Title

Persistent SARS-CoV-2 infection with accumulation of mutations in a patient with poorly controlled HIV infection

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Abstract

A 22-year-old female with uncontrolled advanced HIV infection was persistently infected with SARS-CoV-2 beta variant for 9 months, the virus accumulating >20 additional mutations. Antiretroviral therapy suppressed HIV and cleared SARS-CoV-2 within 6-9 weeks. Increased vigilance is warranted to benefit affected individuals and prevent the emergence of novel SARS-CoV-2 variants.

Background

In the SARS-CoV-2 pandemic, South Africa so far has experienced four distinct waves of infections each driven by different variants. The first wave between June-August 2020 was attributed to a mix of SARS-CoV-2 lineages with low overall diversity; the second wave lasted from November 2020 until February 2021 and was driven by the beta variant of concern (VOC) (B.1.351); the third wave was dominated by the delta VOC (B.1.617.2) and occurred from May until October 2021. The most recent fourth wave, beginning in November 2021, was driven by the omicron VOC (B.1.1.529) [1].

The origins of new divergent variants are not yet understood. One hypothesis is that they arise in severely immunocompromised individuals, such as patients receiving cancer chemotherapy, organ transplant recipients, and people with uncontrolled advanced HIV disease. Failure to clear SARS-CoV-2 due to sub-optimal immune responses results in persistent infections that allow the accumulation of mutations that may confer immune evasive properties [2].

We here describe a case of persistent SARS-CoV-2 infection, lasting for a minimum of 9 months, in a severely immunocompromised person with HIV that had challenges with adherence to antiretroviral therapy. This case report was approved by the Health Research Ethics Committee of Stellenbosch University and the patient provided informed consent.

Case details, methods and results

In mid-September 2021, a female in her 20s was admitted to a tertiary hospital in Cape Town, Western Cape province, South Africa, with a one-week history of sore throat, malaise, poor appetite and dysphagia. The patient was infected with HIV at birth. In January 2021, her antiretroviral therapy (ART) regimen was switched to tenofovir, emtricitabine and efavirenz, but she experienced adherence challenges. In August 2021 she moved from rural KwaZulu-

Natal province, South Africa, to Cape Town. She reported that she had not been vaccinated against COVID-19.

On physical examination, the patient was wasted but had no palpable lymph nodes. She was awake and lucid, with no focal neurological deficits. She was not in respiratory distress, had normal breath sounds with no crackles or wheezes audible and an oxygen saturation of 98% on room air. The cardiovascular and abdominal examinations, renal function, white cell count and liver enzymes were without abnormalities. Her CD4 count was 9 cells/μl and her plasma HIV viral load 4.60 $\log_{10}$ viral RNA copies/ml, indicating advanced HIV infection, poorly controlled by ART.

During a prolonged hospital stay the patient experienced multiple complications requiring treatment. Following adherence counselling, antiretroviral therapy was reinitiated with a new regimen of tenofovir/efavirenz/dolutegravir a week after admission.

A nasopharyngeal swab obtained on 25 September 2021 tested positive by the Alinity m SARS-CoV-2 routine diagnostic assay (Abbott Park, Illinois, U.S.A.); the threshold cycle (Ct) of 16 suggested a relatively high viral RNA load. The sample was serendipitously included in on-going routine genomic surveillance [3], using Oxford Nanopore Technologies (ONT) sequencing on the Nanopore GridION utilising ARCTIC version 3 primers as previously described [4]. The viral sequence belonged to the B.1.351 lineage (GISAID accession: EPI_ISL_5018695). The sequence was flagged for further investigation because beta was at that time responsible for <1% of genomically-confirmed cases, and the Network for Genomic Surveillance in South Africa (NGS-SA) was monitoring closely for evolution of the beta variant.

A second nasopharyngeal swab was obtained a month later on 26 October 2021 again tested positive, with Ct values by the Cepheid GeneXpert SARS-CoV-2 assay (Sunnyvale, California, U.S.A.) of 15.3 for the E-gene and 18.2 for the N-gene targets, suggestive of a persisting high viral RNA load. Genomic sequencing of the virus using Nanopore sequencing again revealed

B.1.351 (GISAID accession: EPI_ISL_6227177). In addition, virus was isolated on Vero E6 cells.

Another month later, on 25 November 2021, the patient's HIV viral load was <50 copies/ml and nasopharyngeal SARS-CoV-2 PCR testing was negative. Unfortunately, a CD4 count was not performed but suppressed HIV replication and clearance of SARS-CoV-2 infection suggest some degree of immune reconstitution at that stage.

On further questioning, she revealed that she had first tested positive for SARS-CoV-2 by PCR on a respiratory sample while still resident in in KwaZulu-Natal in January 2021, eight months prior to presentation at TAH. That sample had been tested on the Allplex™ SARS-CoV-2 assay (Seegene Inc., Seoul, Republic of Korea) with Ct values of 18, 20 and 22 for the E, RdRp and N gene targets, respectively. At the time her CD4 count was 91 cells/μL and her plasma HIV viral load 5.07 $\log_{10}$ viral RNA copies/ml. Sequencing of the archived sample using published methods [1] revealed B.1.351 (GISAID accession: EPI_ISL_6585229).

The three patient genome sequences were analysed against a global reference dataset of 7977 genomes, including 366 from South Africa, using a custom build of the SARS-CoV-2 NextStrain (https://github.com/nextstrain/ncov). The workflow performs alignment of genomes, phylogenetic tree inference, tree dating and ancestral state construction and annotation. The phylogenetic tree (Figure 1) was visualised using ggplot and ggtree.

Phylogenetic analysis confirmed that the infecting virus from all three swabs clustered together on a background of 7977 other SARS-CoV-2 sequences, which confirms persistent infection over at least 9 months rather than re-infection. Over this period, the virus acquired at least 10 mutations in the spike glycoprotein and 11 mutations outside spike over and above the lineage-defining mutations for beta, as shown in Figure 1. The additional spike mutations included six in the spike receptor-binding domain (S371F, N450D, A475V, F490Y, S494P and Q498R); a deletion of amino acids residues 141-143 of the N-terminal domain (NTD) which leads to neutralizing antibody escape [5] and which seems to be frequently observed in chronic

infections; and two substitutions in the S2 domain (D737Y and F888L). Due to a gap in the NTD sequence it is not known whether a further substitution (N30T) in the NTD may have been present from the beginning. We observed a reversion of some of the mutations between the first and second sequences generated in Cape Town, with the spike N30T and spike F888L present in the September sample but not detected in the October one.

Discussion

Our case adds to the evidence that severe immunosuppression associated with uncontrolled HIV infection may lead to chronic SARS-CoV-2 infections [6, 7, 8]. These persistent infections not only allow continued shedding of infectious virions but also lead to the accumulation of mutations, some of which lead to immune escape that may result in emergence of new variants [9, 10]. Therefore, it is important that countries that have a high burden of HIV infection should encourage prompt diagnosis and treatment of HIV infections and compliance with antiretroviral therapy for those already receiving treatment to reduce the risk of persistent SARS CoV-2 infections and continued shedding of infectious virus that pose a threat to controlling the pandemic.

The additional mutations in the receptor-binding domain of the spike glycoprotein (S371F, N450D, A475V, F490Y, S494P and Q498R) in the later genomes are at sites associated with escape from all four classes of neutralizing antibodies [11]. We observed similar mutations at spike positions 475 and 490 in the other case we reported of chronic SARS-CoV-2 infection in association with advanced HIV [7, 10]. It is also notable that these mutations are identical or at the same position as mutations in other variants of concern/interest (Q498R and S371L in omicron; and F490Y in lambda).

The point needs to be made, however, that no genomes identical to or originating from the September or October ones were identified by the wider genomic surveillance. While genomic surveillance efforts may well miss viruses occurring at low frequencies, because of low testing rates and low and patchy coverage of genomic surveillance, a "successful" new variant would

likely increase over time and not escape detection for weeks or months. The history of the detection of the novel Omicron variant here in South Africa supports this notion [1].

There is thus no evidence that the evolved variants from this case successfully spread into the general population. This case, like others before, describes a potential pathway for the emergence of novel variants but it does not prove that any of the variants detected so far did originate from such a persistent infection in a severely immunocompromised host.

This case furthermore highlights the value of well-coordinated and thoroughly established genomic surveillance efforts. Fortuitously, the September sample from this patient was sequenced as part of the NGS-SA effort. It was flagged as warranting further investigation as a beta variant which by that stage had become rare by the sequencing and sequence analysis teams who contacted the diagnostic virologists and those the requesting clinician. Good connections between sequencing laboratories, routine diagnostic laboratories and frontline clinicians are indispensable to identify and investigate such cases.

Once again our experience reinforces previous reports that effective ART is the key to controlling such events. Once HIV replication is brought under control and immune reconstitution commences, rapid clearance of SARS-CoV-2 is achieved, probably even before full immune reconstitution occurs. This underscores the broader point that gaps in the HIV care cascade need to be closed which will benefit other conditions and public health problems, too, including Covid-19 [12].

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Conflict of Interest

All authors have no conflicts of interest to declare.

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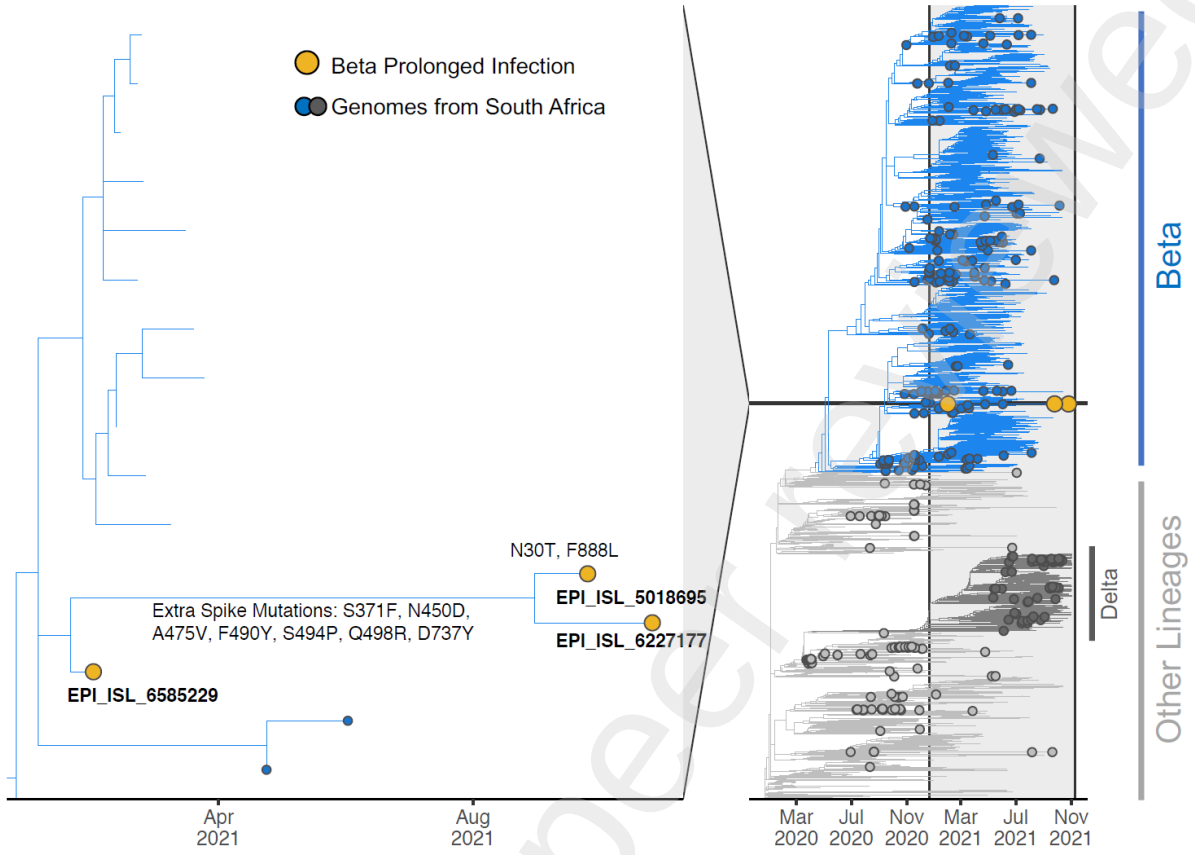

Figure 1: Phylogenetic analysis of three SARS-CoV-2 whole genome sequences from case with prolonged infection with the beta VOC of SARS-CoV-2.

Timed maximum-likelihood phylogenetic tree with patient sequences (yellow) at three time-points (January 2021: hCoV-19/South Africa/CERI-KRISP-K029499/2021, GISAID accession ID: EPI_ISL_6585229; September 2021: hCoV-19/South Africa/Tygerberg_2777/2021, GISAID accession ID: EPI_ISL_5018695; October 2021 hCoV-19/South Africa/Tygerberg_2967/2021, GISAID accession ID: EPI_ISL_6227177) in relation to 336 representative South African and 7641 other global sequences. The zoomed-in view shows the finer phylogenetic relationship between the three patient-derived sequences. Spike mutations accumulated in addition to the known beta mutations are labelled.