T	2008	99.89	100	0.3	ORF1ab			
21789	**C**	**T**	**1841**		**72.3**	**0.3**	**S**	**T76I**	**0.58612**	**4**
21853	**G**	**T**	**2045**		**92**	**0.5**	**S**	**K97N**	**0**	**2**
21855	C	T	2046	99.91	100	0.5	S			
23403	A	G	2527	99.92	99.9	0.2	S			
25505	A	G	2184	99.95	100	0.3	ORF3a			
25906	G	C	1817	99.77	99.9	0.1	ORF3a			
25996	G	T	2178	99.96	99.9	0.1	ORF3a			
27457	**G**	**T**	**1856**		**23.1**	**0.2**	**ORF7a**	**E22STOP**	**−1.8009**	**8**
27610	**C**	**T**	**4033**		**99.9**	**0.3**	**ORF7a**	**H73Y**	**0.85319**	**1**
27708	**G**	**T**	**4227**		**68.5**	**0.3**	**ORF7a**	**A105A**	**0**	**5**
28133	A	T	1483	99.93	99.7	0.3	ORF8			
28368	G	A	1457	99.84	100	0.1	N			
28651	C	T	1886	99.9	99.8	0.2	N			
28869	C	T	1891	99.8	100	0.5	N			

Mutations found in sample 180121 and not in 301120 are shown in bold, with proportions indicated in the 'frequency in 180121 (%)' column. Minor variants comprising over 20% of the reads at any position were sought in the samples. Analysis was performed on cDNA, thus T is called for U in the viral genome. Fitness values are given according to https://github.com/jbloomlab/SARS2-mut-fitness as recorded on 12 September 2023.[35]

This diagnostic shift in NAT from upper respiratory samples in this setting was reported early in the pandemic[39 40] and is frequently described in patients with NHL.[36 37 41]

Our patient showed no detectable SARS-CoV-2 antibodies 50 days after the initial infection. Usually SARS-CoV-2 specific antibodies can be detected from the second week after symptom onset.[42] Patients who have received B-cell depleting therapies show a sharp decrease in B-cell levels 3 days after the first dose of anti-CD 20 antibody and a full recovery takes up to 12 months.[43] B-cell depleted patients are unable to mount a specific humoral response to new infections.[44] The lack of specific antibodies in our case went along with the inability of the patient to clear the virus from blood and his lungs. However, our patient also had hypogammaglobulinaemia meaning that serological tests including tests for SARS-CoV-2 antibodies carry a risk of false negatives. The detection of SARS-CoV-2 in blood is not frequent in patients with COVID-19 (3 of 307, 1% in[45]; 1% in[46]), but viraemia is correlated with more severe disease and increased mortality.[47 48] The long-lasting effect of B-cell depleting therapies has implications on the natural course of SARS-CoV-2 infections as well as on aspects of diagnostics and therapy: such patients are more likely to get infected, more often develop severe symptoms, and are more prone to persistent infections or reinfection.

In our case, we observed a complete and sustained viral clearance from blood after CCP therapy. In a case series reported by Hueso *et al*,[26] 16/17 patients with severe B-cell lymphopenia and prolonged COVID-19 symptoms showed clinical improvement within 48 hours of CCP therapy, and viraemia decreased to levels below the detection limit in all 9 patients that had measurable blood virus levels. Even though large randomised clinical trials have shown that CCP is not effective in immunocompetent hospitalised patients[49 50] and current Infectious Diseases Society of America (IDSA)[51] and National Institutes of Health (NIH) guidelines[52] recommend against its use in this setting, CCP appears as treatment option for immunosuppressed outpatients if certain conditions are met[51] and NIH stated in December 2022 that 'There is insufficient evidence for the Panel to recommend either for or against the use of high-titre CCP for the treatment of COVID-19 in hospitalized or nonhospitalized patients who are immunocompromised'.[52] Our treatment decision in early 2021, in the absence of monoclonal antibodies, is consistent with a recently published suggestion by Furlan *et al* for the management of immune compromised B cell-depleted patients with COVID-19 after rituximab therapy.[36]

It has to be pointed out that our patient was started on 4 weekly IVIG infusions prior to discharge, and that the seroconversion observed 5 weeks after discharge might therefore be a result of transfused SARS-CoV-2 antibodies indicating population level antibodies rather than our patient's immune response to SARS-CoV-2.

This case study also illustrates the power of genome sequencing, not merely for typing and lineage assignment, but in determining the more subtle distinctions present in the viral population as sampled in BALF. The genome sequencing of the samples

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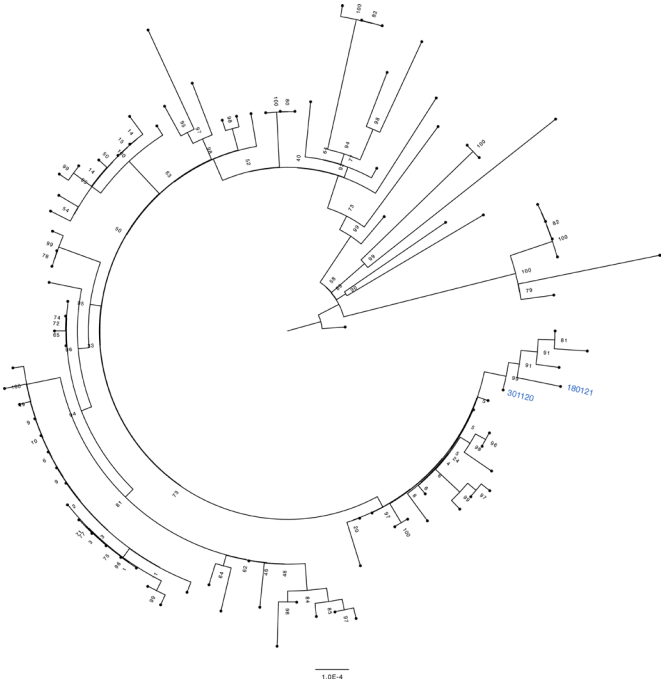

Figure 3 Phylogeny of lineage B.1.221 in Basel-Stadt (samples from 7 September 2020 to 19 March 2021). Analysis based on consensus sequences from GISAID, rooted on the Wuhan-Hu1 reference. For the 180121 sample, single nucleotide variants present in >70% reads are shown. The values at the nodes are the bootstrap values (#1000). Figure 3 by Helena Seth-Smith.

strongly suggests that this is a case of viral replication over weeks in an immunocompromised host. The source of the infection cannot be elucidated, although the B.1.221 lineage was circulating in Basel at the time of the initial diagnosis. By the second episode, the prevalence of this lineage had dropped. Reinfection was initially considered but deemed unlikely given the short time between the two episodes, and can largely be ruled out with the genome analysis. SARS-CoV-2 reinfection has indeed been described after similarly short intervals, and both more and less severe symptoms at reinfection have been described in the literature.[8 53–56] In this context, as well as for tracking the source and spread of new Variants of Concern (VoCs), genomic surveillance of SARS-CoV-2 is critical in our pandemic response.

The homogeneity of the SNVs in the first sample, being present in all reads, suggests that this sample was taken soon after the transmission, before the virus had an opportunity to diversify. That the second sample carried all the mutations of the first, plus others in varying proportions within the viral population, implies that these mutations arose in-host over the intervening 7 weeks between samples. Similarly to our analysis, minority variants have also been identified in other studies on immunocompromised hosts.[15–19 21 22] Two of the novel mutations in sample 180121 were identified in the spike protein, outside the RBD. Neither of these mutations are commonly found in samples from databases (GISAID,[31] epicov.org) or within other characterised immunocompromised hosts.[10 13–15 17–19 22] Based on the available data set (figure 3, online supplemental table S1), there is no evidence for onwards transmission of the virus 180121, but it is possible that the patient was part of a transmission chain before intrahost diversification took place, as all SNVs present in 301120 are shared with the other three closely related sequences.

It is possible to estimate the chronological order in which the mutations occurred, by the proportion of reads possessing each mutation. As such, the mutation at position 27610 likely occurred soon after the sampling on d −50, followed by the mutation at 21853 and so on (table 1). These results strongly suggest ongoing in-host viral replication over the course of the infection. A limitation of the study is the lack of successful repeat sequencing of the 180121 sample, for which the RNA appeared to have degraded. A technical replicate of sample 301121 showed identical results to the first experiment.

The estimated SARS-CoV-2 mutation rate across the population is 26–32 substitutions per genome per year, which translates to 3.5–4 mutations in 7 weeks.[57–59] Yet we see eight mutations arising in this time, which is double the normal rate. This mutation increase within immunocompromised patients has been shown before,[8 10] and such 'adaptive evolution' is also seen in the development of VoCs.[60]

In conclusion, we add this case to the growing body of data on SARS-CoV-2 evolution in immunocompromised hosts. WGS shows an apparently elevated mutation rate

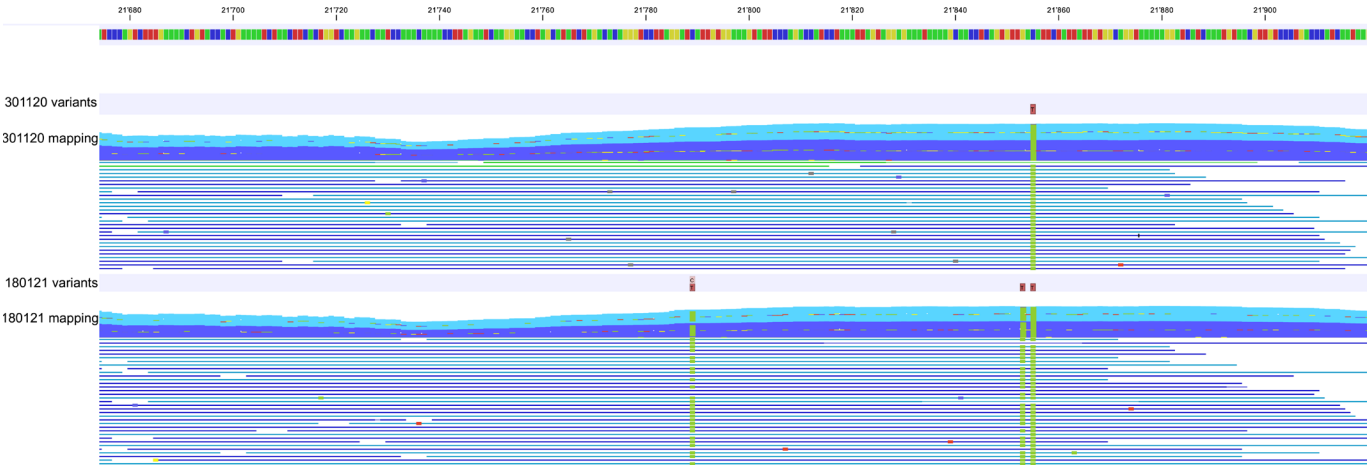

Figure 4 Reads from patient samples mapped against reference strain, and variants called. This example screenshot from CLC Genomics Workbench V20.0.2 shows a region of the S gene. The sequence and position of the reference genome NC_045512.2 is shown across the top. Sample 301120 is shown in the upper panels and possesses the variant at location 21855. Sample 180121 is shown in the lower panels and possesses this single nucleotide variant as well as 21789 and 21853 in a proportion of the reads. Figure 4 by Helena Seth-Smith.

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over the 7 weeks between samples, and sequencing from BALF shows minor variants in proportions which could be used to estimate the chronology of mutations.

Patient's perspective

I am grateful to be alive today and to have gotten rid of the virus. I highly appreciate the fact that new treatment approaches were tried on my case even though they were not established in the early days of the pandemic. Sharing this information and details of such treatments is important.

Learning points

- Consider nucleic acid testing from bronchoalveolar lavage fluid (BALF) instead of nasopharyngeal swabs in cases of high suspicion of SARS-CoV-2 infection, especially in immunocompromised hosts, as nasopharyngeal swabs can provide false-negative results.
- Repeated detection of SARS-CoV2 in the same patient is common. Whole genome sequencing (WGS) of the pathogen can be used to differentiate between reinfection and prolonged infection. The latter is a common finding in immunocompromised patients, especially in patients with lymphoma after B-cell depleting therapies. It can be difficult to clear the virus from such patients.
- During the course of the pandemic, the virus and our knowledge about it evolved quickly, so that available therapies and recommendations needed constant adjustments. Remdesivir and dexamethasone remain the cornerstone of COVID-19 therapy until today. While convalescent plasma (CCP) was the only add on option in early days, monoclonal antibodies later largely replaced them, until resistant Omicron subvariants emerged so that by early 2023 none of the currently available monoclonal antibodies are still recommended in the USA. For immunosuppressed outpatients CCP can still be an option if certain conditions are met.
- WGS can inform on viral evolution: this case does not show convergent evolution with similar cases, but shows the speed of in-host evolution. Viral diversity can be estimated from single nucleotide variant numbers when looking at the population in BALF samples.

Acknowledgements We thank Dr Monika Ebnöther and Dr. Chloe Kaech for insightful clinical case discussions, as well as Dr Peter Koch for laboratory support. We thank Prof. Andreas Buser for providing convalescent plasma. We thank Dr Tim Roloff for supervising the NGS facility with accredited whole genome sequencing of SARS-CoV-2, Prof. Hans Hirsch supervising viral diagnostics, and Dr Fanny Wegner for performing the phylogenetic analysis. We thank Magdalena Schneider, Clarisse Straub, Daniel Gander, Valerie Courtet, and Vincent Hartmann for excellent NGS technical support. COVGAP calculations were performed at sciCORE (http://scicore.unibas.ch/) scientific computing centre at the University of Basel. We gratefully acknowledge the help of data shared via the GISAID Initiative, on which a part of this research is based.

Contributors SV and SO treated the patient and supervised treatment decisions. HS-S and AE performed genome sequencing analysis. SV and HS-S wrote the manuscript. AE and SO edited the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Provenance and peer review Not commissioned; externally peer reviewed.

The datasets generated during the current study can be found on GISAID under the ID EPI_ISL_1389121, strain_name hCoV-19/Switzerland/BS-UHB-42565379/2020 and ENA accession ERS6466100 for sample 301120, and EPI_ISL_1388920 strain name hCoV-19/Switzerland/BS-UHB-2103035433/2021 and ENA accession ERS6465694 for sample 180121.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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