SARS-CoV-2 Vaccinated Breakthrough Infections With Fatal and Critical Outcomes in the Department of Antioquia, Colombia

Andres Cardona

One-Health Genomic Laboratory (Colombia/Wisconsin One-Health Consortium), Universidad Nacional de Colombia

Simon Villegas

One-Health Genomic Laboratory (Colombia/Wisconsin One-Health Consortium), Universidad Nacional de Colombia

Maria Stella Lopez

One-Health Genomic Laboratory (Colombia/Wisconsin One-Health Consortium), Universidad Nacional de Colombia

Maria Angelica Maya

Hospital Universitario de San Vicente Fundación

Celeny Ortiz-Restrepo

Gobernación de Antioquia, Medellin

Olga Hernandez-Ortiz

University of Antioquia

Karl Ciuoderis

One-Health Genomic Laboratory (Colombia/Wisconsin One-Health Consortium), Universidad Nacional de Colombia

Laura Perez

One-Health Genomic Laboratory (Colombia/Wisconsin One-Health Consortium), Universidad Nacional de Colombia

Idabely Betancour

Gobernación de Antioquia, Medellin

Carolina Muñoz

Gobernación de Antioquia, Medellin

Lina Bustamante

Gobernación de Antioquia, Medellin

Andres Mauricio Rangel

University of Antioquia

Jorge Osorio

University of Wisconsin–Madison

Juan Pablo Hernandez-Ortiz (✉ jphernandezo@wisc.edu)

One-Health Genomic Laboratory (Colombia/Wisconsin One-Health Consortium), Universidad Nacional de Colombia

Mark Anderson

Abbott Laboratories, United States

Francisco Averhoff

Abbott Laboratories, United States

Gavin Cloherty

Abbott Laboratories, United States

Research Article

Keywords: COVID-19, Departamento of Antioquia, vaccinated patients, BNT162b2, Ad26.COV2.S, ChAdOx1-S

Posted Date: January 12th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-963938/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The introduction of variants of concern and interest in the Departamento of Antioquia, Colombia, was concomitant with the beginning of the COVID-19 immunization program. Genomic surveillance indicates that none of the emerging variants –alpha, gamma, lambda, mu or delta– were dominant between January and August 2021. The immunization includes CoronaVac, BNT162b2, Ad26.COV2.S and ChAdOx1-S vaccines. By September 10th, 34.43% inhabitants were fully vaccinated. We characterized, SARS-CoV-2 breakthrough infections in 96 patients, 30 with fatal outcomes, 13 with ICU hospitalization and 53 with mild or asymptomatic disease. Even though gamma and mu variants co-circulated at similar levels, the latter was found to be predominant in patients with fatal outcomes and in those with ICU hospitalizations. We found a significant occurrence of the B.1.625 variant in these patients. Genetic substitutions of therapeutic and immunological concern, E484K and N501Y, are consistently observed in 90.1% and 79.5% of these variants, respectively. Evidence suggests that it is less probable to become infected after 60 days post-treatment with BNT162b2 than with CoronaVac. Importantly, we found that advanced age and comorbidities foster conditions for fatal and ICU outcomes in vaccinated patients. Our observations demonstrate the effectiveness of vaccination and identify patients with higher risks of subsequent breakthrough infections.

Introduction

By September 10th, 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had caused more than 226 million known cases of the coronavirus disease (Covid-19) worldwide, with an unfortunate outcome of 4.6 million deaths¹. In response, an unprecedented effort was made to develop, authorize, and deploy vaccines, with more than 5.8 billion doses of several vaccines administered to date. These immunization strategies are mostly directed at the viral spike protein (S), but the emergence of viral variants, particularly of the S gene, threatens their continued efficacy to prevent infection and/or severe disease. Variants of concern (VOC) and variants of interest (VOI) have provided motivation to increase molecular testing and to perform viral genomic surveillance in infected persons, thereby fostering an understanding of the transmissibility and virulence of variants and their ability to evade current vaccines.

In Colombia, VOI and VOC were identified at the beginning of 2021, resulting in an extended wave of infections from March to July of 2021 (around 25 epidemiological weeks (EWs)). Previous infection peaks in Colombia lasted between 4 to 6 EWs. The circulating VOC and VOI include alpha (B.1.1.7), gamma (P.1), lambda (C.37), mu (B.1.621) and delta (B.1.617.2). Parental variants, mostly of the B.1. lineage, did not disappear, and a new B.1.625 variant has been circulating with trivial outcomes. During 2020, genomic surveillance of SARS-CoV-2 was conducted by several independent entities within the country with unconnected results and strategies. The National Institute of Health (INS) initiated a coordinated effort called the Genomic Surveillance Network for SARS-CoV-2, where protocols, sample manipulation and collection, reagents, and bioinformatic algorithms were unified on a nationwide scale. Genomic surveillance at the Departamento of Antioquia has provided 30.1% (N = 1031) of the viral sequences obtained within the country (N = 3425). Real-time information regarding the circulating variants has been identified, e.g. (i) detailed timeline of novel variants in circulation; (ii) the proportion of infections caused by VOC and VOI; (iii) the identification of the mu variant and notification of the international community²; and (iv) the persistent presence of the variant B.1.625.

Antioquia has 6.68 million inhabitants and had reported a total of 740,807 COVID-19 positive patients up to September 2021. The Departamento recorded 15,996 deaths (2.2% mortality), with an average age of 70 years, and 13,259 patients requiring an ICU hospitalization, with an average stay of 13.6 days. The 18th of February of 2021 marked the beginning of the immunization program in Antioquia, and to date, 2.3 million people have completed the vaccination scheme (34.43%); 3.15 million (47.16%) have received the first dose. The vaccines used, by approximate proportion, were 43.19% of BNT162b2 (Pfizer-BioNTech), 35.70% of CoronaVac (Sinovac), 13.60% of ChAdOx1-S (AstraZeneca) and 7.49% of Ad26.COV2.S (Jansen). Administration of the mRNA-1273 (Moderna) vaccine was recently started in Antioquia and other parts of Colombia, however no completed vaccine schedules have been reached yet. As shown in **Figure 1**, the introduction of emergent variants, the extended wave of infections, and the immunization program are contemporary events during the first and second quarters of 2021. For completeness, the progress of the vaccination program in Antioquia is shown in the figure using the dates when increments of one million doses were reached.

Here, we describe 96 patients with breakthrough infections in the Departamento of Antioquia. Among them, 30 had a fatal outcome, 13 required ICU hospitalization, and 53 had mild or asymptomatic disease. Epidemiological, clinical, and viral-genomic characterizations of these patients are provided. The patients were identified within the COVID-19 diagnostic network of Antioquia, and the samples were processed for PCR testing and viral RNA sequencing in the One-Health Genomic Laboratory (OHGL), at the Universidad Nacional de Colombia, and in the Public Health Laboratory of Antioquia (PHLA). Our observations provide support for current strategies to monitor multiple variables proactively, including the determination of risk groups and the possible immune evasion of some variants.

Methods

Specimen collection

Samples (nasal swabs and nasopharyngeal washes) were collected through the COVID-19 diagnostic network of the Departamento de Antioquia, which is regulated and coordinated by the National Institute of Health (INS) and consists of 28 laboratories. The One-Health Genomic Laboratory (OHGL) and the Public Health Laboratory of Antioquia (PHLA) are part of this network, and it is also authorized and endorsed to perform genomic surveillance of SARS-CoV-2 by the INS. The standard operating procedures (SOPs) for sample collection and storage include careful custody of the samples at each laboratory with a mandatory storage at -80°C. Every week, criteria for genomic surveillance are determined and the corresponding samples are sent to the OHGL, where samples are stored at -80°C until processed. Typical criteria for genomic surveillance include hospitalized patients (general hospitalization and intensive care), a history of vaccination, travelers, randomly collected samples, and patients with suspected reinfection. During 2021, the OHGL had processed more than 1,700 samples, including approximately 200 from vaccinated patients. The COVID-19 immunization program in Antioquia currently uses CoronaVac

(Sinovac), BNT162b2 (Pfizer-BioNTech), Ad26.COV2.S (Jansen) and ChAdOx1-S (AstraZeneca) vaccines. Epidemiological and clinical data were collected for each sample, i.e. age, gender, origin, date of onset of symptoms, date of sample collection, associated symptoms, comorbidities, and risk group.

PCR and Ct Calculation

RNA extraction was performed on swab samples and nasopharyngeal washings using the ZR viral kit (Zymo® research, Irvine, CA, USA) following manufacturer's instructions. rRT-PCR was performed in a thermocycler CFX96 (Bio-Rad Laboratories, Inc, USA) using iTaq Universal Probes One-Step Kit (Biorad®) and specific primers/probe targeting Envelope and RNA-dependent RNA polymerase (RdRP) genes³ (**Table S1**). Running conditions were: one cycle at 50°C for 10 min, one cycle at 95°C for 3 min and 40 cycles of 95°C for 15 s and 58°C for 30 s. In addition, for each sample, an amplification of the Ribonuclease P gene (RNaseP) was made as a control for the extraction of viral RNA. A Ct value was obtained from each of the samples according to the rRT-PCR curves; this value represents the cycle in which the samples have a positive amplification and exceed an RFU of 300 for the E and RdRP genes and of 100 for the Ribonuclease gene P. The samples were considered positive when the Ct value was less than 38.

Whole genome sequencing

Samples having a Ct below 27 were selected for sequencing using the nCoV-2019 sequencing protocol v3⁴. cDNA was first made from the RNA (8 µl) and the LunaScript RT Supermix (5X) was used. Then, the multiplex PCR was performed using 218 primers, separated into 2 pools (pool 1 with 110 primers and pool 2 with 108 primers), and the Q5 hot start high fidelity 2X master mix was used (New England Biolabs, CITY, STATE). Two amplifications were conducted for each sample (3 µl of cDNA) and subsequently pooled by mixing 5 µl of each PCR product and 40 µl of nuclease-free water. Each amplicon pool was quantified with the aid of the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). The amplicon pools that had a concentration greater than or equal to 7 ng / µl were used for the end repair. This was conducted using between 50 and 100 ng of DNA and the NEBNext® Ultra™ II End Repair / dA-Tailing Module (New England Biolabs). Then the barcode ligation was made using the Native Barcoding Expansion 96 kit (Oxford Nanopore) and the Blunt / TA Ligase Master Mix (New England Biolabs). Each sample was pooled and purified using AMPure XP beads (Beckman coulter). Next, the AMII was ligated using the Quick Ligation™ Kit (New England Biolabs). DNA purification was performed again with the AMPure XP beads (Beckman coulter). Finally, the purified DNA was mixed with SQB buffer and Loading Beads (LB) and loaded into the Flow Cell (R9).

The assembly of raw NGS data was performed by following the pipeline described for Oxford Nanopore Technologies (ONT) platform⁵. Each sequence obtained was analyzed with the help of Pango-Lineage, which assigns a lineage according to the lineages reported so far⁶. In addition, Nextclade was used for the analysis of each of the mutations of the sequences⁷.

Phylogenetic analysis

We sequenced 267 SARS-CoV-2 genomes from patients in Antioquia - Colombia and downloaded 472 randomly sampled global sequences from GISAID. We aligned the sequences using Nextalign v1.2.1 as part of the Nextclade workflow (<https://github.com/nextstrain/nextclade>), using Wuhan-Hu-1 (NCBI Reference Sequence: NC045512.2) as reference. We manually edited the alignment to remove 5' UTR and 3' UTR using MEGAX software v10.2.6⁸. All Maximum likelihood (ML) phylogenies were inferred using IQ-Tree v2.1.2⁹ with GTR+F+R2 substitution model estimated by means of the Bayesian Information Criterion (BIC) with the ModelFinder function¹⁰. We used both the Shimodaira–Hasegawa approximate likelihood-ratio test (SH-aLRT)¹¹ and ultrafast bootstrap (UFboot)¹² with 1000 replicates each to assess the tree topology and used iTOL v5¹³ to visualize and analyze the resulting phylogenetic tree.

Results

Samples, along with clinical and epidemiological information, from patients and deceased persons with confirmed immunization (partial and complete) that are diagnosed as positive to SARS-CoV-2 by any laboratory of the Antioquia's network are constantly collected by the OHGL and the PHLA. A confirmatory RT-PCR test is carried out on the samples to qualify the Ct and validate the diagnosis. Special attention is made to deceased patients and individuals that required ICU hospitalization. Up to September 2021, we had detected 49,630 breakthrough infections in Antioquia, where 2998 required hospitalization, 1018 need an ICU and 502 died. From those patients more than 200 breakthrough infection samples had been recovered, and 96 had the quality to perform whole genome sequencing. From the latter, 30 patients had a fatal outcome, 13 required ICU, and 53 presented mild or asymptomatic disease. Patients were organized by age with No 1 being the oldest and No 96 the youngest (**Table 1**). The Ct value of the RT-PCR was used to illustrate the impact of age and comorbidities on the outcome of the breakthrough infection (Figure 2A). The probability of getting an infection after n days post vaccination and comparisons for two groups: (i) deceased vs. surviving patients and (ii) Sinovac vs. Pfizer recipients, was also plotted (Figure 2B). Breakthrough infections of other vaccines were of insufficient quantity for analysis, not implying that they offer better protection, but a consequence of the low number of recipients (up to September 2021, Sinovac and Pfizer vaccines were administered to almost 80% of vaccinated recipients).

Analysis of deceased and surviving vaccine recipients with critical disease revealed that 72.09% of the patients had comorbidities and 44.17% were females. Advanced age and comorbidities were determinant factors in the outcome and progression of the disease ($p < 0.01$). The odds ratio (OR) indicates that it is 17.4 more probable to have a fatal outcome for patients older than 60 years of age, while it is 7.3 more probable to succumb from COVID-19 when comorbidities were present. The average age for deceased patients with comorbidities was 81, and 71 for deceased patients without known comorbidities. The average age of ICU survivors with comorbidities was 67, and 59 for ICU survivors without comorbidities. Patients with mild or asymptomatic infection averaged 49 years. The most common comorbidities in patients with fatal outcome were pulmonary arterial hypertension (PAH) (66.7%), diabetes (37.5%) and thyroid diseases (25%). On average these patients each had 3 comorbidities (**Table 1**). In contrast, survivors of critical disease presented with PAH (57.1%) and diabetes (57.1%) as the most prevalent comorbidities and had, on average, 1.5 comorbidities each (**Table 2**). Accounting for the fact that the vaccination program in Colombia for adults older than 70 years used Sinovac in a high percentage, it is not surprising that this vaccine was more frequent in

patients with fatal and ICU outcomes. Tables S2, S3 and S4 summarize information for deceased and ICU patients without comorbidities, patients with mild or asymptomatic disease and patients suspected of being reinfecting (N = 15). Reinfected patients in this report were not confirmed by sequencing due to the poor quality of one of their samples. None of these patients had a fatal outcome and only one required an ICU hospitalization.

Discussion

The introduction of VOC and VOI in Colombia changed the dynamics of COVID-19 infection and generated extra burdens in non-clinical measures to control the ongoing pandemic. A major consequence of lineages with an increased reproduction number was an extended wave of infections that lasted for more than 4.5 months. The emergence of these variants might have not been exclusively responsible for this extended wave, as the country also experienced several episodes of civil unrest during this timeframe. As a consequence, standard social distancing measures, quarantines and other non-clinical measures were difficult to implement, however, it motivated the intensity of the vaccination programs, which started mid-February, and was initially concentrated in high-risk groups (advanced age, healthcare workers, and people with comorbidities). With 34.43% of the population fully vaccinated in the Departamento de Antioquia, the lethality of SARS-CoV-2 decreased from 2.2% in non-vaccinated patients to 0.014% in vaccinated patients¹⁴. The surveillance of patients with a breakthrough infection offers a mechanism to understand the protective capacity of existing vaccines against emerging variants. A total of 96 patient samples were characterized to provide crucial information and to delineate possible strategies to protect patients at risk. We found that advanced age and comorbidities were highly correlated with fatal and ICU outcomes in patients with breakthrough infections ($p < 0.01$). Concomitant with the natural course of the disease, PAH and diabetes were dominant in these patients^{15,16}. Other comorbidities, including thyroid disease, cancer, coronary disease, chronic obstructive pulmonary disease (COPD) and chronic kidney disease (CKD) were present in approximately 25% of the patients. Correlated with the advanced age of these patients, around 40% presented neurological or mental disorders. The average age monotonically increased from patients with mild disease, to critical disease with and without comorbidities to deceased patients with and without comorbidities.

The probability of a breakthrough infection after n days post-vaccination was calculated from the distribution of infections from all patients. We found no correlation between the probability of infection and the outcome of disease, indicated by the similarities between the distributions and the cumulative probabilities in patients with fatal outcome and survivors. For instance, the probability to get infected after vaccination within the first 30 days was 60% and 55% for deceased and survivors, respectively. On the other hand, significant differences are observed when the Pfizer-BioNTech and the Sinovac vaccines were compared. We found that the probability of a breakthrough infection with the Pfizer-BioNTech is higher within the first 60 days after vaccination, i.e. 90% of the patients were infected between day 0 and day 60. The Sinovac vaccine, however, resulted in a flatter distribution that persists for 120 days after vaccination. These observations may indicate that the timeline for protection is, on average, faster for the Pfizer-BioNTech biological than for the Sinovac vaccine.

Examination of the SARS-CoV-2 sequences revealed that the mu variant was present in most of the patients with breakthrough infections with fatal and ICU outcomes. The gamma and the B.1.625 variants were also detected in a considerable number of patients. The elevated participation of the mu variant in these patients is not surprising according to recent reports regarding its diminished neutralization capacity in isolates¹⁷ and pseudo-virus¹⁸. Accounting that we did not observe a dominant emerging variant, some of the breakthrough patients were also infected by persistent non-VOC/VOI variants (mostly of the B.1 group). Some substitutions of therapeutic and immunologic concern were prevalent in the majority of the variants, i.e. the E484K was observed in 90.1%, while the N501Y was present in 79.5%. Other substitutions, like the K417T and the L452R were present in 26.7% and 2.2% of the variants, respectively. Phylogenetic analysis determined the monophyletic nature of the VOC and VOI that circulate in Antioquia and deceased patients and ICU survivors are indicated in the corresponding clade of the variant (Figure 4). It also reinforces the neighboring similarities between the mu and the B.1.625 lineage. In fact, in all sequenced mu and B.1.625 strains the substitutions T95I, E484K, D614G and D950N were present in the spike protein. Furthermore, a close relationship between mu and the other VOCs, such as delta, alpha, gamma, and lambda, was not observed. However, all share pivotal mutations in the Spike protein, such as E484K, D614G, P681H and D950N. These substitutions are of great interest because they have been related to higher rates of infection and the potential escape on the immune response^{19,20,21,22}.

Fifteen of the patients with breakthrough infections were suspected of reinfection (**Table S4**). This classification was made merely on diagnosis, i.e., patients with two positive RT-PCR results with 90 days or more between. Up to September 2021, the OHGL and the PHLA have been unable to corroborate reinfection with sequencing. In most of the cases, one of the samples (either from the first or second infection) did not meet the technical requirements for an acceptable sequence coverage. However, RT-PCR protocols and reagents in Colombia are standardized and the chances are high that the reinfected classification is appropriate. Among these 15 patients, no fatal outcomes were observed and only one patient required an ICU hospitalization. The other patients had mild or asymptomatic disease. The identified viral lineages were gamma (7), mu (4), B.1.625 (2) and parental (2).

Our observations empower the importance of the urgent efforts being taken globally to achieve herd immunity and vaccinate most of the global population. They also validate the effectiveness of the vaccines and highlights the need to stay vigilant and monitor viral evolution and its potential impact on vaccine efficacy. As many countries are preparing themselves for re-immunization campaigns, vaccine manufacturers might also consider updating their formulation to provide coverage against emerging variants. In addition, they leverage the necessity of universal vaccines against beta-coronaviruses and highlight the importance of active surveillance as a platform for pathogen discovery. More importantly, our observations show the importance of the ongoing race between immunization and the potential emergence of viral escape mutants. Our analysis similarly supports the need to maintain molecular testing (in symptomatic, asymptomatic, and vaccinated patients) and viral genome surveillance to establish databases for breakthrough infections.

Declarations

Ethics Statement. The study was approved by the Institutional Review Board of the Corporación de Investigaciones Biológicas (CIB) in Medellín, Colombia, and by the Secretaría Seccional de Salud de Antioquia. Informed consent was obtained from all subjects or family members. Anonymized clinical and epidemiological data was collected and used according to the guidelines presented to the ethical committee. Genomic information was used after clearance

by the Instituto Nacional de Salud (INS), following the National Genomic Surveillance Protocol and after release for public use at the GISAID database (<https://www.gisaid.org/>). All methods were performed in accordance with the relevant guidelines and regulations.

Data availability. All requests for anonymized raw and analyzed data that underlie the results reported in this article will be reviewed by the One-Health Genomic Laboratory (jphernandez@unal.edu.co) to determine whether the request is subject to confidentiality and data protection obligations. Data that can be shared will be released via a material transfer agreement.

Acknowledgements. This work was supported by the Antioquian Corporation/Mining Coalition (Grupo SURA, Grupo Nutresa, Fundación Grupo Bancolombia, Sumicol Grupo Corbeta, Zijin/Continental Gold Ashanti, Gramalote Colombia, Gran Colombia Gold), the Grupo ISA, Fundación Sofía Perez de Soto, Fundación Fraternidad Medellín, the Gobernación de Antioquia and the Universidad Nacional de Colombia, Medellín. The authors thank the laboratories and scientist that are part of the SARS-CoV-2 Genomic Surveillance Program by the Instituto Nacional de Salud and the support by the Abbott Pandemic Defense Coalition.

Author contributions. Study design: J.O. and J.P.H.O. Data collection: A.C., S.V., M.S.L., M.A.M., C.O.-R., O.H.O., K.C., L.P., I.B., C.M., L.B. and J.P.H.O. Data analysis: A.C., S.V., M.S.L., M.A.M., O.H.O., M.A., F.A., G.C., J.O. and J.P.H.O. Data interpretation: A.C., S.V., M.S.L., M.A.M., O.H.O., A.M.R., M.A., F.A., G.C., J.O. and J.P.H.O. Writing: J.O. and J.P.H.O. with comments from all authors.

Competing interests. The authors have declared that no competing interests exist.

References

1. The John Hopkins University COVID-19 surveillance webpage, September 2021 (<https://coronavirus.jhu.edu/>).
2. Laiton-Donato, K. *et al.* Characterization of the emerging B.1.621 variant of interest of SARS-CoV-2. *Infect Genetics Evol* 105038 (2021) doi:10.1016/j.meegid.2021.105038.
3. Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* **25**, 2000045 (2020).
4. Amplicon sequencing protocol for SARS-CoV-2 v3 (<https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye>)
5. Oxford NanoPore - nCoV-2019 novel coronavirus bioinformatics protocol, September 2021, (<https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html>)
6. Proposal for lineage within B.1 with N501Y, E484K, R346K, P681H Spike substitutions, September 2021 (<https://github.com/cov-lineages/pango-designation/issues/57>).
7. NextClade v.1.7.0, September 2021 (<https://clades.nextstrain.org/>).
8. Stecher G, Tamura K, Kumar S. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Mol Biol Evol* 2020;37(4):1237–9.
9. Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020;37(5):1530–4.
10. Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler A von, Jermini LS. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 2017;14(6):587–9.
11. Shimodaira H, Hasegawa M. Multiple Comparisons of Log-Likelihoods with Applications to Phylogenetic Inference. *Mol Biol Evol* 1999;16(8):1114–1114.
12. Hoang DT, Chernomor O, Haeseler A von, Minh BQ, Vinh LS. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol Biol Evol* 2017;35(2):518–22.
13. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 2021;49(W1):gkab301.
14. COVID-19 data and statistics from the Instituto Nacional de Salud de Colombia, September 2021 (<https://www.ins.gov.co/Noticias/Paginas/coronavirus-genoma.aspx>.)
15. Moderbacher, C. R. *et al.* Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* **183**, 996-1012.e19 (2020).
16. Huang, A. T. *et al.* A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* **11**, 4704 (2020).
17. Messali, S. *et al.* A cluster of the new SARS-CoV-2 B.1.621 lineage in Italy and sensitivity of the viral isolate to the BNT162b2 vaccine. *J Med Virol* (2021) doi:10.1002/jmv.27247.
18. Uriu, K. *et al.* Ineffective neutralization of the SARS-CoV-2 Mu variant by convalescent and vaccine sera. (n.d.) doi:10.1101/2021.09.06.459005.
19. Wang, W. B. *et al.* E484K mutation in SARS-CoV-2 RBD enhances binding affinity with hACE2 but reduces interactions with neutralizing antibodies and nanobodies: Binding free energy calculation studies. *Biorxiv* 2021.02.17.431566 (2021) doi:10.1101/2021.02.17.431566.
20. Ferrareze, P. A. G. *et al.* E484K as an innovative phylogenetic event for viral evolution: Genomic analysis of the E484K spike mutation in SARS-CoV-2 lineages from Brazil. *Biorxiv* 2021.01.27.426895 (2021) doi:10.1101/2021.01.27.426895.
21. Leung, K., Shum, M. H., Leung, G. M., Lam, T. T. & Wu, J. T. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Eurosurveillance* **26**, 2002106 (2021).
22. Liu, Y. *et al.* The N501Y spike substitution enhances SARS-CoV-2 transmission. *Biorxiv* 2021.03.08.434499 (2021) doi:10.1101/2021.03.08.434499.

Tables

Table 1. Deceased patients with comorbidities (N = 24)

Patient	à	2	3	4	5	6	8	9	12	13	14	15	16	18	19	20	21	26	27	28	29	33	40	55					
Age	à	96	95	93	92	91	91	88	87	87	86	85	85	84	84	83	83	81	81	80	80	77	73	63					
Gender	à	F	F	M	M	F	M	F	M	F	F	F	M	M	F	M	M	M	F	M	F	M	M	F					
Vaccine	à	sv	sv	sv	sv	sv	sv	sv	sv	sv	sv	sv	sv	sv	pz	sv	sv	sv	sv	sv	sv	sv	pz	sv					
Partial Vaccination	à															x					x					x	x		
Comorbidity	-																												
PAH		x	x	x	x	x	x		x			x		x	x			x	x	x	x	x		x					
Diabetes							x						x	x	x	x			x	x		x		x					
Thyroid disease		x		x								x	x			x		x											
Coronary heart D.							x		x	x								x		x									
Cancer												x	x												x	x			
COPD		x	x	x																				x					
CKD						x	x	x																					
Asthma		x																											
Neurologic/Mental D.		pd	pd	pd			A		tia	A											pd	A		P					
Metabolic Disorders													3x	x															
Other disorders		o		is	dd			cd																			cr		
																								cr					
Smoker														x															
Oxygen dependent		x																											
Total Comorbidities		3	7	2	2	5	4	1	4	1	1	6	3	3	4	1	1	2	5	3	2	4	1	4					

sv: Sinovac vaccine

pz: Pfizer-BioNTech vaccine

pd: psychiatric disorder

A: Alzheimer's disease

P: Parkinson's disease

tia: transient ischemic attack

o: osteoporosis

is: immunosuppressed

dd: digestive disorder

cd: clotting disorder

cr: cirrhosis

ra: rheumatoid arthritis

< > : Average

Note 1: Obesity was not reported in any of these patients

Note 2: In addition to the patients with Cancer, only one patient was reported to be immunosuppressed

Table 2. ICU patients with comorbidities – non-fatal outcome (N = 7)

Patient	à	32	35	45	47	49	56	74	%
Age	à	78	76	69	68	66	62	38	<65.3>
Gender	à	M	M	M	M	F	M	F	-
Vaccine	à	sv	sv	sv	sv	az	az	pz	-
Partial Vaccination	à			x		x	x	x	57.1
Comorbidity									
PAH		x	x			x	x		57.1
Diabetes		x		x		x		x	57.1
COPD			x						14.3
Coronary heart D.					3x				14.3
Smoker									
		x							14.3
Total Comorbidities									
		3	2	1	3	2	1	1	<1.86>

- sv: Sinovac vaccine
- az: AstraZeneca vaccine
- pz: Pfizer-BioNTech vaccine
- < > : Average

Figures

Figure 1

Time progress of the COVID-19 pandemic in the Departamento of Antioquia, Colombia. **A:** incidence according to the symptom onset; **B:** introduction and presence of parental and emerging variants; and **C:** vaccination program with the beginning (February 18th, 2021) and the dates to reach one million doses. The location of Colombia, within South America, and the Department of Antioquia, within Colombia, are included for completeness.

Figure 2

A: Ct value as a function of age for the $N = 96$ patients (circles). Squared symbols indicate the average age and Ct of deceased patients, triangles indicate the averages for the ICU survivors and the pentagon are for the averages of the patients with mild disease. Solid symbols are for patients with comorbidities, while non-solid symbols are for those without comorbidities. **B:** Probability of being infected after n days post-complete vaccination for deceased vs. survivors and for Pfizer-BioNTech vs. Sinovac vaccines.

Figure 3

Parental and emerging variants in the Departamento of Antioquia. **A:** Evolution of the circulating variants according to the genomic surveillance and summary of variants present (N = 1031). **B:** Fraction of variants for the patients with breakthrough infections with fatal outcome. **C:** Fraction of variants for the patients with breakthrough infections with ICU hospitalization.

Figure 4

Phylogenetic Comparisons of the SARS-CoV-2 strains collected in the Departamento de Antioquia (Colombia). Circles represent VOC, VOI, and the B.1.625 lineage. Solid squares indicate the patients with fatal (red) and ICU hospitalization (blue).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [BIVaccinatedSI.docx](#)