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New infections by SARS-CoV-2 variants of concern after natural infections and post-vaccination in Rio de Janeiro, Brazil

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ABSTRACT

After a one-year rollout of the pandemic caused by SARS-CoV-2, the continuous dissemination of the virus has generated a number of variants with increased transmissibility and infectivity, called variants of concern (VOC), which now predominate worldwide. Concerns about the susceptibility of humans that have already been infected before or those already vaccinated to infection by VOC rise among scientists and clinicians. Herein, we assessed the prevalence of different VOC among recent infections at the Brazilian National Cancer Institute (Rio de Janeiro, Brazil). By using a Sanger-based sequencing approach targeting the viral S gene to identify VOC, we have analyzed 72 recent infections. The overall prevalence of VOC was 97%. Among the subjects analyzed, six had been vaccinated with the ChAdOx1-S/nCoV-19 ($n = 4$; one with two doses and three with one dose) or the CoronaVac ($n = 2$; both with 2 doses) vaccine, while five subjects represented reinfection cases, being two of them also part of the vaccinated group (each one with one vaccine type). All vaccinated and re-infected subjects carried VOC irrespective of the vaccine type taken, the number of doses taken, IgG titers or being previously infected during the first wave of the Brazilian pandemic. Importantly, all six vaccinees only had mild symptoms. We present here several examples of how natural infections or vaccination may not be fully capable of conferring sterilizing immunity against VOC

1. Introduction

Genetic diversity is an important source of biological variation and is even more striking in the context of viruses, particularly those carrying RNA genomes. Since the beginning of COVID-19 pandemic the high rates of infection may have favored the appearance of mutations in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), especially in its Spike (S) gene, which encodes the glycoprotein responsible for the virus' interaction with the host cell promoting virus entry and a main target for neutralization antibodies. Genomic surveillance studies have shown that such mutations led to the emergence of SARS-CoV-2 variants around the globe such as B.1.1.7 (UK) and B.1.351 (South Africa) (Rambaut et al., 2020; Tegally et al., 2021). The emergence of variants, especially the ones that harbor mutations in the receptor-binding domain (RBD) of the S protein, are of great concern notably regarding the efficacy of the developed vaccines and as cases of reinfection are being reported (Harrington et al., 2021; Naveca et al., 2021; Tillett et al., 2021). For these reasons, such RBD mutant variants are

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known as variants of concern (VOC). Interestingly, most VOCs share convergent mutations in the S protein, such as E484K and N501Y that may impair antibody neutralization and enhance the affinity of S to its receptor (ACE2), increasing transmissibility (Fratev, 2020; Greaney et al., 2021; Starr et al., 2020).

Brazil has reported its first case of SARS-CoV-2 infection in February 2020, and since then the country has registered over 13 million cases and 340,000 deaths due to COVID-19 (Ministério da Saúde, n.d). The SARS-CoV-2 B1.1.28 has been the predominant strain circulating in the country early in the epidemic. However, the state of Amazonas (Northern Brazil) has recently reported a surge in SARS-CoV-2 cases as well as hospital admissions and deaths due to COVID-19. Whole genome sequencing revealed that this surge was related to a new SARS-CoV-2 VOC called P.1 (Faria et al., 2021). This VOC is a subclade of B1.1.28 and harbors convergent mutations found in the B.1.1.7 and B.1.351 VOCs such as K417T, E484K and N501Y. Voloch et al. (Voloch et al., 2021), have reported another sub-lineage of B1.1.28 first detected in Rio de Janeiro and named P.2. This variant harbor the S mutation E484K but so far has not been considered as a VOC, but instead, a variant of interest (VOI).

Genomic surveillance is of extreme importance to evaluate the impact of new variants of SARS-CoV-2 as new surges become more frequent. Most studies use next generation sequence (NGS) to evaluate new lineages. Albeit being a powerful tool, NGS can be costly and requires a definite number of samples for a single run, which can sometimes delay results. In the present study we determined the lineage of 72 samples collected from recent patients and healthcare workers (HCW) at the Brazilian National Cancer Institute (INCA), Rio de Janeiro, between mid-January and March 2021. Herein we applied a new protocol using Sanger sequencing to determine known VOCs of biological importance based on their mutational signatures and phylogeny. We show for the first time six vaccinees infected with P.1 and P.2 lineages as well as two cases of reinfection by these lineages among vaccinees of the study.

2. Materials and methods

2.1. Sampling

For this study we selected 72 SARS-CoV-2 recently tested positive individuals with nasopharyngeal swabs collected between mid-January and March 2021 with SARS-CoV-2 RT-qPCR Ct values <30. These include both cancer patients and healthcare workers (HCW) from the Brazilian National Cancer Institute (INCA), Rio de Janeiro, Brazil. Of those 72 subjects, six were vaccinated prior the current infection and two out of those six were also reinfection cases. Another three subjects also represented reinfections, showing a previous infection during the first wave of the pandemic in Brazil.

Six HCW included in this study have been fully or partially vaccinated with one of the vaccines available in Brazil, either CoronaVac (Sinovac/Butantã) or ChAdOx1-S/nCoV-19 (AstraZeneca/Oxford/Fiocruz). All six tested positive for SARS-CoV-2 RT-qPCR after at least one vaccine dose and reported mild symptoms during infection.

This study was approved by the Brazilian National Commission for Ethics in Research (CAAE: 30608220.8.0000.5274).

2.2. SARS-CoV-2 infection diagnosis and serological assays

The diagnosis of SARS-CoV-2 infection was performed using RT-qPCR following the U.S. Centers for Disease Control and Prevention protocol (Centers for Disease Control and Prevention, 2020). Most HCW have also had their anti-SARS-CoV-2 IgG responses measured using the SARS-CoV-2 IgG Architect assay (Abbott Laboratório do Brasil LTDA, São Paulo, Brazil) to assess immune responses against natural infection and/or vaccination.

2.3. Spike gene sequencing and variant classification

A region from SARS-CoV-2 S gene (positions 955–2285 based on Wuhan-Hu-1 SARS-CoV-2 S gene Genbank acc# MN908947) which includes most of the variant defining amino acid changes in S (Table 1) from the three most frequent circulating VOCs (B.1.1.7, B.1.351 and P.1) was amplified using the primers 75_LEFT and 78_RIGHT from the ARTIC network nCov-2019 V.3 primer set (Artic Network, Artic Network, n.d) and Platinum Taq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA). The conditions for PCR reactions were 95 °C for 5 min, 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 2 min, followed by a final extension at 72 °C for 5 min. PCR reactions were carried out in a Verity 96-well platform (Thermo Fisher Scientific). PCR positive products were purified and sequenced by Sanger methodology with an automated ABI

Table 1
Signature mutations of SARS-CoV-2 variants of concern/interest analyzed.

SARS-CoV-2 lineage	Mutations outside S gene	Mutations in S gene	Mutations covered by Sanger sequencing
B.1.1.7 (alpha)	orf1a:T1001I	S:del69/70	S:N501Y
	orf1a:A1708D	S:del144/145	S:A570D
	orf1a:I2230T	S:N501Y	S:D614G
	orf1a:del3675/3677	S:A570D	S:P681H
	orf1b:P314L	S:D614G	S:T716I
	Orf8:Q27*	S:P681H	
	Orf8:R52I	S:T716I	
	Orf8:Y73C	S:S982A	
	N:D3L	S:D1118H	
	N:R203K		
	N:G204R		
	N:S235F		
B.1.351 (beta)	ORF1a:T265I	S:D80A	S:K417N
	ORF1a:K1655N	S:D215G	S:E484K
	ORF1a:K3353R	S:del241/243	S:N501Y
	ORF1a:del3675/3677		S:D614G
	ORF1b:P314L		S:A701V
	ORF3a:Q57H		
	ORF3a:S171L		
	E:P71L		
	N:T205I		
P.1 (gamma)	orf1a:S1188L	S:L18F	S:K417T
	orf1a:K1795Q	S:T20N	S:E484K
	orf1a:del3675/3677	S:P26S	S:N501Y
	orf1b:P314L	S:D138Y	S:D614G
	orf1b:E1264D	S:R190S	S:H655Y
	orf3a:S253P	S:K417T	
	orf8:E92K	S:E484K	
	N:P80R	S:N501Y	
	N:R203K	S:D614G	
	N:G204R	S:H655Y	
		S:T1027I	
		S:V1176F	
P.2 (zeta)	orf1a:L3468V	S:E484K	S:E484K
	orf1a:L3930F	S:D614G	S:D614G
	orf1b:P314L	S:V1176F	
	N:A119S		
	N:R203K		
	N:G204R		
	N:M234I		
B.1.617.2 (delta)	ORF1b:P314L	S:T19R	S:L452R
	ORF1b:G662S	S:del157/158	S:T478K
	ORF1b:P1000L	S:D950N	S:D614G
	ORF3a:S26L		S:P681R
	M:I82T		
	ORF7a:V82A		
	ORF7a:T120I		
	ORF8:del119/120		
	N:D63G		
	N:R203M		
	N:D377Y		

3130xl Genetic Analyzer (Thermo Fisher Scientific) using additional inner PCR primers (76_LEFT, 76_RIGHT, 77_LEFT and 77_RIGHT). Results were assembled to the Wuhan-Hu-1 SARS-CoV-2 S gene (Genbank acc# MN908947) and manually edited with Lasergene package (DNAStar, inc. Madison, WI). Access to sequencing data generated in this study is available at GISAID under IDs EPI_ISL_1608103–1608122 and EPI_ISL_2375848–2375899.

Mutational profiles characteristic of the B.1.1.7, B.1.351, P.1 and P.2 VOC/VOI were evaluated manually over the sequences obtained. Maximum likelihood phylogenetic reconstruction was carried out with IQTREE v1.5.5 (Nguyen et al., 2015) using an alignment including sequences belonging to different PANGO lineages selected from GISAID EpiCoV database (Supplementary Table 1). The HKY + I model of nucleotide substitution was selected with ModelFinder (Kalyaanamoorthy et al., 2017). Clade support was assessed with the approximate likelihood-ratio test (aLRT).

3. Results

SARS-CoV-2 S gene sequences were obtained for all 72 patients studied. Maximum likelihood phylogenetic reconstruction (Fig. 1) shows a clade containing the P.1, B.1.1.7 and B.1.351 VOC, but each clustering individually in a highly supported subclade (approximate likelihood-ratio test [aLRT] > 90%). The P.2 variant cluster was not strongly supported, however the sequences branched in a single cluster separate from the remaining lineages. The phylogenetic tree depicts that most of the sequences characterized herein belonged to P.1 (33; 46%) or P.2 (33; 46%) variants, followed by B.1.1.7 (4; 5.5%) and by B.1.1.28 (2; 2.5%), this latter one of the original variants found in the first wave of the pandemic in Brazil.

Samples were manually inspected to identify signatures of SARS-CoV-2 variants that might be present in the region analyzed in order to match their mutational signature profiles with the phylogenetic analysis results. All samples clustered in the P.1 branch harbored the mutations K417T, E484K, N501Y, D614G and H655Y, characteristic of that lineage. Samples that clustered with P.2 shared the mutations E484K and D614G, while the sample clustering with B.1.1.7 had the mutations N501Y, A570D, D614G and P681H (Table 1).

Six subjects of this study (all HCW) were vaccinated prior to the analyzed SARS-CoV-2 infection. The mean age of vaccinated HCW was 46.7 (32–65) years, mean SARS-CoV-2 RT-qPCR Ct value was 24.5 (21.78–29.08) and all six reported mild or no symptoms during infection. Fig. 2 shows a timeline for each of the vaccinees cases, showing their events of vaccination, dates of diagnosed infections, symptoms and IgG tests. Four of the six vaccinees (RJ-IC9512, RJ-IC10172, RJ-IC10369 and RJ-IC10521) were infected with the P.1 variant after vaccination and the remaining two (RJ-IC9838 and RJ-IC9894) were infected with P.2 (Fig. 2). Of those infected with P.1, two had already received the two doses of the CoronaVac vaccine and one had received the two doses of ChAdOx1-S/nCoV-19 vaccine (by participating in the vaccine clinical trials in Brazil during 2020) before infection, while the remaining subject had received only one dose of ChAdOx1-S/nCoV-19 prior to infection. Regarding the P.2 infections, both cases had been vaccinated only with the first dose of the ChAdOx1-S/nCoV-19 vaccine prior to infection (Fig. 2).

Five subjects represented reinfections that had been infected in the first wave of the Brazilian pandemic in mid-2020. Two of them were also part of the vaccinated group described above (Fig. 2); one of them received two doses of CoronaVac (subject RJ-IC10369) and the other, one dose of ChAdOx1-S/nCoV-19 (subject RJ-IC9838). The three remaining reinfection cases were not vaccinated. Four of the five reinfection cases were among HCW, while the remaining reinfection (non-vaccinated) occurred in a pediatric cancer patient.

Interestingly, despite evident IgG seroconversion in October 2020 after vaccination, RJ-IC9512 had an episode of infection by P.1 variant in February 2021 (Fig. 2). On the other hand, one of the reinfection cases (RJ-IC9838) had no detectable IgG titers after primo-infection, and was re-infected by a P.2 virus.

4. Discussion

Addressing the SARS-CoV-2 circulating variants is crucial to understanding their impact in immune escape and infection spread, as well as the efficacy of the vaccines available. In this study we propose a Sanger-based sequencing protocol to identify and assess the distribution of SARS-CoV-2 variants. We were able to define the lineages both based on phylogenetic analysis and by identifying signature mutations, evidencing that this is a robust method for genotyping SARS-CoV-2 known variants.

The present report points out to differences in the distribution of SARS-CoV-2 lineages at the Brazilian National Cancer Institute in the first wave of infections as compared to recent samples from December 2020 to March 2021. We have previously shown that in the first half of 2020 most of the patients and HCW at INCA were infected by lineage B.1.1.28 (Siqueira et al., 2021), however this was not the case for our recent samples where lineages P.1 and P.2 have risen and are now predominant. None of the subjects included in this study had any travel history to Manaus, epicenter of P.1, indicating that the P.1 lineage is circulating at high frequency in Rio de Janeiro. The shifts in the prevalence of different SARS-COV-2 lineages seen in our case series compared to the first wave of the Brazilian pandemic parallel in all aspects the scenario proposed for the epidemiological situation in Rio de Janeiro (Rede Genômica Fiocruz, Rede Genômica Fiocruz, n.d), with a rapid but transitory rise of P.2 strains, followed by an outgrowth of P.1 strains. This suggests that our series comprised of cancer patients and their healthcare providers are representative of the general population of the city with respect to the circulation and prevalence of SARS-CoV-2 lineages. Although our study is limited by the fact that only partial S gene sequences and not full genomes are generated from the viruses analyzed for SARS-CoV-2 variant identification, the mutation profiles covered by our strategy are sufficient to unequivocally distinguish between each VOC, VOI or parental strain.

In this survey, we report for the first-time six cases of vaccinees infected with the emergent P.1 and P.2 lineages. Of note, our study shows cases of infection in persons vaccinated with either CoronaVac or ChAdOx1-S/nCoV-19, the two vaccines firstly available in Brazil. Although the two infections of people vaccinated with CoronaVac have occurred soon after the administration of the second dose (Fig. 2), partial protection was already expected after the first dose. We provide several examples of infections by SARS-CoV-2 novel strains after vaccination with either vaccine in widespread use in Brazil. One of the subjects vaccinated with CoronaVac (RJ-IC10369) showed a case of reinfection almost a year after the primo-infection and only a few days after taking the second dose of the vaccine, showing that neither the previous natural infection nor vaccination was able to protect this subject from reinfection. A second case of reinfection after vaccination, RJ-IC9838, has an IgG negative test after primo-infection and was infected several months later by a P.2 virus. This is consistent with recent evidence that asymptomatic or oligo-symptomatic primo-infections may fail to generate detectable humoral or T-cell responses and make subjects fully susceptible to reinfections by similar or distinct SARS-CoV-2 variants (Kang et al., 2021; Legros et al., 2021).

One of the subjects studied (RJ-IC9512) was fully vaccinated with ChAdOx1-S/nCoV-19 (through the ChAdOx1-S/nCoV-19 phase III clinical trial conducted in Brazil in 2020) five months before infection even though he had an IgG positive test with high titers (155 Index (S/C) RLU) one month after vaccination regimen was completed. Moreover, he had another IgG test performed the day after he tested positive for SARS-CoV-2 infection, and the IgG titers were even higher than before (332 Index (S/C) RLU). It has been shown that anti-SARS-CoV-2 antibodies might last at least six months after infection (Lumley et al., 2021), which was indeed observed in this case. Here we show that even previously

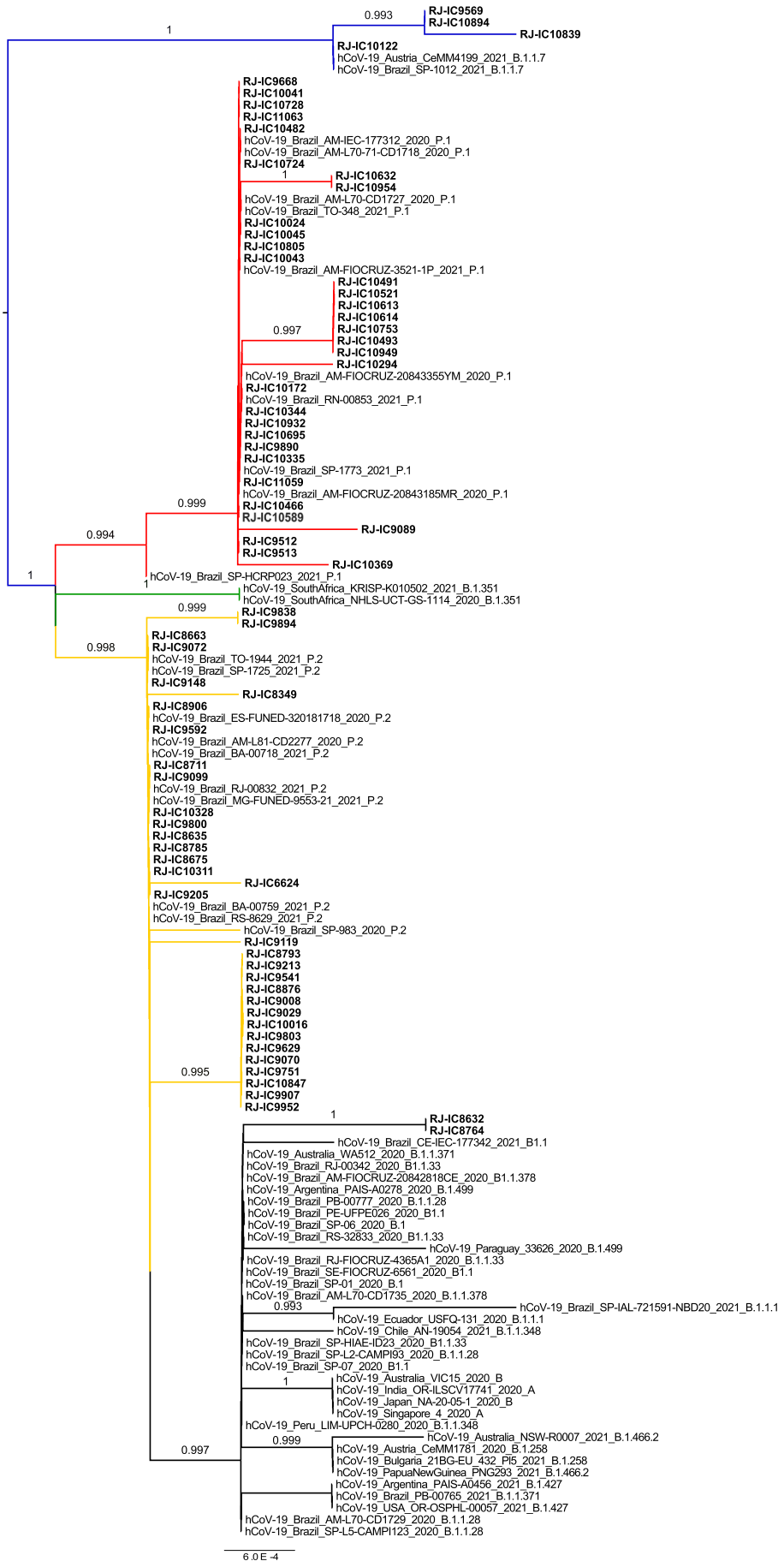

Fig. 1. Maximum likelihood phylogenetic reconstruction comprising SARS-CoV-2 S gene sequences generated in this study and sequences from different PANGO lineages available at GISAID Database. Phylogenetic analysis was conducted for lineage classification using partial SARS-CoV-2 S gene sequences (positions 955–2285 based on Wuhan-Hu-1 SARS-CoV-2 S gene, Genbank acc# MN908947). Lineage B.1.1.7 is show in blue, B.1.351 in green, P.1 in red and P.2 in yellow. Clinical samples characterized herein are highlighted in bold and only aLRT values greater than 0.7 are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

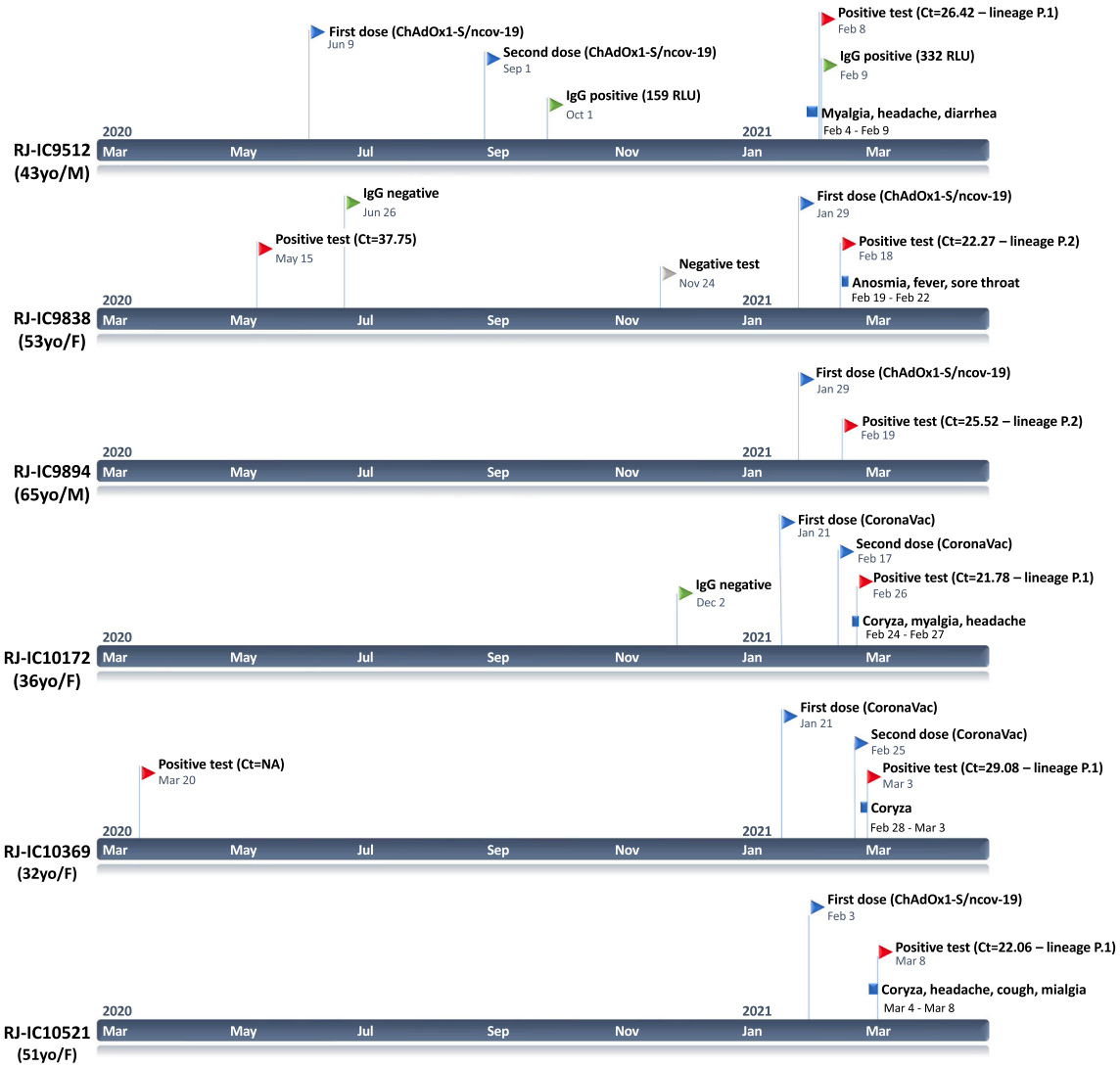

Fig. 2. Timeline of events for the vaccinees cases included in the study. Sample ID, subject's age (years-old, yo) and gender are indicated in each timeline. Events are depicted as follows: vaccination scheme is shown is blue flags, RT-qPCR positive tests (CDC RT-PCR protocol) with their Ct values (in parentheses) in red flags, IgG tests (S/C RLU in parentheses) in green flags, negative tests shown in gray flags and symptoms and their time frame depicted as blue boxes (absence of blue boxes represent asymptomatic cases). Two of the vaccinees (RJ-IC9838 and RJ-IC10369) are also cases of reinfection. NA, not available. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

infected and fully vaccinated subjects with high anti-S IgG titers are susceptible to infection by VOC, highlighting that immune responses induced by either natural infection or vaccination may not be sufficient to prevent infection by SARS-CoV-2 variants. Our study is limited by the fact that only total IgG titers were evaluated among our subjects, and it is known that total IgG correlate only partially to neutralizing antibody activity and to immunity against infection/reinfection (Babiker et al., 2021; Tang et al., 2020). Other assays, including neutralizing antibodies and cellular immunity, have not been performed, although it has been shown that neutralizing antibodies directed against ancestral viruses of the pandemic have reduced effectiveness against VOCs (Collier et al., 2021; King et al., 2021; McCallum et al., 2021).

All of our infected vaccinees and reinfection cases were HCW, except for one pediatric cancer patient. Although HCW are by definition a risk group, there is no other clinical or epidemiological evidence that would make those subjects of our case series more susceptible to reinfection or infection post-vaccination. No specific outbreaks took place at our clinical center during the survey, and HCW were otherwise healthy subjects. The only non-HCW analyzed here, a pediatric inpatient with acute lymphocytic leukemia, could be more susceptible to reinfection due to her immunosuppressive condition.

SARS-CoV-2 VOC/VOI were responsible for all reinfection and post-vaccination infection cases described herein. Although evidence suggest that this is an intrinsic feature of VOC/VOI or of subjects under high-risk of infection, we cannot completely rule out the theoretical possibility that non-VOC/VOI are also able to infect subjects under those circumstances, as our data was completely biased towards the occurrence of VOC/VOI. In fact, recent data suggest that non-VOC can also infect vaccinated subjects (Magalis et al., 2021).

Both vaccines approved for use in Brazil have the important role of preventing development of severe COVID-19 disease, however prevention of infection is only partial. We show herein six vaccinees infected with VOC P.1 and VOI P.2 after vaccination. These two lineages harbor the E484K mutation, which is also present in the South African VOC B.1.351, and which has been associated with immune evasion and escape neutralization by vaccine-induced humoral responses (Garcia-Beltran et al., 2021; Jangra et al., 2021).

In summary, we showed several examples of how natural SARS-CoV-2 infections or vaccination may not be fully capable of conferring sterilizing immunity against novel viral VOC/VOI. All vaccinated and re-

infected subjects carried VOC/VOI irrespective of the vaccine type taken, the number of vaccine doses taken, IgG titers or being previously infected during the first wave of the Brazilian pandemic. The data herein presented highlight the potential future challenges in controlling SARS-CoV-2 dissemination and the chronification of the COVID-19 pandemic.

Author contributions

Conceptualization, L.R.G, J.D.S and M.A.S.; methodology, L.R.G, J. D.S and B.M.A; formal analysis, L.R.G, J.D.S; resources, C.C, J.A, M.A.S and J.P.B.V; data curation, L.R.G, J.D.S, B.M.A. and M.M.G; writing—original draft preparation, L.R.G, J.D.S and M.A.S; writing—review and editing, L.R.G, J.D.S, B.M.A, M.M.G, A.C.P.M.P, C.C., J.A, J.P. B.V and M.A.S; project administration, M.A.S; funding acquisition, M.A. S and J.P.B.V. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

none.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2021.104998.

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