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CASE REPORT

Detection of SARS-CoV-2 Alpha variant in a severely immunocompromised HIV-1-infected patient in the omicron era

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SUMMARY

Persistence of detectable viral RNA does not depend on the symptomatic status of the patients. Here we describe the case of a strongly immunocompromised patient living with a prolonged SARS-CoV-2 Alpha variant infection without showing any symptoms. The importance of our findings is that the persistent infection with an old SARS-CoV-2 strain, in an immunocompromised host, may allow recombination events generating new viral variants whose pathogenicity cannot be predicted. Our observation calls for the urgent need for continuous monitoring of SARS-CoV-2 genomic evolution in immunocompromised patients.

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INTRODUCTION

It has recently been shown that, upon infection, the persistence of detectable viral RNA varies greatly among individuals and does not depend on symptomatic status (Zapor, 2020). Moreover, RNA virus population in a host is not represented by a single sequence, but comprises a number of replicating quasi-species (Sun *et al*., 2021; Caccuri *et al*., 2022). Thus, prolonged infections, especially in immunocompromised patients, allow long-term viral replication, supporting the emergence of potential new variants (Voloch *et al*., 2021).

SARS-CoV-2 variants have emerged and spread globally since autumn 2020. All Variants of Concern (VOCs) are characterized by a typical mutational pattern in the S gene, the most variable part of the coronavirus genome, that largely contributed to virus adaptation. The first SARS-CoV-2 VOC, defined as Alpha variant, emerged at the end of 2020 in United Kingdom (Fiorentini *et al*., 2021), spread all over the world, and started to disappear in May 2021. In Italy, the last case of the Alpha VOC was identified in December 2021. Indeed, starting in September 2021 this variant was replaced by the emerging Delta variant, which appeared, at that time, to be the unique VOC circulating in the Brescia area (Bertelli *et al*., 2021). Therefore, here we present the case of a completely immunocompromised patient having an asymptomatic and unrecognized prolonged SARS-CoV-2 infection with the Alpha variant diagnosed at the end of February 2022.

Key words:
Immunocompromised host, HIV, Persistent SARS-CoV-2 infection.

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CASE REPORT

Herein we describe the case of an HIV-1-infected 26-year-old woman who was admitted to the Brescia Civic Hospital on February 22, 2022, with no previous history of SARS-CoV-2 infection. The patient was infected by the circulating recombinant form 01_AE HIV-1 and in the last five years experienced several virological failures, probably due to poor antiretroviral therapy adherence. On the admission day, laboratory testing was remarkable for leukopenia 1.78 K/µL (normal 4.0-10.8 K/µL), CD4 T cell count 1 cells/µL (normal 273-1882 cells/µL), CD4% of 0.4% (normal 28.5-65.6%), CD8 T cell count 95 cells/µL (normal 177-783 cells/µL), CD8% of 33.4% (normal 10.5-37.7%), erythrocyte sedimentation rate 120 mm/h (normal 2-37 mm/h), lactate dehydrogenase 440 u/L (normal 135-225 u/L), aspartate transaminase 500 u/L (normal 18-34 u/L), alanine transaminase 212 µ/L (normal 10-35 µ/L), gamma-glutamyltransferase 354

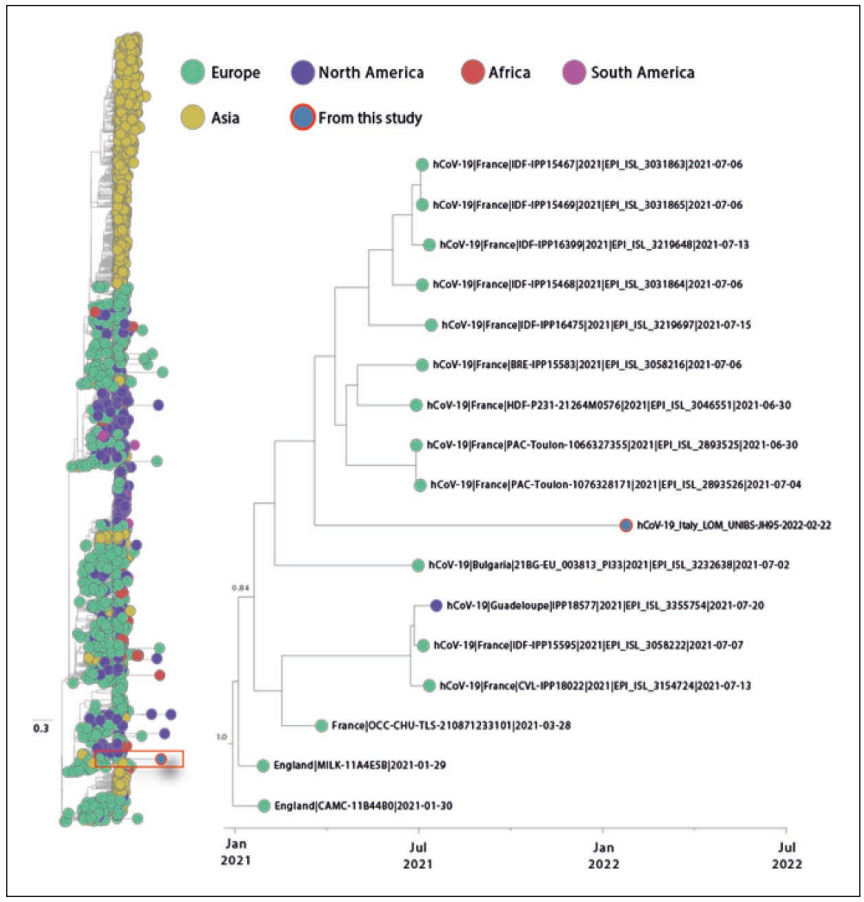

Figure 1 - Genomic characterization of SARS-CoV-2 persistent strain in an immunocompromised patient. *Time-scaled maximum likelihood (ML) tree including the new isolate obtained in this study plus n=2,553 SARS-CoV-2 strains belonging to the Alpha variant of concern collected up to March, 2022. New isolate is highlighted in red (blue circle). On the right side, a zoom of the new isolate clade obtained from the immunocompromised patient. Branch support is shown at key nodes.*

u/L (normal 6-42 µ/L), HIV viral load 1,339,481 copies/mL. On admission, the patient was also subjected to nasopharyngeal swab (NPS) for SARS-CoV-2 and was diagnosed positive for the infection. The patient received the last dose of anti-SARS-CoV-2 vaccine in August 2022. The antibody titer against the SARS-CoV-2 spike protein was 36.1 u/mL.

The NPS of the patient was included in our genomic surveillance program of SARS-CoV-2 positive samples implemented through Sanger sequencing. The sequence obtained presented the Alpha lineage specific mutations including the ones localized at the spike protein, such as HV69-70del, Y144del, E484K, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, together with other new amino acid substitutions (C15S, L176F, DPFLGV138-143del, Y145del, T299I, P348L, Q498K). A genetic characterization by whole-genome sequencing was also performed, confirming that the new strain identified belongs to the Alpha VOC.

To put the new sequence in a global context, we constructed a phylogenetic tree to explore the relationship of the sequenced genome with those of other isolates of this lineage. We retrieved 2,553 SARS-CoV-2 genome sequences from this lineage (collected up to March 2022) with associated lineage date and country of collection from GISAID, using the Augur pipeline from Nextstrain as previously described (Giovanetti *et al.*, 2021). Sequence alignment, editing, and phylogenetic inferences were done as previously described (Caccuri *et al.*, 2020; Messali *et al.*, 2021).

Our phylogeny combined with the lineage assignment (https://github.com/hCoV-2019/pangolin) showed that the viral sequence obtained from the immunocompromised patient belonged to the Alpha variant and clustered together with strong support with strains isolated from European countries between June and July 2021 (*Figure 1*).

Due to lack of the patient's sample at the time of infection, it was not possible to establish the presumable time of infection with SARS-CoV-2 from phylogenetic analyses or from the patient's history.

DISCUSSION

Various authors have shown that many SARS-CoV-2 infections among HIV-infected people are asymptomatic (Berenguer *et al.*, 2021; Kowalska *et al.*, 2021). Here we show that immunocompromised individuals may live with a silent infection for many months without showing any symptoms. As previously shown (Caccuri *et al.*, 2022), this outcome may be due to strong control of the infection supported by the innate immune response, which overcomes the lack of

adaptive immunity. Immunocompromised patients do not develop a strong antibody response following either natural infection or vaccination (CDC, 2022), thus persistently-infected patients may undergo re-infection. This event may increase the complexity of intra-host quasi-species population favoring the accumulation of critical amino acid changes, as well as recombination events, which are common among coronaviruses (Graham *et al*., 2010). Recombinant SARS-CoV-2 genomes containing a hybrid spike protein derived from different variants have been already described (Lacek *et al*., 2022; Sekizuka *et al*., 2022). These phenomena may induce the generation of new SARS-CoV-2 lineages which may spread, become more contagious or cause severe COVID-19 disease.

Even if the patient's NPS was collected in February 2022, our phylogenetic analysis locates the clusterization of the newly-identified sequence in the first months of 2021, when the circulating VOC in the Brescia area was the Alpha variant (Bertelli *et al*., 2020). This evidence prompted us to hypothesize a persistent Alpha variant infection, even if the possibility of an intra-host evolution of a wild-type strain, as already described (Caccuri *et al*., 2022), cannot be completely ruled out. However, the importance of our findings is that reinfection, in the omicron era, of an immunocompromised patient already infected with an old SARS-CoV-2 strain, may allow recombination events generating new viral variants whose pathogenicity cannot be predicted.

Collectively, our findings underline the fundamental importance of continuous monitoring of SARS-CoV-2 genomic evolution in immunocompromised patients. This will help to prevent the emergence and the spread of new viral variants.

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Conflict of interest

No conflict of interest to declare.

Data availability statement

The newly sequenced data obtained in this study was deposited in Global Initiative on Sharing All Influenza Data (GISAID), accession number: EPI_ISL_11789931.

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