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THÈSE DE DOCTORAT

Soutenue à Aix-Marseille Université
en cotutelle avec l'Université Libanaise
le 21 Novembre 2024 par

Dalal NOUR

Caractérisation génotypique des lignées circulantes du SARS-CoV-2 et cinétique des titres d'anticorps anti-Spike chez les adultes vaccinés au Liban

Discipline
Biologie santé

Spécialité
Microbiologie Alimentaire et Médicale (Université Libanaise).
Microbiologie (Université Aix-Marseille).

École doctorale
Ecole doctorale des Sciences et Technologie (Université Libanaise).
ED62- Ecole doctorale Sciences de la Vie et de la Santé (Université Aix-Marseille).

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Affidavit

I, undersigned, Dalal NOUR, hereby declare that the work presented in this manuscript is my own work, carried out under the scientific supervision of Monzer HAMZE, Philippe COLSON, and Rayane RAFEI, in accordance with the principles of honesty, integrity, and responsibility inherent to the research mission. The research work and the writing of this manuscript have been carried out in compliance with both the French national charter for Research Integrity and the Aix-Marseille University charter on the fight against plagiarism.

This work has not been submitted previously either in this country or in another country in the same or in a similar version to any other examination body.

Place Tripoli (Lebanon), date 11 juillet 2024



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List of publications and/or Patents and participation in conferences

1) List of publications and/or patent carried out as part of the thesis project:
    1. First author in "The Role of Lebanon in the COVID-19 Butterfly Effect: The B.1.398 Example", published in ***Viruses journal*** (https://doi.org/10.3390%2Fv14081640)
    2. First author in "Evaluation of SARS-CoV-2 anti-Spike antibody levels and breakthrough infection risk among vaccinated adults in North Lebanon" published in ***PLoS ONE journal*** (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0302579)
    3. Third author in "A cross-sectional study revealed a low prevalence of SARS-CoV-2 infection among asymptomatic University students in Tripoli, North Lebanon", submitted to the ***Journal of Pathogens***.

2) Participation in conferences and summer schools during the thesis period:
    1. Oral presentation: Lebanese University – Doctoral School of Sciences and Technology – Azm Center for Research in Biotechnology (September 17th, 2021, Tripoli, Lebanon).
    2. Oral Online presentation: Aix Marseille University – (March 8th, 2024).
    3. Workshop: Biomedical Electrophysiology Day – Lebanese University-Doctoral School of Sciences and Technology – Azm Center for Research in biotechnology (March 2nd, 2024, Tripoli, Lebanon).
    4. Workshop: 23rd Annual Meeting of the Lebanese Society for Infectious Diseases and Clinical Microbiology. Antimicrobial Resistance: The Evolution Pandemic (September 15th, Dbayeh Lebanon).
    5. Training delivered by the Lebanese University:
        - Apport du laboratoire dans la surveillance des infections liées aux soins
        - Stratégies de prévention et de lutte
        - Epidémiologie des infections liées aux soins
        - Risques infectieux liés à l'environnement hospitalier
        - Introduction à Python
    6. Online training delivered by Aix Marseille University:
        - For non-French speakers: Promote your scientific production 2: Manage your

researcher profiles (July 7th – July 8th, 2022).

- For non-French speakers: Scientific Publication: 2. Writing an article (June 16th 2022).
- Du chaos des idées à la thèse réussie : formation intensive pour réussir votre rédaction de thèse (November 20th – November 24th, 2023).
- English speakers: Open Science: 1. Scientific publication issues (October 9th, 2023).
- English speakers: Open Science: 2. How to deposit in HAL my first publications or conference proceedings? (January 16th 2023).
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- Intégrité Scientifique dans les métiers de la recherche 17 oct.2022 - 6 janv.2023 FUN Campus (October 17th, 2022 – January 6th, 2023).
- L'intelligence économique pour les acteurs de la recherche : sensibilisation (March 9th, 2023).
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- English speakers: Gender equality in the academic and professional settings: the influence of gender stereotypes - CONFERENCE (December 2nd, 2022).
- Les fondamentaux de la recherche d'emploi avec l'APEC (July 7th, 2023).
- Parcours Entrepreneuriat : comment se lancer ? De l'idée à l'entreprise (December 1st, 2022).
- Témoignage d'un Docteur : de la thèse au financement de l'Innovation (October 13th, 2022).
- Initiation au logiciel R, Statistiques et Analyse multivariée (February 2nd 2023 – June 30th 2023).
- Word pour documents longs - Produire sa thèse (February 6th 2023).

Résumé

A partir de fin 2019, le monde entier a été perturbé par l'apparition d'une nouvelle maladie à coronavirus, la COVID-19, détectée pour la première fois dans la ville du Wuhan, en Chine, puis propagée à travers le monde, aboutissant à une pandémie mondiale. Cette maladie est causée par un virus à ARN de polarité positive, simple brin, avec un taux de mutation élevé, appelé coronavirus 2 du syndrome respiratoire aigu sévère (SRAS-CoV-2), qui a rapidement donné naissance à différentes lignées, provoquant des épidémies superposées ou successives. Plusieurs vaccins contre la COVID-19 ont été conçus. Le Liban, petit pays de la région de la Méditerranée orientale, a été fortement touché par la COVID-19, comptant plus d'un million de personnes infectées au 23 juin 2024, tandis que le nombre de séquences libanaises dans la banque GISAID ne dépassait pas 0,26 % du nombre de cas documentés. De plus, les réponses immunitaires générées après la vaccination ou l'infection ont été peu étudiées. Dans ce contexte, les travaux de cette thèse ont été divisés en deux axes. Le premier est un axe épidémiologique génomique, caractérisant les lignées circulantes du SRAS-CoV-2 au Liban par séquençage du génome entier entre Juillet 2020 et Février 2021. Notre première étude génomique a montré que, pendant cette période, B.1.398 était la lignée prédominante au Liban. Il est intéressant de noter que le Liban a joué le rôle d'un diffuseur potentiel de cette lignée dans différentes régions du monde. Notre deuxième étude a utilisé l'approche de « quantitative reverse transcription polymerization chain reaction » (RT-qPCR) pour détecter les variants Alpha et Delta et les sous-variants Omicron au cours des trois premières années de la pandémie au Liban, présentant cette approche comme réalisable, précise et peu coûteuse. Le deuxième axe de cette thèse a porté sur l'évaluation des anticorps anti-spike SRAS-CoV-2 dans une population d'adultes vaccinés au Nord-Liban. Notre étude a retrouvé que l'âge était inversement corrélé à la réponse immunitaire, et que le vaccin BTN162B2 était plus efficace que le vaccin AZD1222 en prévention de l'infection. En conclusion, cette thèse a fourni de nouvelles informations aux données libanaises concernant l'épidémiologie, la génomique et le statut sérologique pour les infections à SRAS-CoV-2. Néanmoins, des investigations et des suivis plus approfondis sont nécessaires pour mieux caractériser et compléter rétrospectivement et prospectivement la connaissance des lignées circulantes du SARS-CoV-2 et l'état immunitaire de la population vis-à-vis de ce virus au Liban.

Mots-clés : COVID-19, SRAS-CoV-2, lignées, Liban, épidémiologie génomique, variants, anticorps anti-spike, vaccinés

Abstract

From the end of 2019, the entire world was disrupted by the emergence of a new coronavirus disease, COVID-19, first detected in the city of Wuhan, China, and that then spread across the world, resulting in a global pandemic. This disease is caused by a single-stranded, positive-sense RNA virus with a high mutation rate, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which rapidly gave rise to different lineages, causing superimposed or successive epidemics. Several vaccines against COVID-19 have been designed. Lebanon, a small country in the Eastern Mediterranean region, has been heavily affected by COVID-19, with more than a million people infected as of June 23, 2024, when the number of Lebanese sequences in the GISAID did not exceed 0.26% of the number of documented cases. In addition, the immune responses generated after vaccination or infection have been scarcely studied. In this context, this thesis works was divided into two axes. The first is a genomic epidemiological axis, characterizing the circulating lineages of SARS-CoV-2 in Lebanon by whole genome sequencing between July 2020 and February 2021. Our first genomic study showed that, during this period, B.1.398 was the predominant lineage in Lebanon. It is interesting to note that Lebanon played the role of a potential disseminator of this lineage in different regions of the world. Our second study used the "quantitative reverse transcription polymerization chain reaction" (RT-qPCR) approach to detect Alpha and Delta variants and Omicron sub-variants during the first three years of the pandemic in Lebanon, presenting this approach as feasible, precise, and inexpensive. The second axis of this thesis focused on the evaluation of anti-SARS-CoV-2 spike antibodies in a population of vaccinated adults in North Lebanon. Our study found that age was inversely correlated with the immune response, and that the BTN162B2 vaccine was more effective than the AZD1222 vaccine in preventing infection. In conclusion, this thesis provided new insights to Lebanese data regarding epidemiology, genomics and serological status for SARS-CoV-2 infections. Nevertheless, more in-depth investigations and monitoring are necessary to better characterize and complete retrospectively and prospectively the knowledge of the circulating lineages of SARS-CoV-2 and the immune state of the population regarding this virus in Lebanon.

Keywords: COVID-19, SARS-CoV-2, lineages, Lebanon, genomic epidemiology, variants, anti-spike antibodies, vaccinated.

Acknowledgments

Three years ago, I had the chance to embark on a new journey. It was long and arduous, but many wonderful people were there to steer me in the right direction. As I approach the conclusion of my undertaking, I wish to take the time to thank everyone who helped and stood by me throughout this endeavor.

First, I wish to express my sincerest gratitude to my supervisors **Prof. Monzer HAMZE** and **Prof. Philippe COLSON,** and my co-supervisors **Prof. Rayane RAFEI** and **Prof. Mohamad Bachar ISMAIL**, for providing me the opportunity to engage with such a thought-provoking subject. The success of my research was greatly facilitated by their constant guidance, excellent supervision, readiness and excellent communication. Moreover, I want to thank each one of them for their consideration of the challenging moments and difficulties that I confronted during this journey, and their constant support to help me during these times. Besides I would like to thank **Prof. Marwan OSMAN**, for his valuable advice, his help and support in conducting this work.

I would also like to thank the **"Fondation Mérieux, Réseau GABRIEL"** for their funding, which made this work possible.

Additionally, I would like to convey my gratitude to the members of the jury from near and far, for their invaluable guidance and insight on numerous aspects of my thesis: **Prof. Mehmet ÖZDEMİR, Dr. Sylvie PILLET, Dr. Mira CHAAR,** and **Prof. Ghazi TADMOURI.** A special thanks goes to the president of the jury.

A deep thanks to the **LMSE** laboratory staff, for their invaluable assistance. I would like to deeply thank **Taha ABDOU**, **Assmaa ALLOUCH**, **Nazih LAZKANI**, **Sarah AMRIEH,** and **Dalal KASIR**, for their willingness to share their expertise and experience at a moment's notice, and their readiness to offer assistance whenever required, that were instrumental in enabling me to complete my work. I was very grateful to be part of the **IHU-Méditerranée Inféction** laboratory, and would like to thank especially **Mme. Marielle BEDOTTO**, for being there and helping me in my lab work.

A special thanks goes to my friend and colleague **Layla TAJER,** for exchanging her ideas and experience with me and helping in completing this work. Additionally, I would like to express my deep thanks to my friend, **Lina TABCHE**, for being an inspiring spring of ideas in designing the figures in my literature review, adding her own touch, and bringing the paper to the next level.

Moreover, I would like to express my deepest thanks and gratitude to my dearest **mother**, **father**, **brothers,** and **sisters,** who believed in me and supported me during this long

journey. I would like also to deeply thank my **husband's family**, who were my second family, for their encouragement and constant support during the many years of this work.

I would like to convey my deepest love to my little family, my beloved husband, **Rabih**, and my two little daughters **Hoda** and **Nour**, for being by my side, supporting and motivating me to believe in myself and achieve my goals. I am blessed to have the best caring and supportive families.

Abbreviation list

ACE2: Angiotensin Converting Enzyme 2

ARDS: Acute Respiratory Distress Syndrome

CD: Connector Domain

CDC: the Centers for Disease Control and Prevention

CH: Central Helix

COVID-19: COronaVirus Disease 2019

CP: Convalescent Plasma

Ct: Cycle threshold

CT: Cytoplasmic Tail

Del: Deletion

DNA: DesoxyriboNucleic Acid

E: Envelop protein

EUA: Emergence Use Authorization

FDA: U.S. Food and Drug Administration

FP: Fusion Peptide

FPPR: Fusion-Peptide Proximal Region

GISAID: Global Initiative on Sharing Avian Influenza Data

hCoV: human CoronaVirus

HR1: Heptad Repeat 1

HR2: Heptad Repeat 2

ICU: Invasive Care Unit

ID: Inhibitory Dilution

Kb: Kilobase

KSA: Kingdom of Saudi Arabia

LFU: Loss to Follow-Up

M: Membrane protein

mABs: monoclonal AntiBodies

MERS: Middle East Respiratory Syndrome

Mpro: Main protease

mRNA: messenger RNA

N: Nucleocapsid protein

NAAT: Nucleic Acid Amplification Tests

ORF: Open Reading Frame

PANGOLIN: The Phylogenetic Assignment of Named Global Outbreak Lineages

PLpro: Papain-Like protease

Pp1ab: Polyprotein 1ab

R_0: basic Reproductive number

RdRP: RNA dependent RNA polymerase

RNA: RiboNucleic Acid

R_t: effective Reproductive number

RTC: Replication/Transcription Complex

S: Spike protein

SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus

SGTF: S Gene Target Failure

SGTL: S Gene Target Late amplification

TAG-VE: Technical Advisory Group on SARS-CoV-2 Virus Evolution

TM: Transmembrane Segment

UK: United Kingdom

US: United States

VBM: Variant Being Monitored

VOC: Variant Of Concern

VOHC: Variant Of High Consequence

VOI: Variant Of Interest

VRBPAC: FDA's Vaccines and Related Biological Products Advisory Committee

VUM: Variant Under Monitoring

WGS: Whole Genome Sequencing

WHO: World Health Organization

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Introduction

Four years ago, on December 31, 2019, a new infectious coronavirus disease (COVID-19) was first detected in Wuhan City, China, and then spread across the world, causing a global pandemic, declared by the world health organization (WHO), on March 11, 2020 [1, 2]. It was caused by the severe acute respiratory syndrome 2 (SARS-CoV-2), a novel coronavirus, belonging to the same *Betacoronavirus* genus of previous SARS and middle east respiratory syndrome (MERS), but marked by its higher levels of transmissibility [3].

Its genome is a single-stranded, positive sense RNA, with six major open reading frames (ORF1a, ORF1b, S, M, E, and N), encoding 16 non-structural proteins (Nsp), essential for the replication, translation, and assembly of new virions; and four structural protein: spike protein (S) implicated in the viral attachment to the host cell via angiotensin-converting enzyme 2 (ACE2) receptor, and being the major target for vaccines and treatment; membrane protein (M) and nucleocapsid protein (N) that are implicated in the genome packaging and viral assembly; and envelope protein (E) involved in assembly, shaping and releasing of new viruses [4-7]. The remaining OFRs (ORF 3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10), encode the accessory proteins, affecting the immune evasion of the virus and its pathogenesis [4, 8]. The gold standard diagnostic tool for SARS-CoV-2 is reverse transcription polymerization chain reaction (RT-PCR), targeting different viral genes, including N, E, S, ORF1ab, and RdRp [9, 10].

SARS-CoV-2's genome was altered by the occurrence of continuous mutations, ending with the apparition of new viral lineages with new viral capacities [11]. Whole genome sequencing (WGS) is the gold standard technique for tracking emerging viral lineages [12]. Epidemiological surveys tracking new variants resulted in a rising list of SARS-CoV-2's genome sequences, that are stored in the global initiative of sharing avian influenza data (GISAID), available at https://gisaid.org/ and other international databases. Regardless of the usefulness and the validity of WGS, tools like quantitative RT-PCR (RT-qPCR), targeting specific mutations can constitute a rapid, accurate, and cost-effective substitution, especially in low-income countries [13].

Variants of concern (VOCs): Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.2 alias for B.1.1.28.2), Delta (B.1.617.2) and Omicron (B.1.1.529), were identified as descendant lineages having increased transmissibility or important change in the epidemiology of the disease, and/or increased virulence or change in the clinical manifestation of the disease, and/or decreased validity of used diagnostic tools, vaccines or treatments or in the public health social measures [14]. Many sub-lineages branched out from the Delta variant (AY.1 to AY.133) and were

identified in different geographical regions [15]. Besides, the Omicron variant has split into many subvariants, including BA.1 (B.1.1.529.1), BA.2 (B.1.1.529.2), BA.3 (B.1.1.529.3), BA.4 (B.1.1.529.4) and BA.5 (B.1.1.529.5) [11, 16]. Currently, no circulating variants are fitting with the VOC's characters according to the WHO [17].

In light of the rapid spread of COVID-19, with the lack of effective treatment, medical companies were racing to create vaccines, hoping to procure prevention of the disease. In this purpose, many types of vaccines have been developed, including: mRNA-based vaccines, viral-vector-based vaccines, live attenuated or inactivated viruses, viral protein subunits, and DNA vaccines [18]. Nonetheless, the FDA-approved vaccines for 2023-2024, are only those with updated formulas, including a monovalent component related to the Omicron variant XBB.1.5 (XBB.1 is a recombinant of two BA.2 lineages BA.2.75 and BJ.1 (BA.2.10.1)) [19, 20]. Only three vaccines have launched such updated formula, namely, the two mRNA vaccines Comirnaty® (BNT162b2, Pfizer/BioNTech), and Spikevax® (mRNA-1273) - Moderna vaccine as well as the protein sub-unit vaccine Nuvaxovid® (NVX-CoV2373) – Novavax [19]. FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) recommend that manufacturers design a monovalent JN.1-lineage vaccine for 2024-2025 [21].

In Lebanon, COVID-19 was first detected in February 21, 2020 [22]. Hereafter, 1,239,904 people were infected by the virus and the number of related deaths reached 10,947, as of June 23, 2024 [23]. Even though the disease has wildly spread in the country, tracking SARS-CoV-2 lineages is still under-estimated, since the GISAID database counted only 3,289 Lebanese viral sequences (corresponding to 0.26% of the total notified SARS-CoV-2 cases in Lebanon), as of June 30, 2024. Several previous Lebanese studies have investigated SARS-CoV-2 circulating lineages, showing that the country was dominated by B.1, B.4, and B.1.1 lineages during the first month of the pandemic [24]. Subsequently, between March and December 2020, B.1.398 was the prevalent national lineage, followed by the Alpha variant (B.1.1.7) and B.1 lineage [25], and this by analyzing 58 sequences. From December 2020 to May 2021, Merhi *et al.* tested 905 Lebanese sequences and revealed that the Alpha variant was completely superseded by the Delta variant by May 2021 [26]. A further investigation in health care workers (HCW) detected BA.1 and BA.1.1 as predominant lineages between December 2021 and January 2021, while the Delta variant was detected in only 6% of the total 250 tested nasopharyngeal samples [27]. The last investigation was implemented during the flu season and revealed the domination of the BA.5 sub-lineages from September to October 2022 [28]. Consequently, additional lineages, namely BA.2 sub-lineages were among the prevalent ones in December 2022 and January 2023 [28]. Finally, as of February 2023, the recombinant BA.2 lineage XBB (resulting from the recombination of BJ.1 (BA.2.10.1.1) and BM.1.1.1

(BA.2.75.3.1.1.1)) prevailed in the country [28]. On the other hand, a published Lebanese study has tested an RT-qPCR targeting the 69-70del in the S gene and 106-108del in the nsp6 gene, to detect the Alpha variant, emphasizing the importance of this approach in rapid screening implementation [29].

COVID-19 vaccines were introduced to Lebanon on February 14, 2021, and as of November 26, 2023, the percentage of the total population vaccinated with a complete primary series of a COVID-19 vaccine was 35%, while only 15% have taken at least one booster dose of a COVID-19 vaccine [23]. A previous study followed the effectiveness of primary (two doses of BTN162b2 or Sputnik V vaccines) and booster (addition of a third dose of BTN162b2 to the previous primary vaccination) vaccine regimens, in a collection of samples taken from employees at the National Airline company in Lebanon, during the periods of the Delta wave (between August and December 2021) and the Omicron wave (in January and February 2022) [30]. The results have shown that the protection provided from previous infection coupled with vaccine intake was higher than that provided by vaccination only. Besides, BTN162b2 was associated with higher anti-spike IgG levels than the Sputnik V vaccine [30]. BTN162b2 was more effective than Sputnik V in protecting against the Delta variant infection. While a significant decrease in the BTN162b2 protection was observed in the Omicron variant infection when Sputnik V didn't procure any protection. Moreover, the booster BTN162b2 didn't procure protection against Omicron infection [30].

In the present Ph.D. thesis, we addressed two aspects of SARS-CoV-2: genomic epidemiological and immunological. From a genomic epidemiological perspective: we aimed to discover the circulating SARS-CoV-2 lineages and track the variants in Lebanon during the first three years of the pandemic. From an immunological perspective, we evaluated the immune response associated with a 2-dose vaccine admission among the Lebanese population in North Lebanon.

In this context, this manuscript is divided into four main chapters:

Chapter I: involves a review paper addressing the passage of SARS-CoV-2 from a viral entity to different emerging variants. In this review, we presented the epidemiological global status of COVID-19. Afterward, the structure of SARS-CoV-2's genome as well as its notable mutations were addressed. We then detailed the VOCs and the variants of interest (VOI), their key mutations, and their implications in affecting the viral characteristics including virulence, transmissibility, and responses to vaccination, treatment, and diagnosis. The review will be submitted to The Journal of Pathology, Microbiology and Immunology - the APMIS journal.

Chapter II: fits within the first genomic epidemiological axe of our study, investigating the circulating SARS-CoV-2 lineages in Lebanon between July 2020 and February 2021, using

WGS. The sequenced genomes were added to the GISAID database. We then selected the prevalent national lineage in our study period, B.1.398, to investigate its transmission within Lebanon and between Lebanon and other countries. This part of the study is published in *Viruses* Journal (doi:10.3390%2Fv14081640).

In **Chapter III** we tested a more feasible and cost-effective approach to search for VOCs and Omicron subvariants during the first three years of the COVID-19 pandemic in Lebanon. Our results were then compared to the Lebanese SARS-CoV-2's genomes presented in the GISAID database. The article will be submitted to the Journal of Medical Microbiology.

Finally, **Chapter IV** treated the immunological axe of our study, evaluating the SARS-CoV-2 anti-Spike antibody levels and breakthrough infection risk among vaccinated adults in North Lebanon. The article is published in *PLoS ONE* journal (doi:10.1371%2Fjournal.pone.0302579).

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. Epub 2020/01/28. doi: 10.1016/S0140-6736(20)30183-5. PubMed PMID: 31986264; PubMed Central PMCID: PMCPMC7159299.

2. The World Health Organization (WHO). Coronavirus disease (COVID-19) pandemic [July 7, 2024]. Available from: https://www.who.int/europe/emergencies/situations/covid-19#:~:text=Cases%20of%20novel%20coronavirus%20(nCoV,pandemic%20on%2011%20March%202020.

3. Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E. A Novel Coronavirus Emerging in China - Key Questions for Impact Assessment. N Engl J Med. 2020;382(8):692-4. Epub 2020/01/25. doi: 10.1056/NEJMp2000929. PubMed PMID: 31978293.

4. Cao C, Cai Z, Xiao X, Rao J, Chen J, Hu N, *et al*. The architecture of the SARS-CoV-2 RNA genome inside virion. Nat Commun. 2021;12(1):3917. Epub 2021/06/26. doi: 10.1038/s41467-021-22785-x. PubMed PMID: 34168138; PubMed Central PMCID: PMCPMC8225788.

5. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, *et al*. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020;9(1):221-36. Epub 2020/01/29. doi: 10.1080/22221751.2020.1719902. PubMed PMID: 31987001; PubMed Central PMCID: PMCPMC7067204.

6. Bianchi M, Benvenuto D, Giovanetti M, Angeletti S, Ciccozzi M, Pascarella S. Sars-CoV-2 Envelope and Membrane Proteins: Structural Differences Linked to Virus Characteristics? Biomed Res Int. 2020;2020:4389089. Epub 2020/07/01. doi: 10.1155/2020/4389089. PubMed PMID: 32596311; PubMed Central PMCID: PMCPMC7261327 publication of this paper.

7. Rajpal VR, Sharma S, Sehgal D, Singh A, Kumar A, Vaishnavi S, *et al*. A comprehensive account of SARS-CoV-2 genome structure, incurred mutations, lineages and COVID-19 vaccination program. Future Virol. 2022. Epub 2022/06/25. doi: 10.2217/fvl-2021-0277. PubMed PMID: 35747328; PubMed Central PMCID: PMCPMC9203033.

8. Zandi M, Shafaati M, Kalantar-Neyestanaki D, Pourghadamyari H, Fani M, Soltani S, *et al*. The role of SARS-CoV-2 accessory proteins in immune evasion. Biomed Pharmacother. 2022;156:113889. Epub 2022/10/21. doi: 10.1016/j.biopha.2022.113889. PubMed PMID: 36265309; PubMed Central PMCID: PMCPMC9574935.

9. Snehlata.; Teja KBM, B. Chapter 1 - Application of CRISPR-Based Diagnostic Tools in Detecting SARS-CoV-2 Infection. COVID-19: Tackling Global Pandemics through Scientific and Social Tools2022.

10. Shafie MH, Antony Dass M, Ahmad Shaberi HS, Zafarina Z. Screening and confirmation tests for SARS-CoV-2: benefits and drawbacks. Beni Suef Univ J Basic Appl Sci. 2023;12(1):6. Epub 2023/01/18. doi: 10.1186/s43088-023-00342-3. PubMed PMID: 36647397; PubMed Central PMCID: PMCPMC9833029.

11. Centers for Disease Control and Prevention (CDC). SARS-CoV-2 Variant Classifications and Definitions 2020 [January 2, 2024]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html.

12. Vogels CBF, Breban MI, Alpert T, Petrone ME, Watkins AE, Ott IM, *et al*. PCR assay to enhance global surveillance for SARS-CoV-2 variants of concern. medRxiv. 2021. Epub 2021/03/25. doi: 10.1101/2021.01.28.21250486. PubMed PMID: 33758901; PubMed Central PMCID: PMCPMC7987060.

13. Munoz-Gallego I, Melendez Carmona MA, Martin Higuera C, Viedma E, Delgado R, Folgueira MD. Rapid screening of SARS-CoV-2 variants, a key tool for pandemic surveillance. Sci Rep. 2023;13(1):11094. Epub 2023/07/09. doi: 10.1038/s41598-023-37866-8. PubMed PMID: 37422474; PubMed Central PMCID: PMCPMC10329708.

14. The World Health Organization (WHO). Weekly epidemiological update - 25 February 2021 2021 [July 7, 2024]. Available from: https://www.who.int/publications/m/item/covid-19-weekly-epidemiological-update.

15. Rambaut A, Holmes EC, O'Toole A, Hill V, McCrone JT, Ruis C, *et al*. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol. 2020;5(11):1403-7. Epub 2020/07/17. doi: 10.1038/s41564-020-0770-5. PubMed PMID: 32669681; PubMed Central PMCID: PMCPMC7610519.

16. Sah R, Rais MA, Mohanty A, Chopra H, Chandran D, Bin Emran T, *et al*. Omicron (B.1.1.529) variant and its subvariants and lineages may lead to another COVID-19 wave in the world? -An overview of current evidence and counteracting strategies. Int J Surg Open. 2023;55:100625. Epub 2023/05/31. doi: 10.1016/j.ijso.2023.100625. PubMed PMID: 37255735; PubMed Central PMCID: PMCPMC10192062.

17. The World Health Organization (WHO). WHO COVID-19 dashboard [July 9, 2024]. Available from: https://data.who.int/dashboards/covid19/variants.

18. Soleimanpour S, Yaghoubi A. COVID-19 vaccine: where are we now and where should we go? Expert Rev Vaccines. 2021;20(1):23-44. Epub 2021/01/14. doi: 10.1080/14760584.2021.1875824. PubMed PMID: 33435774; PubMed Central PMCID:

PMCPMC7898300.

19. U.S. Food and Drug Admission (FDA). COVID-19 Vaccines for 2023-2024 2024 [July 9, 2024]. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines-2023-2024.

20. CoV lineages/Pango designation, 2022 [July 11, 2024]. Available from: https://github.com/cov-lineages/pango-designation/issues/1058.

21. U.S. Food and Drug Admission (FDA). Updated COVID-19 Vaccines for Use in the United States Beginning in Fall 2024 2024 [cited July 9, 2024]. Available from: https://www.fda.gov/vaccines-blood-biologics/updated-covid-19-vaccines-use-united-states-beginning-fall-2024.

22. Health LMoP. Novel Coronavirus 2019-Lebanon [June 25, 2024]. Available from: https://www.moph.gov.lb/en/Pages/2/24870/novel-coronavirus-2019.

23. The World Health Organization (WHO). WHO COVID-19 dashboard-Lebanon [June 25, 2024]. Available from: https://data.who.int/dashboards/covid19/deaths?m49=422&n=o.

24. Feghali R, Merhi G, Kwasiborski A, Hourdel V, Ghosn N, Tokajian S. Genomic characterization and phylogenetic analysis of the first SARS-CoV-2 variants introduced in Lebanon. PeerJ. 2021;9:e11015. Epub 2021/10/07. doi: 10.7717/peerj.11015. PubMed PMID: 34611501; PubMed Central PMCID: PMCPMC8447710.

25. Fayad N, Abi Habib W, Kandeil A, El-Shesheny R, Kamel MN, Mourad Y, *et al*. SARS-CoV-2 Variants in Lebanon: Evolution and Current Situation. Biology (Basel). 2021;10(6). Epub 2021/07/03. doi: 10.3390/biology10060531. PubMed PMID: 34198622; PubMed Central PMCID: PMCPMC8232177.

26. Merhi G, Trotter AJ, de Oliveira Martins L, Koweyes J, Le-Viet T, Abou Naja H, *et al*. Replacement of the Alpha variant of SARS-CoV-2 by the Delta variant in Lebanon between April and June 2021. Microb Genom. 2022;8(7). Epub 2022/07/26. doi: 10.1099/mgen.0.000838. PubMed PMID: 35876490; PubMed Central PMCID: PMCPMC9455693.

27. AlKalamouni H, Abou Hassan FF, Bou Hamdan M, Page AJ, Lott M, Matthews M, *et al*. Genomic surveillance of SARS-CoV-2 in COVID-19 vaccinated healthcare workers in Lebanon. BMC Med Genomics. 2023;16(1):14. Epub 2023/01/29. doi: 10.1186/s12920-023-01443-9. PubMed PMID: 36707851; PubMed Central PMCID: PMCPMC9880935.

28. Kodsi IA, Rayes DE, Koweyes J, Khoury CA, Rahy K, Thoumi S, *et al*. Tracking SARS-CoV-2 variants during the 2023 flu season and beyond in Lebanon. Virus Res. 2024;339:199289. Epub 2023/12/01. doi: 10.1016/j.virusres.2023.199289. PubMed PMID: 38036064; PubMed Central PMCID: PMCPMC10704499.

29. Abdel Sater F, Younes M, Nassar H, Nguewa P, Hamze K. A rapid and low-cost protocol for the detection of B.1.1.7 lineage of SARS-CoV-2 by using SYBR Green-based RT-qPCR. Mol Biol Rep. 2021;48(11):7243-9. Epub 2021/10/07. doi: 10.1007/s11033-021-06717-y. PubMed PMID: 34613565; PubMed Central PMCID: PMCPMC8493944.

30. Moghnieh R, El Hajj C, Abdallah D, Jbeily N, Bizri AR, Sayegh MH. Immunogenicity and Effectiveness of Primary and Booster Vaccine Combination Strategies during Periods of SARS-CoV-2 Delta and Omicron Variants. Vaccines (Basel). 2022;10(10). Epub 2022/10/28. doi: 10.3390/vaccines10101596. PubMed PMID: 36298460; PubMed Central PMCID: PMCPMC9607825.

Chapter I: SARS-CoV-2: from virus to variants

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was the etiological agent of the new coronavirus disease COVID-19 appeared in 2019, in Wuhan City, China, and spread thereafter all across the world causing a global pandemic [1]. Carrying a single-stranded, positive-sense RNA-virus, of approximately 29.9 kb, SARS-CoV-2 includes four structural proteins, namely spike (S), nucleocapsid (N), membrane (M) and envelope (E), 16 nonstructural proteins (Nsp) and eight accessory proteins [2]. Likely to other RNA viruses, SARS-CoV-2 evolves rapidly, due to the apparition of successive mutations in its genome, resulting in the emergence of a variety of descendant lineages [3]. The ones with major changes or suspected effects on transmissibility, severeness of the infection, or in the validity of diagnostics, and therapies, were considered as variants of concern (VOCs) and variants of interest (VOIs) [4].

The previously identified VOCs by the world health organization (WHO) are the Alpha (B.1.1.7) and Beta (B.1.351) variants, which were considered as VOCs on December 18, 2020, after their first documentation on September and May 2020 at the United Kingdom (UK) and South Africa, respectively [5]. Subsequently, the Gamma variant (P.1 or B.1.1.28.1) was isolated in Brazil in November 2020 and stratified as VOC on January 11, 2021 [5]. Afterward, a fourth VOC, Delta (B.1.617.2), was identified as VOC on May 11, 2021 [5]. Omicron (B.1.1.529) was the last identified VOC, on November 26, 2021, after being detected in many countries during that month [5]. WHO also identified a series of VOI, between March 2021 and August 2022, namely, Epsilon (B.1.427 and B.1.429), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37) and Mu (B.1.621) [6]. As of May 3, 2024, three circulating VOIs are identified, namely the three Omicron subvariants: EG.5, BA.2.86 (23I), and JN.1 [7].

In this review, we presented the general structure of SARS-CoV-2 and the mechanism of its cell entry, focusing on the role of the S protein. We then listed the main diagnostic, therapeutics, and vaccines developed to detect, treat, and prevent COVID-19 infection. Afterward, we mentioned the three nomenclature systems of SARS-CoV-2' descendent lineages. Moreover, we described the notable mutations altering SARS-CoV-2's genome. We next detailed the previous VOCs and VOIs identified by the WHO, their key mutations, and their effects on the different viral characteristics.

References

1. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022;23(1):3-20. Epub 2021/10/07. doi: 10.1038/s41580-021-00418-x. PubMed PMID: 34611326; PubMed Central PMCID: PMCPMC8491763.

2. Jahirul Islam M, Nawal Islam N, Siddik Alom M, Kabir M, Halim MA. A review on structural, non-structural, and accessory proteins of SARS-CoV-2: Highlighting drug target sites. Immunobiology. 2023;228(1):152302. Epub 2022/11/27. doi: 10.1016/j.imbio.2022.152302. PubMed PMID: 36434912; PubMed Central PMCID: PMCPMC9663145 competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

3. Eskier D, Karakulah G, Suner A, Oktay Y. RdRp mutations are associated with SARS-CoV-2 genome evolution. PeerJ. 2020;8:e9587. Epub 2020/08/04. doi: 10.7717/peerj.9587. PubMed PMID: 32742818; PubMed Central PMCID: PMCPMC7380272.

4. Outbreak.info. Variants [July 11, 2024]. Available from: https://outbreak.info/.

5. (WHO) TWHO. Historical working definitions and primary actions for SARS-CoV-2 variants 2023 [June 6, 2024]. Available from: https://www.who.int/publications/m/item/historical-working-definitions-and-primary-actions-for-sars-cov-2-variants.

6. The World Health Organization (WHO). Previously circulating VOIs [June 6, 2024]. Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants/previously-circulating-vois.

7. The World Health Organization (WHO). Tracking SARS-CoV-2 variants [July 4, 2024]. Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants.

Review Paper

A review on SARS-CoV-2: from virus to variants

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This review is prepared to be submitted to the Journal of Pathology, Microbiology, and Immunology - the APMIS journal

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Abstract

Since its description at the end of 2019, COVID-19 continues to be an evolving disease, owing to the wide frequency of mutations in its etiological agent, SARS-CoV-2. Spike protein constitutes the host of most notable mutations, namely in its N-terminal domain (NTD), and its receptor-binding domain (RBD), responsible for viral binding to the host. These mutations were associated with improvement of transmission, replication, virulence, immune evasion, and reduction in the efficiency of certain diagnosis tests and treatments, leading to the emergence of variants under monitoring (VUMs), variants of interest (VOIs), and variants of concern (VOCs). In this review we briefly describe the general structure of SARS-CoV-2, highlighting the role of the Spike protein in different viral mechanisms. Afterward, we present the main mutations affecting SARS-CoV-2's genome. We also reviewed the previous VOIs identified by the WHO as well as the five VOCs: Alpha, Beta, Gamma, Delta, and Omicron, their mutations, and their associated effect on health outcomes.

Keywords: SARS-CoV-2, COVID-19, Spike protein, mutation, VOI, VOC.

1. Overview:

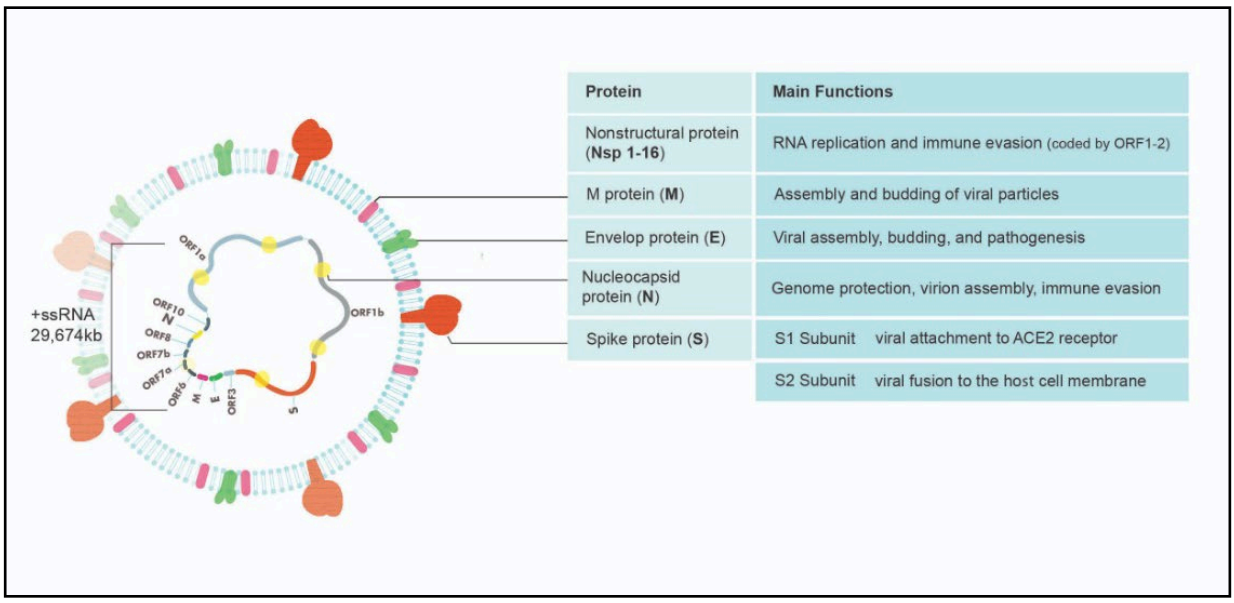
1.1 First identification of the virus and epidemiology: December 2019 was marked by the emergence of a new infectious respiratory disease, causing initially a cluster of unknown pneumonia cases in Wuhan City, China [1]. After that, this large spreading disease rapidly crossed the state's borders and took on the world, under the name of Coronavirus Disease 2019 (COVID-19) [2]. Its etiological agent was a novel Coronavirus, as whole genome sequencing results have shown [1], named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses [3]. Subsequently, the world health organization (WHO) declared this new emerging disease a Global pandemic, on March 11, 2020 [4]. As of May 26, 2024, more than 775 million cases of COVID-19, and more than 7 million deaths have been recorded across the world [5]. However, COVID-19 didn't impact global public health only, this disease has literally upset the entire world fixture, with changes in daily habits, hygiene basics, social distancing, wearing masks in public areas, and imposing lockdowns across the globe [6].

1.2 History and potential origins of the virus: The first description of human coronaviruses dates back to the 1960s, with the discovery of HCoV-229E and HCoV-OC43 [7]. However, coronaviruses did not engross attention until late 2002, when a serious outbreak of Coronavirus in China was attributed to a virus named SARS-CoV [8]. Two other human Coronaviruses were identified in 2004 and 2005, NL63 and HKU1 respectively [9]. Moreover, in 2012, a new epidemic of Middle East Respiratory Syndrome (MERS) appeared in the Kingdom of Saudi Arabia (KSA), sharing similar clinical manifestations of SARS-CoV, but less transmissibility [8]. The origin of human Coronaviruses is not yet definitive, but all of the previous strains are initially zoonotic [10]. Concerning SARS-CoV-2, more than one potential origin has been discussed, including zoonotic origin from bats and pangolin, and a laboratory derivation suggestion was also discussed [10-13]. Retrospective studies about the geographical detection of SARS-CoV-2 have shown that COVID-19 might be originated in another region than its first documentation in Wuhan city, China in December 2019 [14], including Barcelona, Spain [15], France [16], Brazil [14], the United States (US.) [17], and Italy [18, 19]. These investigations have reported positive SARS-CoV-2 samples or antibodies against the virus before its recorded detection date.

1.3 SARS-CoV-2 structure, replication, and transmission mechanisms: SARS-CoV-2 belongs to the Coronaviridae family, marked by their large single-stranded positive-sense RNA genome, of 27-32 kb, and flanked by untranslated regions: 5'-cap structure and 3'-poly(A) tail [20,

21]. SARS-CoV-2 is classified as lineage B in the genus Beta-Coronavirus, including SARS-CoV and MERS-CoV [22]. Its genome is approximately 29.9kb. It is composed of 14 open reading frames (ORFs) [23], encoding four structural proteins: envelope (E), spike (S), membrane protein (M) present on the surface of the virus, and nucleocapsid (N) packing the virus's genome, as well as 16 nonstructural proteins (Nsp1-16), that are instrumental in RNA replication and immune evasion of the virus [24, 25]. S is a heavily glycosylated protein, with a large ectodomain visible on the viral surface and a transmembrane domain, allowing it to be anchored to the viral membrane [26]. It is a trimer composed of an N-terminus signal domain and two subunits: S1 responsible for viral attachment and S2 responsible for viral fusion to the host cell membrane [26]. To mediate cell fusion, the S protein is cleaved by a cellular protease, such as transmembrane serine protease 2 (TMPRSS2) [27, 28]. S1 is divided into the N-terminal domain, (NTD), receptor binding domain (RBD), and C-terminal domains, and S2 is composed of fusion peptide (FP), fusion-peptide proximal region (FPPR), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane segment (TM) and the cytoplasmic tail (CT) [26]. The RBD and NTD are the essential targets of monoclonal and polyclonal antibodies because of their high immunogenicity [29].

SARS-CoV-2 enters into its host cell by the attachment of the RBD of S glycoprotein to the angiotensin-converting enzyme 2 (ACE2) receptor, present in the oral epithelial cells, the tongue, the minor salivary glands [30, 31] and in nasal epithelial cells of the upper respiratory tract [32]. After reaching its target cell, SARS-CoV-2 uses the cell's machinery to begin the translation of the first two-thirds of its genome from 5'-cap (ORF1a and ORF1ab), resulting in two polyproteins Pp1a and Pp1ab [33], which are cleaved by two viral proteases: papain-like protease (PLpro) and main-protease (Mpro), to produce the Nsp [34]. Nsp12 includes the RNA-dependent RNA polymerase (RdRp), which forms a complex along with Nsp7 and Nsp8 [35-37] to constitute the replication/transcription complex (RTC), required for the synthesis of viral RNA molecules [38]. The remaining one-third of the genome at the 3'-poly(A) end includes four ORFs, encoding structural proteins that play a curtail role in viral assembly and elaboration of new mature viruses, as well as other ORFs encoding several accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10), that are involved in viral survival, infection, and transmission [25, 39]. **Figure 1** presents the viral structure of SARS-CoV-2, essential genome parts and their main functions [21, 23-25, 40-43]



Protein	Main Functions
Nonstructural protein (**Nsp 1-16**)	RNA replication and immune evasion (coded by ORF1-2)
M protein (**M**)	Assembly and budding of viral particles
Envelop protein (**E**)	Viral assembly, budding, and pathogenesis
Nucleocapsid protein (**N**)	Genome protection, virion assembly, immune evasion
Spike protein (**S**)	S1 Subunit viral attachment to ACE2 receptor
	S2 Subunit viral fusion to the host cell membrane

Figure 1 Schematic representation of the SARS-CoV-2 virus particle and its genome. SARS-CoV-2 is a single-stranded, positive sense RNA-virus, with four structural proteins: membrane (M), nucleocapsid (N), envelope (E), and spike (S) protein and 16 non-structural proteins (Nps). The role of each protein is mentioned in the attached table.

A person can become infected with COVID-19 through the inhalation of respiratory droplets from an infected individual (including aerosols generated through sneezing, coughing, speaking, singing, or breathing) or through direct contact with infected droplets through the eyes, mouth, or nose [44]. The infection progresses in four stages: the asymptomatic stage, the infection of the upper respiratory tract, and the invasion of the lower respiratory tract, ending with the possibility of developing acute respiratory distress syndrome (ARDS) [32]. When the virus reaches the nasal epithelial cell, even though the viral load is still relatively low, the patient becomes highly contagious and may develop a limited immune response [14]. Afterward, the virus gets to the upper respiratory tract, resulting in developing symptoms such as fatigue, fever, dry cough, and body pain, along with increasing in the immune response [45]. SARS-CoV-2 reaches then the lower respiratory tract to infect type 2 alveolar epithelial cells after binding to the ACE2 receptor, to begin replication, and thus increasing the viral load [14]. This step may result in a loss in type 1 and 2 pneumocytes and diffuse alveolar damage progressing in ARDS, in one out of five cases [46-48]. In the most severe cases, the lower respiratory tract is attacked by the virus, culminating in respiratory failure, multi-organ failure, and eventually death [49, 50].

1.4. Diagnosis: For current infection diagnosis, the centers for disease control and prevention (CDC) recommends using either nucleic acid amplification tests (NAAT), such as the polymerase

chain reaction (PCR) or antigen tests [51]. The gold standard method for COVID-19 identification is real-time reverse transcription polymerase chain reaction (RT-PCR) [52]. Specific genes, including N, E, S, ORF1ab, and RdRp genomic regions, are targeted for viral detection [53].

1.5. Therapies: Regarding treatments of SARS-CoV-2, numerous medicines have been promoted to treat COVID-19 symptoms, and were approved by the U.S. food and drug administration (FDA) [54]. However, in some cases, an unapproved drug or an unapproved use of an approved drug could be authorized by the FDA under what is called the emergency use authorization (EUA) [55]. The drugs include antiviral drugs, immune modulator drugs, and monoclonal antibodies (mAbs) [56].

Concerning the first type, an intravenous antiviral drug: remdesivir (Veklury®), targeting the viral RdRp, was approved by the FDA to be used whether in hospitalized or non-hospitalized adults and certain children [55, 57]. Besides, an oral antiviral pill containing two medications: nirmatrelvir and ritonavir (Paxlovid® tablets), was also approved by the FDA to be used in children aged 12 years and above [55, 58]. On the other hand, in some hospitalized adult cases, COVID-19 can be treated by the use of immune modulators: baricitinib (Oluminant®), and tocilizumab (Actemra®) [55]. Baricitinib Janus kinase inhibitor agreed by the FDA for hospitalized adults, while it has FDA EUA for children, in case both age groups require various respiratory support levels [54]. Moreover, the tocilizumab belonging to the interleukin-6 inhibitor class, has been approved by the FDA for adults while it has only FDA EUA for children, assuming that both ages were already undergoing systemic corticosteroid and requiring various levels of respiratory support [54]. The array extends to include the cytotoxic T-Lymphocyte-Associated Antigen 4 Agonists, like abatacept (Orencia®), and the tumor necrosis factor-alpha inhibitors, like infliximab (Remicade®), which are both recommended to use for certain hospitalized patients [54]. Concerning mAbs, their role is to neutralize the virus and block its entry into target cells. However, the continuous genomic mutations of SARS-COV-2 variants may cause resistance to the mAbs therapies [56]. The convalescent plasma (CP) has also received EUA from the FDA to treat hospitalized and non-hospitalized patients with COVID-19 who have immunosuppressive diseases or receive immunosuppressive therapy [54]. Furthermore, antibiotics are typically not recommended for treating viral infections unless there is a proven or strongly suspected bacterial infection alongside the viral infection [54].

1.6. Vaccination: The increasing number of COVID-19 cases worldwide in the absence of an

effective treatment, has led drug companies to accelerate in creating an effective vaccine to prevent the continuous spread of the disease. Many vaccine types were developed including: mRNA-based vaccines, viral-vector-based vaccines, live attenuated or inactivated viruses, viral protein subunits, and DNA vaccines [59]. Certain vaccines have received FDA approval to protect against the SARS-CoV-2 virus [60]. For instance, the mRNA vaccine Comirnaty® **(**BNT162b2, Pfizer/BioNTech FDA EUA authorized), contains mRNA with codon-optimized sequences for efficient expression of the SARS-CoV-2 S antigen, coupled with stabilizing mutations [61, 62]. This vaccine boasts a remarkable 95% efficacy in safeguarding against infection [63]. Its immunogenicity persists for approximately two months and the vaccine is suitable for individuals aged 16 years and older [63]. However, it is contraindicated for patients suffering from severe allergic reactions and immunocompromised patients [63]. Similarly, the Spikevax® (mRNA-1273) - Moderna (FDA EUA authorized) vaccine operates on the same principle, employing mRNA to instruct cells to produce the SARS-CoV-2 S antigen [63, 64]. It has an efficacity of 94.5%, differing slightly from BNT162b2. Eligible recipients must be 18 years and older and the vaccine's immunogenicity lasts approximately three months [63]. Both BNT162b2 and Moderna have updated their vaccines to target multiple variants, each offering three formulas: the first being a monovalent vaccine focusing on the S protein of the original SARS-CoV-2, the second, a bivalent vaccine targeting both the S protein of the original SARS-CoV-2 and the Omicron BA.4/BA.5 lineage, and the latest 2023-2024 formula designed to accommodate Omicron XBB.1.5 lineage [65].

The adenovirus vector is another vaccine type, such as Vaxzevria® (AZD1222) - AstraZeneca/University of Oxford, Sputnik V® (Gam-COVID-Vax) - Gamaleya Research Institute, and Janssen COVID-19 Vaccine® (Ad26.COV2.S) - Johnson & Johnson vaccines [61]. Those vaccines utilize modified adenoviral DNA to produce SARS-CoV-2 S proteins in the recipient cells.

On the other hand, the inactivated virus vaccines such as CoronaVac® (Sinovac Biotech) and BBIBP-CorV® (Wuhan Sinopharm/Chinese Academy of Science) vaccine, utilize a virus that has been rendered harmlessly through processes such as such as heating, radiation exposure, or chemical treatment commonly with substances like formaldehyde or alkylating agents [66].

Finally, an example of the subunit vaccines is the Nuvaxovid® (NVX-CoV2373) – Novavax (FDA EUA authorized) [67]. The subunit vaccines use synthetic peptides or recombinant proteins

derived from the target pathogen [66]. These proteins contain specific antigenic fragments without any of the pathogen's disease-causing products [66]. Similar to the BNT162b2 and Moderna vaccines, Novavax has introduced its latest 2023-2024 formulation, targeting the Omicron XBB.1.5 lineage [65]. This updated version differs from past formulations in that it is a monovalent vaccine, focusing only on the S protein from the Omicron variant XBB.1.5. This is in contrast to previous versions, which included a monovalent vaccine targeting only the original SARS-CoV-2 S protein and a bivalent vaccine that targeted both the original SARS-CoV-2 S protein and the BA.4/BA.5 S proteins [65]. Those three types of vaccine (adenovirus vector, inactivated, and subunit vaccines) are eligible only for adults [68], and their side effects are similar to those of mRNA [69]. **Table 1** shows COVID-19 vaccines, their types, FDA approval, efficiency, indications, limitations, and the presence of updated formulas.

Table 1: COVID-19 vaccines: their types, FDA approval, efficiency, indications, limitations, and the presence of updated formulas.

Vaccine (Name)	Manufacturer	Type	FDA approval	efficiency	indications	Limitations	Updated vaccine	References
Comirnaty	Pfizer/BioNTech	mRNA	FDA Authorized	95%	Individuals 16 years and older	Contraindicated for severe allergic reactions and immunocompromised patients	2023-2024 formula (Omicron XBB.1.5)	[61, 62, 65, 70]
Spikevax	Moderna	mRNA	EUA Authorized	94.5%	Individuals 18 years and older	Contraindicated for allergic reactions and immunocompromised patients	2023-2024 formula (Omicron XBB.1.5)	[63-65]
Vaxzevria	AstraZeneca/University of Oxford	Adenovirus Vector	Not approved	67%	Adults	Contraindicated for individuals with a history of capillary leak syndrome or severe allergic reactions	None reported for updated formulas targeting specific variants	[61, 68, 69, 71]
Sputnik V	Gamaleya Research	Adenovirus	Not	91.6%	Adults	Contraindicated for	None	[61, 68, 69, 72]

	Institute	Vector	approved			allergic reactions and immunocompromised patients	reported for updated formulas targeting specific variants	
Janssen COVID-19 Vaccine	Janssen Biotech	Adenovirus Vector	EUA Authorized	75%	Adults	Rare cases of thrombosis with thrombocytopenia syndrome (TTS) reported	None reported for updated formulas targeting specific variants	[61, 68, 69, 73]
CoronaVac	Sinovac Biotech	Inactivated Virus	Not Approved	51%	Adults	Contraindicated for allergic reactions and immunocompromised patients	None reported for updated formulas targeting specific	[68-70, 74]

							variants	
BBIBP-CorV	Wuhan Sinopharm/Chinese Academy of Science	Inactivated Virus	Not Approved	65%	Adults	Contraindicated for allergic reactions and immunocompromised patients	None reported for updated formulas targeting specific variants	[68-70, 75]
Nuvaxovid	Novavax	Subunit	EUA Authorized	90%	Adults	Contraindicated for individuals with a history of severe allergic reactions to any component of the vaccine	2023-2024 formula (Omicron XBB.1.5)	[65-70]

Currently, BNT162b2, Spikevax® (mRNA-1273), and NVX-CoV2373 are the only FDA-approved vaccines for 2023-2024, because they updated their formulas including a monovalent component related to the Omicron variant XBB.1.5 [76]. Moreover, the recommendation of FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) for the design of new vaccines in 2024-2025 is to include a monovalent JN.1-lineage vaccine [77].

2. SARS-CoV-2 nomenclature systems: After its propagation around the world, the SARS-CoV-2's genome has been subjected to many mutations, leading to the apparition of different variants of the virus [78]. In light of these continuous mutations, as well as the advanced tools in sequencing and molecular epidemiology, an increasing number of viral sequences are being added day by day to the Global Initiative on Sharing Avian Influenza Data (GISAID), that has grouped 16,775,408 hCoV-19 genome sequences, as of June 9, 2024 [79]. This required a rigorous method of classification and nomenclature for new sequences, to standardize global data. In this context, three nomenclature systems have been used [80].

The first system is the GISAID, where naming is based on clades defined by the statistical distribution of genome distances in phylogenetic clusters and merging the smaller groups that share the same marker variant into one major clade [80]. The nomenclature utilizes letters referring to marker mutations. Non-synonymous substitutions are marked by letters, while synonymous substitutions are identified by numbers [80]. Available SARS-CoV-2 sequences are classified into 11 clades namely: G, GH, GK, GV, GR, GRA, GRY, L, O, V, and S [81]. S, L, and O were first identified [82]. Subsequently, the G and V clades branched out of the L clade. The marker mutations in the G clade are C241T, C3037T, A23403G, and S-D614G [80]. G split into GR and GH followed by GV [80]. As of September 2020, GR generated GRY clade (Alpha variant). Hereafter, in late 2020, the GK clade (Delta variant) was derived from the G clade [80]. GR was also split into GRA after the first quarter of 2021, and the circulation of the two clades GRY and GRA was still considered relatively high after GK [83].

The second nomenclature system is Nextstrain, based on year-letter genetic clades i.e. the clade's name begins with the year of its first identification followed by a capital letter starting with the letter A for each year and respecting the alphabet order. A new clade is defined by signature mutations, a global frequency above 20% for more than two months, and it should conserve at least two mutations with its ancestral clade [84]. The first two identified clades were 19A and 19B, marked by C8782T and T28144C mutations [84]. Till now, 37 Nextstrain clades are available [85]. This nomenclature system helps to follow determinants of diversity at a large level and sheds light on clades persisting for more than several months and having significant

geographical spread [80].

The last one, phylogenetic assignment of named global outbreak lineages (Pangolin) tool, is a more dynamic and rational nomenclature feature that helps to investigate patterns of outbreaks and global spread of SARS-CoV-2 [80, 86]. It is based on the assignment of lineages, a group of sequences detected in a particular region with evidence of their transmission in this region, based on phylogenetic information and metadata related to the sequences [80]. The name begins with a letter, followed by a numerical suffix indicating the descent lineages, with a maximum of three hierarchic levels. Lineages A and B are designated as original lineages at the phylogenetical root of this classification [86, 87]. Special rules are added to name recombinant lineages, which are given a new top-level lineage prefix that begins with an X, without a numerical suffix. Recombinant lineages are considered special ancestor lineages because they don't have a single determined ancestor. Nonetheless, non-recombinant decedent lineages include numerical suffixes, and after reaching three hierarchic levels, the next available top-level prefix is given to the corresponding lineage [87].

Last but not least, WHO has tracked the genome changes affecting the virus characteristics, mainly the transmissibility and severity associated with COVID-19, as well as the performance of diagnostics, vaccines, and therapeutics, resulting in three types of variants: variants under monitoring (VUMs), variants of interest (VOIs) and variants of concern (VOCs) [88]. VUM is defined as a variant harboring mutations that may affect its properties and early signals of growth advantage globally or only in a single WHO region, compared with other circulating variants. This is applicable only if the variant has no clear evidence of phenotypic or epidemiological impact [88]. In the case where the variant available sequences are few to determine the growth advantage, the concerned variant can be assigned as VUM if it carries a large number of mutations in known antigenic sites coupled with evidence of community transmission in two or more countries within a period of two to four weeks [88]. Concerning the VOI, it should meet two conditions: genetic changes having predicted or known effects on the virus characteristics along with identified growth advantage in more than one WHO region, compared with circulating variants as well as increasing relative prevalence and increasing number of cases or other epidemiological impacts indicating an emergent global public health risk [88]. As for VOC, in addition to the parameters of a VOI, it should be proved with a moderate or high level of confidence according to the WHO technical advisory group on SARS-CoV-2 virus evolution (TAG-VE) that this variant is associated with higher disease severity, or impact on COVID-19 epidemiology requiring crucial public health interventions, or significant decrease in vaccines performance [88].

On the other hand, the US. SARS-CoV-2 Interagency Group (SIG) uses four types of variant

classification for SARS-CoV-2: VOI, VOC, VUM previously named as variant being monitored (VBM) [89], and an additional group called variant of high consequence (VOHC) [90]. Alongside the characteristics of a VOC, a VOHC has clear evidence of a significant decrease in prevention and medical countermeasures including diagnostic failure, a substantial reduction in vaccine effectiveness and therapeutic susceptibility, and increased severeness and hospitalizations. No such variant has appeared till now [90].

3. Main mutations in SARS-CoV-2's genome and their important consequences: All of the SARS-CoV-2's structural proteins were exposed to mutations, that have altered many features in the virus [91]. Nonetheless, most of these mutations have occurred in the S protein, the essential viral component for host cell fusion and the target for host antibody production, vaccines, and antibody-based therapeutics [92-94]. The mutations occurring in the RBD were the most critical ones since they affect the host affinity to the virus, the transmission and pathogenesis of the virus, and immune responses against SARS-CoV-2. These mutations include N501Y, E484K/Q, L452R, K417N/T, N439K, Y453F, S477N, T478K, F490S and S494P [94-96].

The first mutation of SARS-CoV-2's genome, D614G, was detected in late January 2020. It consists of a substitution of an aspartic acid (D) by a glycine amino acid (G) at the 614th position, in the chain of the C-terminal domain of the S1 subunit of S protein [93, 97, 98], corresponding to a replacement of A nucleotide into G nucleotide at the position 23,403 in the Wuhan reference strain [97]. The mutation did not affect the affinity of S protein for the ACE2 receptor because it is located outside the RBD [98]. Since March 2020, the new mutation has rapidly spread globally, causing a significant impact on the pandemic, resulting in the "G clade" designation by the GISAID nomenclature system [97]. The new resulting strain was detected at an increasing rate in different regions, starting from Europe to North America and Oceania and later in Asia, to dominate consequently worldwide [97, 98]. Experimental evidence has shown that this strain was associated with more infectivity and higher viral load according to clinical data compared with the original strain [97]. Subsequently, the S protein confronted other mutations, including N501Y corresponding to a substitution of asparagine (N) with tyrosine amino acid (Y) at position 501, located in the RBD of the S protein [99]. It was detected in the United Kingdom (UK), South Africa and Brazil [100]. As N501Y occurred in the RBD, it was associated with enhanced viral affinity to ACE2, reaching four times higher than the wild-type strain [91, 100, 101]. This mutation improved both replication and transmission of the virus [92, 102].

Another mutation first detected in the South African variant and later in the Brazilian variant was the E484K mutation [103], consisting of a replacement of glutamic acid (E) with lysine

(K) amino acid in position 484 in the RBD of S protein [104]. This mutation is associated with antibody neutralization escape affecting antibody-based treatments and vaccine efficacity [102].

Regarding the NTD mutations, 69-70del in the S protein was related to higher viral replication [96]. Besides, the reduction of antibody neutralization capacities was associated with deletions in positions 141-146 and 242-244 amino acids, as well as L18F and D253Y substitutions and other NTD mutations [96, 105]. Q675H/R and P681H/R were related to improvement in S1/S2 cleavage in the human cell [92]. And the list goes on.

4. Variant of Concern (VOC) main mutations and consequences: First, the VOCs were named based on the region of their first apparition. Indeed, four variants appeared successively across the world, the first one in September 2020 in the UK, referring to the B.1.1.7 variant in the Pangolin nomenclature [106, 107]. This variant appeared two months after the first S protein mutation D614G [14]. After that, other mutations led to the apparition of the South African variant in May 2020, the Brazilian variant in November 2020, and the Indian variant in October 2020, corresponding to B.1.351, P.1, and B.1.617.2 Pangolin lineages respectively [78]. Afterward, in May 2021, the WHO announced a new system of nomenclature using Greek letters, to accord the names Alpha, Beta, Gamma, and Delta to the variants B.1.1.7, B.1.351, P.1, and B.1.617.2 [86, 108]. Subsequently, a sudden increase in COVID-19 cases has been recorded in Gauteng province in South Africa [78] and was associated with the apparition of a new VOC, Omicron, in November 2021 [78]. Data have confirmed all VOCs were more transmissible than the wild-type virus [102, 109-116].

Alpha variant: A few months after its first detection, in Kent, UK on September 20, 2020, the Alpha variant (GRY GISAID clade, 20I/501Y.V1 Nextstrain clade, B.1.1.7 Pangolin lineage) became prevalent worldwide [117-119]. This variant was the first to be designated as a VOC on December 18, 2020 [119]. However, according to the WHO, as of March 9, 2022, it was considered a previous VOC [119]. The Alpha variant has been reported in approximately 189 countries [117]. Nevertheless, there have been no observations of this variant after September 2021 [117].

The B.1.1.7 variant exhibits a total of 23 mutations alongside D614G and N501Y mutations inherited from the ancestral mutant [120]. Among these, 13 are non-synonymous mutations, namely T1001I, I2230T, and A1708D in the ORF1ab; Q27stop, Y73C, and R52I in ORF8; P681H, A570D, T716I, D1118H, and S982A in S protein; and S235F and D3L in the N protein [120]. Additionally, there are synonymous mutations, including T26801C in the M gene; C14676T, C5986T, C913T, T16176C, and C15279T in ORF1ab [120]. Furthermore, three deletions were identified: Y144del and H69-V70del in S protein, and SGF3675-3677del in

ORF1ab [120].

The H69-V70del in the S gene affects diagnostic tests since it hinders the binding of the oligonucleotide probe to its target sequence during PCR test, which leads to target failure in the TaqPath COVID-19 RT-PCR Combo Kit, resulting in S gene target failure (SGTF) [121]. SGTF is associated with higher viral loads [121]. Some samples also exhibit S gene target late amplification (SGTL), characterized by significantly higher Ct values than other tests targeting N and ORF1ab genes [121]. Therefore, this deletion complicates the diagnosis process, making it more challenging [121]. This genetic alteration is also associated with immune escape in immunocompromised persons and an enhanced capacity for viral infectivity in laboratory settings [122, 123].

Another important mutation in the Alpha variant is N501Y acting as a critical contact point within the RBD as mentioned previously [124]. This mutation amplifies the virus's capability to bind strongly to human ACE2 [124], significantly contributing to viral proliferation and infectivity [125]. On the other hand, the P681H mutation is located close to the furin cleavage site in the S protein, a well-acknowledged area crucial for both infection process and transmission [124, 126]. B.1.1.7 was associated with 36-75% higher transmissibility [124, 127, 128] and 10-13% higher secondary attack rate [129].

Regarding the host immune response, the Alpha variant demonstrates efficacy in upregulating crucial viral RNA and proteins within airway epithelial cells, surpassing earlier variants [117]. The increased expression of Orf9b, Orf6, and N proteins causes a delay in the early host innate immune response [117]. These proteins, recognized as innate immune antagonists, slow down the regular functioning of the host's innate immune system [117]. On the other hand, some studies have shown that the B.1.1.7 variant retains its vulnerability to neutralization, although with a negligible decrease in effectiveness when tested against CP and vaccinated individuals [130]. Neutralizing titers revealed a slight reduction but were still above the levels expected to confer protection [131]. Moreover, no significant effect on the neutralizing activity was detected using Moderna, BNT162b2, AZD1222, Novavax, and Bharat vaccines [132-135]. In addition, the protection from the disease hasn’t shown significant changes with BNT162b2, AZD1222, and Novavax [132-134]. Nonetheless, the evidence of vaccines' capacity to prevent infection with the Alpha variant was limited, and AZD1222 was related to decreased protection [132, 135]. Moreover, certain mAbs targeting the RBD of the S protein exhibit reduced efficacy against this variant, while others are only minimally affected [136]. In summary, these findings indicate that the B.1.1.7 variant was improbable to pose a notable threat to existing vaccines or significantly raise the risk of reinfection [130, 136].

Concerning the infection status, a case-control study has shown that the Alpha variant is

linked to a more severe manifestation of COVID-19 compared to the wild type [137]. However, a connection between the Alpha variant and higher mortality rates may not exist [137]. In contrast, another study demonstrated that it is correlated with an elevated risk of both hospitalization and mortality when compared to the wild-type virus [138]. The risk of transmission was 45% to 71% higher for the Alpha variant than the wild-type virus (as calculated in 15 studies) [39]. On the other hand, the basic reproduction number R_0 (that describes the contagiousness or transmissibility of SARS-CoV-2) was 75% to 78% higher in the Alpha variant than in the non-VOC, and the reported effective reproduction number R_t was from 1.1 to 2.8. R_0 represents the expected number of cases that can be infected by one case in a population when everyone is susceptible to infection, and Rt is the number of new cases generated in a population at a certain period of immunity [139]. A meta-analysis study estimated that the hazard ratio of hospitalization risk, ICU admission risk, and mortality risk were 1.53, 1.74, and 1.37 higher in the Alpha variant than the wild type, respectively [140].

Beta variant: After being considered as VOC since December 18, 2020, the Beta variant (GH GISAID clade, 20H/501Y.V2 Nextstrain clade, B.1.351 and descendant Pangolin lineages) was classified as previous VOC by March 9, 2022 [119]. This variant harbors 21 mutations, including 9 mutations in the S protein. Alongside N501Y, it has 2 key mutations in the RBD (E484K, K417N) [141]. The genome includes other important mutations in the NTD (L18F, D80A, D215G, LAL242–244del, R264I, and A701V) [141].

The Beta variant was associated with higher infection severity than the Alpha variant [142]. In a random investigation of RT-qPCR of 18,355 COVID-19 samples in Qatar, the Alpha variant was associated with 2.65 higher Ct values than the Beta variant [143]. The lower Ct value is related to a higher infectiousness level, which can be associated with a higher viral charge and longer duration of the infection [143]. In addition, the Beta variant was associated with higher hospitalization rates, infection severity, and mortality rates [144]. Moreover, 501Y.V2 manifested 1.5 times more transmissibility and 21% higher immune escape than previous circulating lineages [114]. An *in vitro* investigation has shown that mAbs and CP were less effective against the Beta variant than the Alpha variant [114]. Additionally, regarding immune response modulation, it has been revealed that the E484K mutation in the Beta variant is crucial in reducing sensitivity to CP [145]. Wibmer *et al.* have noticed an effective escape of antibody neutralization associated with 501Y.V2 lineage, after using two major classes of antibodies targeting a highly antigenic and immunodominant site in the RBD [146]. This immune escape was detected with antibodies against NTD structures as well, and this was related to the three amino acid deletions in this domain [146]. Wibmer *et al.* have also tested the neutralization action of polyclonal plasma extracted from patients with PCR-confirmed

SARS-CoV-2 infection, against 501Y.V2 lineage. Notably, neutralization activity was detected in only half of the samples, and the highest inhibitory dilution (ID_{50}) was associated with plasma obtained from participants having developed a severe infection [146]. Sera recuperated from individuals vaccinated with Moderna and BNT162b2 were also tested against 501Y.V2, and showed significantly reduced neutralization capacity [147]. In addition, an assessment of the neutralizing activity of convalescent serum and vaccine serum taken from individuals vaccinated with Wuhan Sinopharm or Sinovac Biotech, B.1.351 showed two times higher resistance to neutralization of CP and 2.5 to 3.5 more resistance to vaccine serum than the wild-type virus. While B.1.1.7 manifested low resistance [148]. Similarly, L18F mutation in the Beta variant's genome is associated with enhanced NTD binding antibody escape and an increased viral replication rate [113, 149]. K417N mutation is a double moderator related to boosted binding affinity to ACE2 [150, 151] and reinforced immune escape from class 1 neutralizing antibodies [102, 152].

Gamma variant: The SARS-CoV-2 Gamma variant (GRl501Y.V3 GISAID clade, 20J (V3) Nextstrain clade, P.1 Pangolin lineage), belonging to the B.1.1.28 lineage, was first detected in Manaus, Brazil, in December 2020 [118, 119, 153]. After that, it quickly spread to Brazil and many other countries [153, 154]. This variant was designated as a VOC on January 11, 2021, and a previous VOC on June 7, 2022 [119]. The P.1 variant has been reported in 91 countries as of 29 August 2021 [155], and its prevalence decreased after July 2021 due to its replacement by the Delta variant [156]. This variant contains four synonymous mutations: T733C, C2749T, C12778T, and C13860T in the ORF1ab [157]. Moreover, there are several non-synonymous mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I and V1176F in the S protein; S1188L, K1795Q, and E5665D the in ORF1ab; P80R in the N protein and E92K in the ORF8 [157]. Besides, one deletion in the ORF1ab and one insertion in the N protein (SGF3675-3677del and ins28269-28273, respectively) have also been identified [157].

Concerning diagnostics, there are no significant challenges in the detection of this variant by nucleic acid, antibody, and viral antigen tests [158]. As for severity, it has been estimated that the hazard ratio of hospitalization risk, ICU admission risk, and mortality risk was 1.71, 1.94, and 1.06 higher in the Gamma variant than the wild type, respectively [16]. Besides, P.1 exhibited higher virus titers and increased severity than the preceding variants [159]. Additionally, this variant was related to an increased mortality rate in women and people belonging to the 20-49 age group [158].

On the other hand, the Gamma variant is 1.4 to 2.2 times more transmissible than previous strains [160]. An enhanced capacity of immune response evasion was linked to the Gamma

variant as well [160]. The N501Y, K417T, and E484K mutations found in the RBD, increase the ACE2 affinity and the antibody circumvention, thereby increasing pathogenesis [161]. Thus, the Gamma variant seems more resistant to plasma treatment than other lineages (B1.1.7, B.1.351, B.1.429, B.1.617.2, and B.1.427), due to the E484K mutation especially [158]. Regarding the mAbs therapy, a resistance to the casirivimab and the bamlanivimab + etesevimab was detected in the P.1 variant. This seems to be associated with the K417N, E484K mutations and K417T, E484K, and N501Y mutations respectively [158]. The K417T mutation also decreases the effectiveness of the vaccine and contributes to reinfections [158]. On the other hand, only the Moderna vaccine became somewhat less active [158].

Delta variant: After its classification as VOI on April 4, 2021, Delta was then assigned by WHO as the fourth VOC on May 11, 2021 (G/478K.V1 GISAID clade, 21A, 21I, 21J Nextstrain clade, B.1.617.2 and descendant Pangolin lineages) [119]. Afterward, this variant has spread worldwide to become the only VOC since September 2021 [162]. However, on June 7, 2022, it was considered a previous VOC [119]. This variant was marked by higher infectiousness than other pre-Omicron variants [163, 164]. With overall 23 mutations in its genome, B.1.617.2 carries 10 mutations in the S protein (T19R, G142D, R158G, L452R, T478K, D614G, P681R, D950N, and two deletions at the positions 156 and 157) [92, 165]. The most critical mutations are L452R, T478K, and P681R [165]. Notably, the E484G mutation present in B.1.617.1 and B.1.617.3 lineages does not appear in the Delta variant genome, which carries instead T478K unique mutation [166]. In an *in silico* molecular analysis, this last mutation seemed to amplify the affinity of RBD to ACE2 and thus the virus entry to the cell [167]. T478K was related to host antibody evasion in an *in vitro* study [167]. On the other hand, L452R creates a rise in the electrostatic potential of the RBD making it positive, which is associated with enhancing the affinity of RBD to ACE2 and thus increases viral transmission [168]. In addition, this mutation is associated with reduced cell-mediated immunity, contributing to an improvement in viral replication [169]. As for the P681R mutation, it was identified for the first time in the B.1.617 lineage [165]. Its location within the furin cleavage site was found to increase significantly the level of cleaved S2 subunit, thus expanding viral fusion and transition to infect new cells [27, 165]. An *in vitro* investigation has shown that this association was significantly higher in the mutant carrying D614G/P681R mutations than in the mutant carrying D614G only [170]. Furthermore, the carriage of D614G and P681R mutations was related to a decrease up to 1.5-fold of the neutralizing activity of three mAbs against RBD, when compared with D614G alone [170]. Similarly, D614G/P681R mutants developed significant resistance to antibodies recuperated from sera of two doses-BTN162B2 vaccinated persons [170].

Liu *et al.* investigated the neutralization activities of mAbs, convalescent, and vaccine sera

in B.1.617.1 and B.1.617.2 lineages [171]. Their results showed a reduction in neutralizing activity of B.1.617.2 in five antibodies in common with B.1.617.1 among 20 tested mAbs. Besides, B.1.617.2 showed a 5-fold decrease in neutralizing activity for six of the mAbs. While an increased neutralization was detected in one of the antibodies [171]. When compared with Victoria virus (SARS-CoV-2/human/AUS/VIC01/2020: a strain isolated in the early stages of the pandemic in Australia), B.1.617.2 showed a neutralization reduction of 2.8-fold with B.1.1.7 convalescent serum, 6-fold and 2.9-fold with B.1.351 and P.1 convalescent sera respectively [171]. They have also tested the protective effect of previous infections from the Delta variant. Neutralization of CP from individuals previously infected with B.1.351 and P.1 was practically absent, suggesting that these individuals present a higher risk of being reinfected by the Delta variant. While B.1.1.7 may procure a good protection [171]. As for the vaccine efficacity, protection from B.1.617.1 and B.1.617.2 was tested using serum from individuals receiving two doses of the BNT162b2 or AZD1222 vaccine. Geometric mean neutralization titers against B.1.617.2 were reduced 2.5-fold for the BNT162b2 vaccine serum and 4.3-fold for the Oxford-AstraZeneca vaccine, compared with Victoria virus [171]. Concerning the severity of infection associated with the Delta variant, a retrospective cohort study in Canada has shown that the adjusted elevation in risk associated with the Delta variant was significantly higher than in patients infected with N501Y-positive VOCs, corresponding to 108% for hospitalization, 234% for ICU admission and 132% for death [172]. Furthermore, a study built in Scotland has shown that the hospital admission rate was approximately doubled in patients infected with the Delta variant compared with the Alpha variant [173]. Likewise, Delta variant was associated with higher hospitalization rates, and mortality rates and a more severe infection [144]. Besides, according to studies conducted in the UK, differences in symptoms were detected with Delta variant infection: loss of smell and cough were not common, while headache, fever, runny nose, and sore throat were more frequent. Delta infections were also associated with some more serious symptoms including hearing problems, gastrointestinal disabilities, and blood clotting leading to tissue deaths [174].

Many sub-lineages were derived from the Delta variant and classified using a dynamic nomenclature AY.X grouping sub-lineages from AY.1 to AY.133, across different regions around the world [86]. The AY.1 sub-lineage, also known as the Delta Plus variant, has been reported in England [175]. It carries the mutation K417N in its S protein, previously found in the Beta variant [175, 176]. This mutation is associated with enhancement of antibody evasion and ACE2 binding affinity, making this variant more infective [166, 175, 176]. Compared with other VOCs, the Delta Plus variant is more transmissible with a higher affinity to epithelial lung cells [176]. A resistance to mAbs bamlanivimab and etesevimab was detected in the Delta-Plus

variant whereas the Delta variant was resistant to bamlanivimab only [175]. This enhanced resistance may be related to the K417N mutation in the Delta-Plus variant [175]. On the other hand, another sub-lineage of the Delta variant was detected in Vietnam and termed Delta-V. It was suggested to be a hybrid virus because it carries some mutations of the Alpha variant within its S protein [175]. A comparison between the Delta variant, Delta-Plus, and Delta-V subvariants has shown a similar decrease in antibody neutralization as the Delta variant after BNT162b2 vaccination [175]. Moreover, the viral entry to a host cell and the neutralization sensitivity were relatively similar between the Delta variant and its descendent sub-lineages [175]. However, the AY.4.2 sub-lineage showed higher transmissibility than the ancestral lineage [177].

Omicron variant: The Omicron variant (GISAID clade GR/484A, Nextstrain clade 21K, B.1.1.529 Pangolin lineage) was classified as VOC on November 26, 2021, after being considered as VUM on November 24 of the same year [119]. It then was designated as a previous VOC on March 14, 2023 [119]. Since WHO designated Omicron as a VOC, global reports have tallied over 130 million cases and 500,000 fatalities, contributing to a 44% surge in the average count of COVID-19 infections [178]. WHO reports that Omicron is probably present in most countries, even though it hasn't been detected yet [179]. When compared to the reference strain, Omicron displays a minimum of 50 identified mutations [180]. 32 mutations have altered the S protein including A67V, 69-70del, T95I, G142D, VYY143-145del, 211I, L212Idel, ins214EPE, G339D, S371L, S373P, S375F, K417 N, N440K, G446S, S477 N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F [181].

Each of these mutations contributes to the pathogenicity in its way. For example, G339D, S477N, and N501Y increase the binding affinity of the S protein with the ACE2 receptor [95, 182]. On the other hand, S373P, N440K, G446S, Q493R, G496S, Y505H, T478K, and D614G contribute to increasing the chance and the rate of the infection [183-187]. D614G, the most predominant mutation, also increases the viral transmissibility and stability [188]. These mutations also play an important role in the resistance against the coronavirus treatment. S477N increases the resistance to the neutralization by mAbs while E484K escapes neutralization by CP [186, 189]. Accordingly, some studies revealed that Omicron can escape from CP treatment and double shots of vaccine intake [190]. In addition, the S gene mutations especially the 69-70del have contributed to many false negative RT-PCR test results, failing to target the S gene [160]. **Figure 2** presents the S protein structure of the five previously circulating VOCs, highlighting S protein mutations, and their attributed effects.

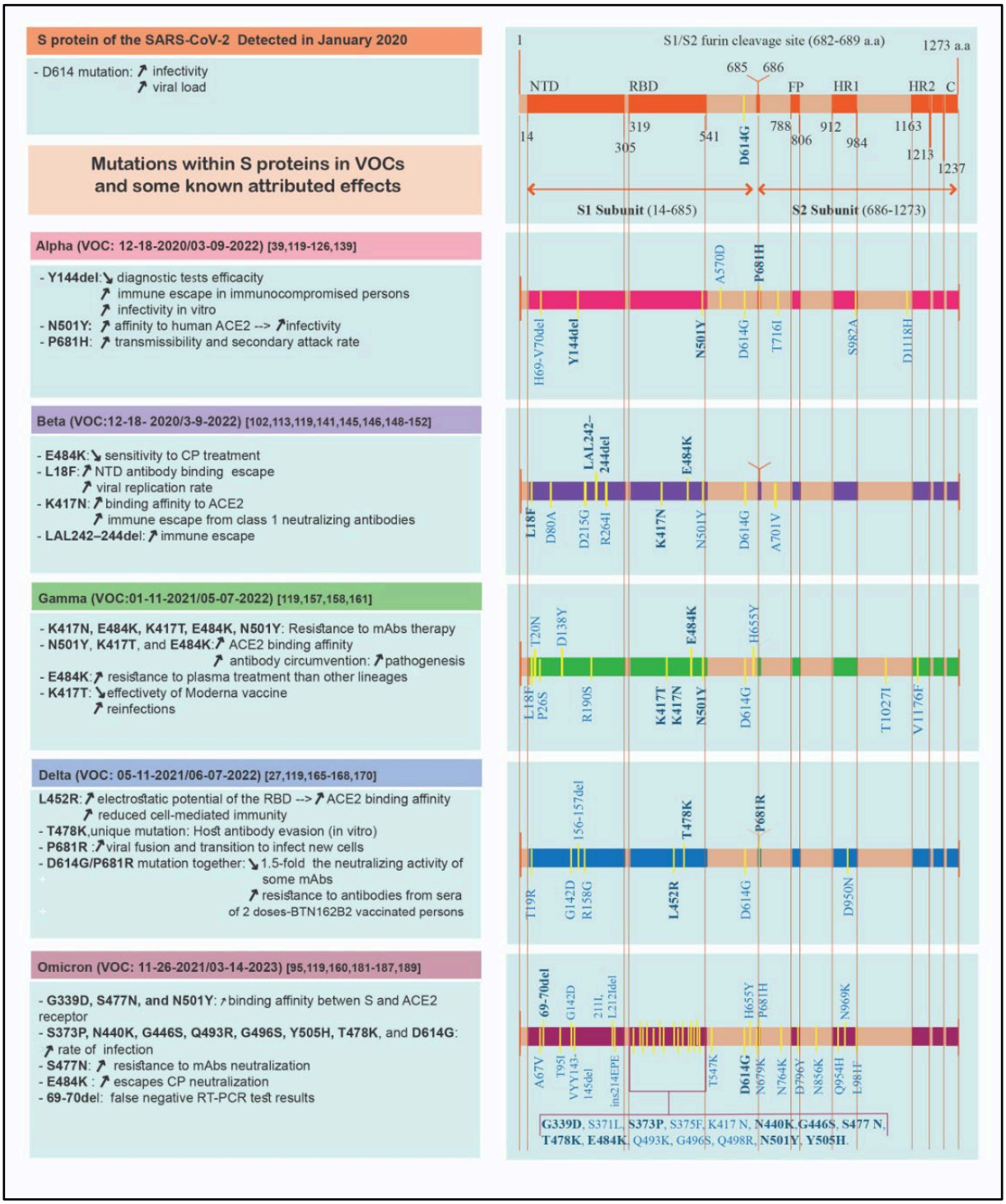


Figure 2 Schematic representation of the S protein sequences of the five VOCs, Alpha, Beta, Gamma, Delta, and Omicron highlighting S protein mutations and presenting the attributed effects. Dates of identification of each VOC by the WHO are also mentioned.

Omicron variant has several subvariants: BA.1 (B.1.1.529.1), BA.2 (B.1.1.529.2), BA.3 (B.1.1.529.3), BA.4, BA.5, and their sub-lineages [191, 192]. These five subvariants share many similar mutations in the S gene including G142D, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y55H, D614G, H655Y, T95I,

N679K and P681H in the S1 subunit and N764K, D796Y, O954H and N969K in the S2 subunit. However, each subvariant is distinguished from the other by specific mutations. BA.1 contains A67V, 69-70del, 143-145del, 211I, L212Idel, ins214EPE, S371L, G446S, G496S and T547K in the S1 subunit and N856K and L981F in the S2 subunit. Regarding BA.2, it contains T9I, L245, V213G, D405N, R408S, S371F, T376A and 25-27del in the S1 subunit. BA.3 also accumulates 69-70del, A88V and NSP6 in the S1 subunit. BA.4 and BA.5 have similar mutations as BA.2 next to 69-70del, L452R, F486V and the original Q493 (from the wild type) [193, 194]. The BA.5 Omicron strain has been detected as the predominant and highly contagious subvariant worldwide [192]. For these subvariants, BA.2 is distinguished from BA.1 and BA.1.1 by not containing the 69-70del mutation, thereby not exhibiting the characteristic SGTF [195]. BA.4 and BA.5 have also tested positive for SGTF [196].

Many sub-lineages were associated with significant impact, for example, the ones that emerged from BA.2, including BA.2.74, BA.2.75, BA.2.76, and BA.2.12.1, with BA.2.75 and BA.2.12.1 being major subvariants of BA.2 [197]. BA.2.75, known additionally as "Centaurus," was first identified in India [198]. It later spreads to about 20 countries, with a significant increase in cases in India. This rise has led to predictions that BA.2.75 will continue to pervade globally, particularly in Asia [199]. Subsequently, it was proven that the transmissibility of the BA.2.75 sub-lineage is very high, rivaling that of BA.5 [200]. In addition, BQ.1 and its subvariants originate from BA.5. Accordingly, BQ.1 and BQ.1.1 exhibit similar mutations in the S protein as BA.5. BQ.1 includes additional three mutations: K444T, N460K, and Q493R, while BQ.1.1 carries an additional R346T substitution [201]. Due to their heightened transmissibility, BQ.1 and BQ.1.1 underwent rapid expansion, displacing the previously dominant BA.5 in many countries and leading to 50% of infections worldwide in January 2023. By early December 2022, the proportion of infections attributed to BQ.1 and BQ.1.1 surged from approximately 2% in mid-September to 52.22%, and it was involved in 60.92% and 60% of infections in the US and the UK, respectively [202]. Furthermore, the XBB subvariant is a recombinant of the BA.2.10.1 and BA.2.75 variants, and subsequent descendant lineages have given rise to XBB.1 and XBB.2 for example [203-205], with XBB.1.9.1, XBB.1.9.2, XBB.2.3, XBB.1.16, and XBB.1.5 being VUM according to WHO [191]. XAG is also a recombination of two Omicron subvariants: BA.1.1 and BA.2.23, and was initially identified in Rio Grande do Sul, Brazil in April 2022 [206].

Regarding transmissibility between Omicron subvariants, BA.2 tends to be 30–50% more transmissible than BA.1 [207], while BA.4 and BA.5 are 36% more infectious than BA.2 [208]. Concerning vaccination, it has shown a moderate effect against BA.1 and BA.2, while BA.4 and BA.5 indicated a 7-fold decrease in neutralizing antibody production compared to BA.1

[208]. Even though, the WHO still recommends taking booster doses, to decrease mortality and hospitalization rates [209].

Esper *et al.* have indicated that the Omicron variant is less severe than the previous variants (Alpha, Gamma, and Delta), but people still get highly infected with Omicron despite their vaccination compared to other variants [210]. Several studies demonstrated that infection with Omicron is less likely to develop severe clinical outcomes, hospitalization, or death than Delta variant infection [211-216]. Chen and its colleagues have predicted that Omicron BA.1 would be 10 times more contagious than the wild-type virus and 2.8 times more infectious than the Delta variant, essentially due to N440K, T478K, and N501Y mutations in its RBD [217]. In addition, the Omicron variant was estimated to be 2–3 times more transmissible than the Delta variant by Ito *et al.* [218]. Also, a systematic review conducted by Relan *et al.* concludes that infection with Omicron had a 61% lower risk of death and 56% lower risk of hospitalization including ICU admission, oxygen therapy, and non-invasive and invasive ventilation compared to Delta infection. BA.1 and BA.2 have approximately the same risk of hospitalization [219].

The majority of the available SARS-CoV-2 sequences since February 2022, were derived from the Omicron variant [220]. New emerging lineages over time are more likely to derive from the latter, although previous VOCs or new variants may be sources of novel sequences as well [220]. The new variant tracking system developed by WHO on 15 March 2023, classified the Omicron sub-lineages separately as VUMs, VOIs, or VOCs, thus allowing the comparison of new descendant lineages with Omicron parent lineages (BA.1, BA.2, BA.4 and BA.5) [220].

According to the WHO, no VOCs were detected after Omicron [221]. Recently, as of May 10, 2024, JN.1 (a descendent lineage of B.A.2.86 also known as 24A) and its descendants KP.2 and KP.3 are highly predominant in the US. Moreover, JN.1 descendent lineages are the current circulating VUMs, including JN.1, KP.2, KP.3 and JN.1.18. Concerning KP.2 (known additionally as JN.1.11.1.2), it has contributed to about 25% of new COVID-19 infections in the US. The proportion of illnesses attributed to KP.2 has surged from approximately 3.9% in the week of March 30 to around 24.9% by the week of April 27, 2024, according to the CDC [221]. The SGTF in PCR testing can indicate that the Omicron subvariant is likely to be the JN.1 [221].

5. Variant of Interest (VOI) main mutations and consequences: VOIs were also named by the WHO using Greek letters till 15 March 2023, where Greek letters were given only to VOCs, and VOIs followed the scientific nomenclature of Nextstrain and Pangolin [222]. Five VOIs were identified in March 2021, namely Epsilon (GH/452R.V1 GISAID clade, 21C Nextstrain clade, B.1.427 and B.1.429 Pangolin lineages), Zeta (GR/484K.V2 GISAID clade, 20B/S.484K Nextstrain clade, B.1.1.28.2 or P.2 Pangolin lineage), Eta (G/484K.V3 GISAID

clade, 21D Nextstrain clade, B.1.525 Pangolin lineage), Theta (GR/1092K.V1 GISAID clade, 21E Nextstrain clade, P.3 Pangolin lineage) and Iota (GH/253G.V1 GISAID clade, 21F Nextrain clade, B.1.526 Pangolin lineage). Kappa (G/452R.V3 GISAID clade, 21B Nextstrain clade, B.1.617.1 Pangolin lineage) was assigned as VOI in April 2021, followed by Lambda (GR/452Q.V1 GISAID clade, 21G Nextstrain clade, C.37 Pangolin lineage) in June 2021, and then by Mu (GH GISAID clade, 21H Nexstrain clade, B.1.621 Pangolin lineage) in August 2022 [223]. These variants were then considered as previous VOIs [223].

Epsilon's first documentation was in the US in March 2020 [223]. Its key S protein mutations are L452R, W152C, S13I, and D614G [224]. This variant presented increased transmissibility up to 18.6-24% in comparison with wild-type circulating variants [225]. It was also related to a depletion in the neutralizing activity of convalescent and post-vaccination sera. [225]. Hereafter, the Zeta variant first appeared in Brazil in April 2020, with eight main S protein mutations: L18F, T20N, P26S, F157L, E484K, D614G, S929I and V1176F [224]. Data showed a potential decrease in the effectiveness of neutralization using convalescent and post-vaccination sera or mAbs treatment in this variant [226]. Subsequently, the Kappa variant was identified in India in October 2020 [223]. Its key S protein mutations L452R, E484Q, D614G, and P681R are related to harmful antibody neutralization activity and increased transmission [227]. Afterward, the US witnessed the apparition of the Iota variant in November 2020 [223]. Its S protein is attacked mainly by E484K, D614G, and A701V, affecting the antibody neutralization efficacy [227]. Thenceforth, the Eta variant was found in many countries including the UK and Nigeria in December 2020 [223, 224]. Many significant mutations were detected within the S protein of this variant, which are A67V, 69-70del, 144del, E484K, D614G, Q677H, and F888L [228]. Eta may be related to a decrease in antibody neutralization by convalescent and post-vaccination sera and mAbs treatment [226]. Still, in December 2020, the Lambda variant detected in Peru [223] carried a novel 7-aminoacid deletion in the NTD (246-252del) and 6 mutations in the S protein, including two novel ones in the RBD, namely, L452Q and F490S [229]. It has been found that the L452Q mutation was related to enhanced binding affinity to the ACE2 receptor, while F490S has shown a reduced effect of the BNT162b2 vaccine [230, 231]. Subsequently, the Theta variant was first identified in the Philippines in January 2021 [223]. Its key S protein mutations E484K, N501Y, D614G, and P681H were implicated in the modulation of transmission and antibody neutralization activity [227]. Also in January 2021, the Mu variant was isolated in Colombia [223]. It harbors T95I, Y144S, Y145N, and 146N insertion in the NTD, R346K, E484K, N501Y in the RBD and D614G, P681H, and D950N in other regions of S [232, 233]. 146N insertion was detected to straighten the binding affinity to the ACE receptor [234]. Whereas, E484, N501Y, and D614G

found in previous VOCs, are implicated in the same mechanisms of increasing ACE2 binding affinity and reducing antibodies neutralization [233, 235]. **Figure 3** presents the S protein structure of the eight mentioned previous VOIs, with the key S protein mutations of each variant, as well as some attributed effects.

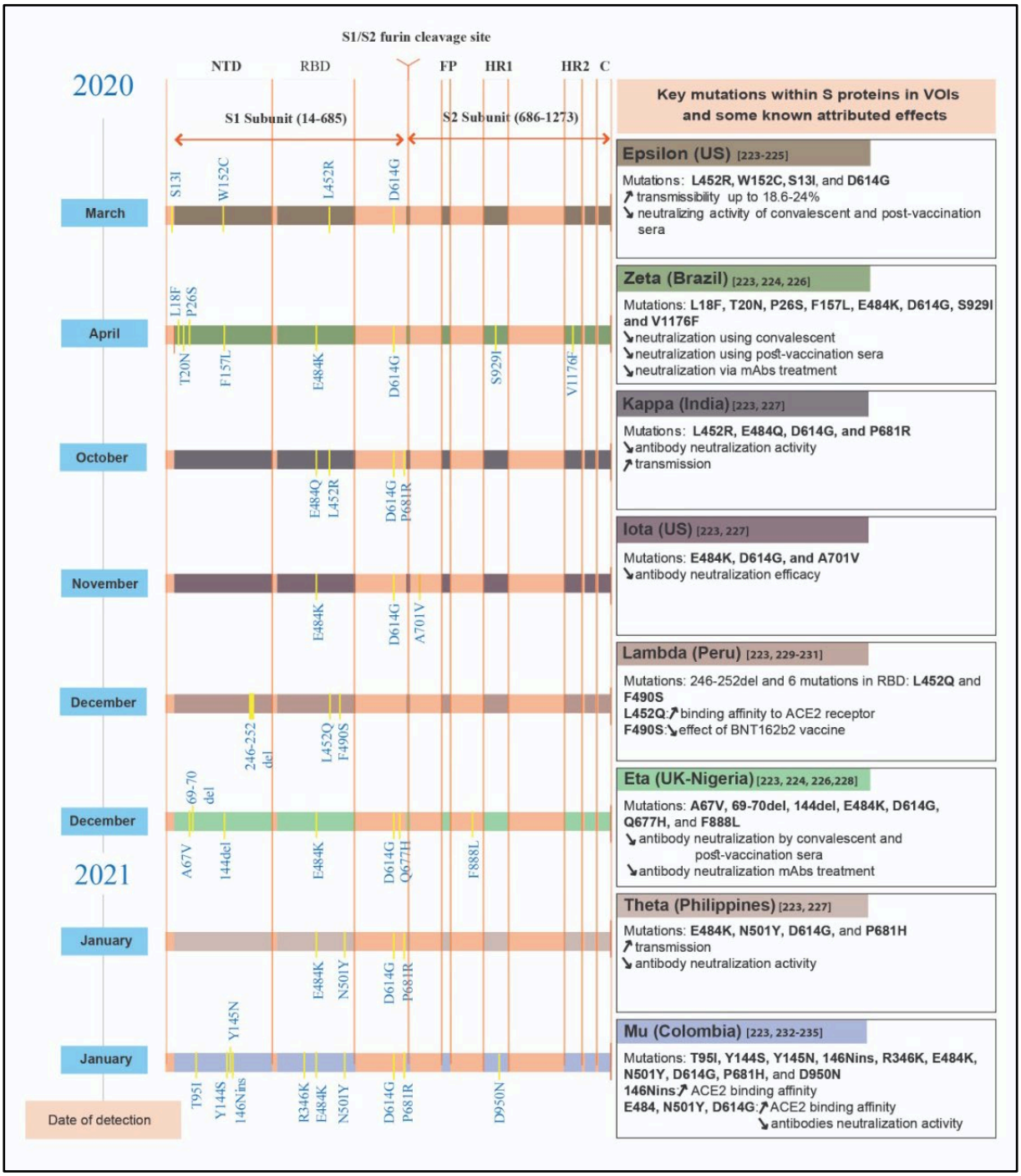


Figure 3 Schematic representation of the S protein sequences of the eight VOIs, Epsilon, Zeta, Kappa, Iota, Lambda, Eta, Theta, and Mu, highlighting S protein key mutations and their effects. Dates and countries of each variant isolation are also mentioned.

The current circulating VOI, as of December 2, 2024, is the Omicron subvariant: JN.1 [203].

6. Conclusion: After its first identification in Wuhan, China, SARS-CoV-2 has shown enormous abilities to mutate, elaborating new strains continuously. S protein was the most important viral part attacked by serious mutations, affecting infectivity, transmissibility, virulence, diagnosis, immune and therapeutic escape of the virus, and giving rise to VUMs, VOIs, and VOCs. Even though the emergency status has ended, and no VOC could be detected on this date, COVID-19 is still a pandemic, and SARS-CoV-2 continues generating new variants. The last VOI identified by the WHO, JN.1, was derived from Omicron. New variants and recombination can emerge in the future, thus constant effort to develop efficient vaccines and therapies is still needed in the hope of getting rid of this disease.

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. Epub 2020/01/28. doi: 10.1016/S0140-6736(20)30183-5. PubMed PMID: 31986264; PubMed Central PMCID: PMCPMC7159299.

2. The World Health Organization (WHO). Naming the coronavirus disease (COVID-19) and the virus that causes it 2020 [June 9, 2024]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it.

3. Coronaviridae Study Group of the International Committee on Taxonomy of V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-44. Epub 2020/03/04. doi: 10.1038/s41564-020-0695-z. PubMed PMID: 32123347; PubMed Central PMCID: PMCPMC7095448.

4. The World Health Organization (WHO). WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 2020 [June 9, 2024]. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.

5. The World Health Organization (WHO). WHO COVID-19 dashboard [July 11, 2024]. Available from: https://data.who.int/dashboards/covid19/.

6. The World Health Organization (WHO). Advice for the public: Coronavirus disease (COVID-19) 2023 [January 7, 2024]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public.

7. Vougogiannopoulou K, Corona A, Tramontano E, Alexis MN, Skaltsounis AL. Natural and Nature-Derived Products Targeting Human Coronaviruses. Molecules. 2021;26(2). Epub 2021/01/21. doi: 10.3390/molecules26020448. PubMed PMID: 33467029; PubMed Central PMCID: PMCPMC7831024.

8. Hu B, Ge X, Wang LF, Shi Z. Bat origin of human coronaviruses. Virol J. 2015;12:221. Epub 2015/12/23. doi: 10.1186/s12985-015-0422-1. PubMed PMID: 26689940; PubMed Central PMCID: PMCPMC4687304.

9. Scientist T. A Brief History of Human Coronaviruses 2020 [Januar 1, 2024]. Available from: https://www.biofiredx.com/blog/history-of-coronavirus/.

10. Holmes EC, Goldstein SA, Rasmussen AL, Robertson DL, Crits-Christoph A, Wertheim JO, *et al.* The origins of SARS-CoV-2: A critical review. Cell. 2021;184(19):4848-

56. Epub 2021/09/05. doi: 10.1016/j.cell.2021.08.017. PubMed PMID: 34480864; PubMed Central PMCID: PMCPMC8373617.

11. Abulsoud AI, El-Husseiny HM, El-Husseiny AA, El-Mahdy HA, Ismail A, Elkhawaga SY, *et al.* Mutations in SARS-CoV-2: Insights on structure, variants, vaccines, and biomedical interventions. Biomed Pharmacother. 2023;157:113977. Epub 2022/11/13. doi: 10.1016/j.biopha.2022.113977. PubMed PMID: 36370519; PubMed Central PMCID: PMCPMC9637516.

12. Salahshoori IM-A, N.; Seyfaee, A.; Nasirabad, N. M.; Dehghan, Z.; Faraji, M.; Ganjkhani, M.; Babapoor, A.; Shadmehr, Z. S.; Hamrang, A. Overview of COVID-19 Disease: Virology, Epidemiology, Prevention Diagnosis, Treatment, and Vaccines. biologics. 2021;1(1). doi: https://www.mdpi.com/2673-8449/1/1/2#.

13. Tang D, Comish P, Kang R. The hallmarks of COVID-19 disease. PLoS Pathog. 2020;16(5):e1008536. Epub 2020/05/23. doi: 10.1371/journal.ppat.1008536. PubMed PMID: 32442210; PubMed Central PMCID: PMCPMC7244094.

14. Hao YJ, Wang YL, Wang MY, Zhou L, Shi JY, Cao JM, *et al.* The origins of COVID-19 pandemic: A brief overview. Transbound Emerg Dis. 2022;69(6):3181-97. Epub 2022/10/12. doi: 10.1111/tbed.14732. PubMed PMID: 36218169; PubMed Central PMCID: PMCPMC9874793.

15. Chavarria-Miro G, Anfruns-Estrada E, Martinez-Velazquez A, Vazquez-Portero M, Guix S, Paraira M, *et al.* Time Evolution of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Wastewater during the First Pandemic Wave of COVID-19 in the Metropolitan Area of Barcelona, Spain. Appl Environ Microbiol. 2021;87(7). Epub 2021/01/24. doi: 10.1128/AEM.02750-20. PubMed PMID: 33483313; PubMed Central PMCID: PMCPMC8091622.

16. Carrat F, Figoni J, Henny J, Desenclos JC, Kab S, de Lamballerie X, *et al.* Evidence of early circulation of SARS-CoV-2 in France: findings from the population-based "CONSTANCES" cohort. Eur J Epidemiol. 2021;36(2):219-22. Epub 2021/02/07. doi: 10.1007/s10654-020-00716-2. PubMed PMID: 33548003; PubMed Central PMCID: PMCPMC7864798.

17. Basavaraju SV, Patton ME, Grimm K, Rasheed MAU, Lester S, Mills L, *et al.* Serologic Testing of US Blood Donations to Identify Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)-Reactive Antibodies: December 2019-January 2020. Clin Infect Dis. 2021;72(12):e1004-e9. Epub 2020/12/01. doi: 10.1093/cid/ciaa1785. PubMed PMID: 33252659; PubMed Central PMCID: PMCPMC7799215.

18. Apolone G, Montomoli E, Manenti A, Boeri M, Sabia F, Hyseni I, *et al.* Unexpected

detection of SARS-CoV-2 antibodies in the prepandemic period in Italy. Tumori. 2021;107(5):446-51. Epub 2020/11/13. doi: 10.1177/0300891620974755. PubMed PMID: 33176598; PubMed Central PMCID: PMCPMC8529295.

19. La Rosa G, Mancini P, Bonanno Ferraro G, Veneri C, Iaconelli M, Bonadonna L, *et al.* SARS-CoV-2 has been circulating in northern Italy since December 2019: Evidence from environmental monitoring. Sci Total Environ. 2021;750:141711. Epub 2020/08/25. doi: 10.1016/j.scitotenv.2020.141711. PubMed PMID: 32835962; PubMed Central PMCID: PMCPMC7428442.

20. Brian DA, Baric RS. Coronavirus genome structure and replication. Curr Top Microbiol Immunol. 2005;287:1-30. Epub 2004/12/22. doi: 10.1007/3-540-26765-4_1. PubMed PMID: 15609507; PubMed Central PMCID: PMCPMC7120446.

21. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol. 2020;92(4):418-23. Epub 2020/01/23. doi: 10.1002/jmv.25681. PubMed PMID: 31967327; PubMed Central PMCID: PMCPMC7167049.

22. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, *et al.* A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395(10223):514-23. Epub 2020/01/28. doi: 10.1016/S0140-6736(20)30154-9. PubMed PMID: 31986261; PubMed Central PMCID: PMCPMC7159286.

23. Yan W, Zheng Y, Zeng X, He B, Cheng W. Structural biology of SARS-CoV-2: open the door for novel therapies. Signal Transduct Target Ther. 2022;7(1):26. Epub 2022/01/29. doi: 10.1038/s41392-022-00884-5. PubMed PMID: 35087058; PubMed Central PMCID: PMCPMC8793099.

24. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. Front Cell Infect Microbiol. 2020;10:587269. Epub 2020/12/17. doi: 10.3389/fcimb.2020.587269. PubMed PMID: 33324574; PubMed Central PMCID: PMCPMC7723891.

25. Wu CR, Yin WC, Jiang Y, Xu HE. Structure genomics of SARS-CoV-2 and its Omicron variant: drug design templates for COVID-19. Acta Pharmacol Sin. 2022;43(12):3021-33. Epub 2022/01/22. doi: 10.1038/s41401-021-00851-w. PubMed PMID: 35058587; PubMed Central PMCID: PMCPMC8771608.

26. Zhang J, Xiao T, Cai Y, Chen B. Structure of SARS-CoV-2 spike protein. Curr Opin Virol. 2021;50:173-82. Epub 2021/09/18. doi: 10.1016/j.coviro.2021.08.010. PubMed PMID: 34534731; PubMed Central PMCID: PMCPMC8423807.

27. Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, *et al.* Cell entry mechanisms of

SARS-CoV-2. Proc Natl Acad Sci U S A. 2020;117(21):11727-34. Epub 2020/05/08. doi: 10.1073/pnas.2003138117. PubMed PMID: 32376634; PubMed Central PMCID: PMCPMC7260975.

28. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11(1):1620. Epub 2020/03/30. doi: 10.1038/s41467-020-15562-9. PubMed PMID: 32221306; PubMed Central PMCID: PMCPMC7100515.

29. Aziz MW, Mukhtar N, Anjum AA, Mushtaq MH, Ashraf MA, Nasir A, *et al.* Genomic Diversity and Evolution of SARS-CoV-2 Lineages in Pakistan. Viruses. 2023;15(7). Epub 2023/07/29. doi: 10.3390/v15071450. PubMed PMID: 37515139; PubMed Central PMCID: PMCPMC10386162.

30. Ferrer MD, Barrueco AS, Martinez-Beneyto Y, Mateos-Moreno MV, Ausina-Marquez V, Garcia-Vazquez E, *et al.* Clinical evaluation of antiseptic mouth rinses to reduce salivary load of SARS-CoV-2. Sci Rep. 2021;11(1):24392. Epub 2021/12/24. doi: 10.1038/s41598-021-03461-y. PubMed PMID: 34937855; PubMed Central PMCID: PMCPMC8695582.

31. Wang Z, Lv J, Yu P, Qu Y, Zhou Y, Zhou L, *et al.* SARS-CoV-2 treatment effects induced by ACE2-expressing microparticles are explained by the oxidized cholesterol-increased endosomal pH of alveolar macrophages. Cell Mol Immunol. 2022;19(2):210-21. Epub 2022/01/06. doi: 10.1038/s41423-021-00813-6. PubMed PMID: 34983944; PubMed Central PMCID: PMCPMC8724656.

32. Parasher A. COVID-19: Current understanding of its Pathophysiology, Clinical presentation and Treatment. Postgrad Med J. 2021;97(1147):312-20. Epub 2020/09/27. doi: 10.1136/postgradmedj-2020-138577. PubMed PMID: 32978337; PubMed Central PMCID: PMCPMC10017004.

33. Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, *et al.* Structural insights into SARS-CoV-2 proteins. J Mol Biol. 2021;433(2):166725. Epub 2020/11/28. doi: 10.1016/j.jmb.2020.11.024. PubMed PMID: 33245961; PubMed Central PMCID: PMCPMC7685130.

34. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses - drug discovery and therapeutic options. Nat Rev Drug Discov. 2016;15(5):327-47. Epub 2016/02/13. doi: 10.1038/nrd.2015.37. PubMed PMID: 26868298; PubMed Central PMCID: PMCPMC7097181.

35. Sola I, Almazan F, Zuniga S, Enjuanes L. Continuous and Discontinuous RNA Synthesis in Coronaviruses. Annu Rev Virol. 2015;2(1):265-88. Epub 2016/03/10. doi: 10.1146/annurev-virology-100114-055218. PubMed PMID: 26958916; PubMed Central

PMCID: PMCPMC6025776.

36. Snijder EJ, Decroly E, Ziebuhr J. The Nonstructural Proteins Directing Coronavirus RNA Synthesis and Processing. Adv Virus Res. 2016;96:59-126. Epub 2016/10/08. doi: 10.1016/bs.aivir.2016.08.008. PubMed PMID: 27712628; PubMed Central PMCID: PMCPMC7112286.

37. Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat Commun. 2019;10(1):2342. Epub 2019/05/30. doi: 10.1038/s41467-019-10280-3. PubMed PMID: 31138817; PubMed Central PMCID: PMCPMC6538669.

38. Chen J, Malone B, Llewellyn E, Grasso M, Shelton PMM, Olinares PDB, *et al.* Structural Basis for Helicase-Polymerase Coupling in the SARS-CoV-2 Replication-Transcription Complex. Cell. 2020;182(6):1560-73 e13. Epub 2020/08/14. doi: 10.1016/j.cell.2020.07.033. PubMed PMID: 32783916; PubMed Central PMCID: PMCPMC7386476.

39. Lowery SA, Sariol A, Perlman S. Innate immune and inflammatory responses to SARS-CoV-2: Implications for COVID-19. Cell Host Microbe. 2021;29(7):1052-62. Epub 2021/05/23. doi: 10.1016/j.chom.2021.05.004. PubMed PMID: 34022154; PubMed Central PMCID: PMCPMC8126603.

40. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271-80 e8. Epub 2020/03/07. doi: 10.1016/j.cell.2020.02.052. PubMed PMID: 32142651; PubMed Central PMCID: PMCPMC7102627.

41. Mu J, Xu J, Zhang L, Shu T, Wu D, Huang M, *et al.* SARS-CoV-2-encoded nucleocapsid protein acts as a viral suppressor of RNA interference in cells. Sci China Life Sci. 2020;63(9):1413-6. Epub 2020/04/16. doi: 10.1007/s11427-020-1692-1. PubMed PMID: 32291557; PubMed Central PMCID: PMCPMC7154568.

42. Voss D, Pfefferle S, Drosten C, Stevermann L, Traggiai E, Lanzavecchia A, *et al.* Studies on membrane topology, N-glycosylation and functionality of SARS-CoV membrane protein. Virol J. 2009;6:79. Epub 2009/06/19. doi: 10.1186/1743-422X-6-79. PubMed PMID: 19534833; PubMed Central PMCID: PMCPMC2705359.

43. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J. 2019;16(1):69. Epub 2019/05/28. doi: 10.1186/s12985-019-1182-0. PubMed PMID: 31133031; PubMed Central PMCID: PMCPMC6537279.

44. The World Health Organization (WHO). Coronavirus disease (COVID-19): How is it

transmitted? 2021 [April 30, 2024]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/coronavirus-disease-covid-19-how-is-it-transmitted?gad_source=1&gclid=CjwKCAjwrcKxBhBMEiwAIVF8rB1PsOaca6T3srzwftYEqA-n_1Kj--rgKtbsAhEe6L-1Wd0R0zhjsBoCkHcQAvD_BwE.

45. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, *et al.* Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020;323(18):1843-4. Epub 2020/03/12. doi: 10.1001/jama.2020.3786. PubMed PMID: 32159775; PubMed Central PMCID: PMCPMC7066521.

46. Bain CC, MacDonald AS. The impact of the lung environment on macrophage development, activation and function: diversity in the face of adversity. Mucosal Immunol. 2022;15(2):223-34. Epub 2022/01/13. doi: 10.1038/s41385-021-00480-w. PubMed PMID: 35017701; PubMed Central PMCID: PMCPMC8749355.

47. Borges V, Isidro J, Trovao NS, Duarte S, Cortes-Martins H, Martiniano H, *et al.* SARS-CoV-2 introductions and early dynamics of the epidemic in Portugal. Commun Med (Lond). 2022;2:10. Epub 2022/05/24. doi: 10.1038/s43856-022-00072-0. PubMed PMID: 35603268; PubMed Central PMCID: PMCPMC9053228.

48. Kikkenborg Berg S, Dam Nielsen S, Nygaard U, Bundgaard H, Palm P, Rotvig C, *et al.* Long COVID symptoms in SARS-CoV-2-positive adolescents and matched controls (LongCOVIDKidsDK): a national, cross-sectional study. Lancet Child Adolesc Health. 2022;6(4):240-8. Epub 2022/02/11. doi: 10.1016/S2352-4642(22)00004-9. PubMed PMID: 35143771; PubMed Central PMCID: PMCPMC8820960.

49. Barizien N, Le Guen M, Russel S, Touche P, Huang F, Vallee A. Clinical characterization of dysautonomia in long COVID-19 patients. Sci Rep. 2021;11(1):14042. Epub 2021/07/09. doi: 10.1038/s41598-021-93546-5. PubMed PMID: 34234251; PubMed Central PMCID: PMCPMC8263555.

50. D'Onofrio V, Keulen L, Vandendriessche A, Dubois J, Cartuyvels R, Vanden Abeele ME, *et al.* Studying the clinical, radiological, histological, microbiological, and immunological evolution during the different COVID-19 disease stages using minimal invasive autopsy. Sci Rep. 2022;12(1):1360. Epub 2022/01/27. doi: 10.1038/s41598-022-05186-y. PubMed PMID: 35079048; PubMed Central PMCID: PMCPMC8789771.

51. Centers for Disease Control and Prevention (CDC). COVID-19 Testing: What You Need to Know 2024 [May 28, 2024]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/testing.html#types-of-tests.

52. Yuce M, Filiztekin E, Ozkaya KG. COVID-19 diagnosis -A review of current methods.

Biosens Bioelectron. 2021;172:112752. Epub 2020/10/31. doi: 10.1016/j.bios.2020.112752. PubMed PMID: 33126180; PubMed Central PMCID: PMCPMC7584564.

53. Snehlata.; Teja KBM, B. Chapter 1 - Application of CRISPR-Based Diagnostic Tools in Detecting SARS-CoV-2 Infection. COVID-19: Tackling Global Pandemics through Scientific and Social Tools2022.

54. National Institutes of Health (NIH). Coronavirus Disease 2019 (COVID-19) Treatment Guidelines 2023 [October 18, 2023]. Available from: https://www.covid19treatmentguidelines.nih.gov/.

55. U.S. Food and Drug Admission (FDA). Know Your Treatment Options for COVID-19 2023 [May 25, 2024]. Available from: https://www.fda.gov/consumers/consumer-updates/know-your-treatment-options-covid-19.

56. U.S. Food and Drug Admission (FDA). Coronavirus (COVID-19) | Drugs 2023 [cited June 9, 2024]. Available from: https://www.fda.gov/drugs/emergency-preparedness-drugs/coronavirus-covid-19-drugs.

57. Yin W, Mao C, Luan X, Shen DD, Shen Q, Su H, *et al.* Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Science. 2020;368(6498):1499-504. Epub 2020/05/03. doi: 10.1126/science.abc1560. PubMed PMID: 32358203; PubMed Central PMCID: PMCPMC7199908.

58. Centers of Disease and Control (CDC). COVID-19 Treatment and Preventive Medication 2024 [May 26, 2024]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/your-health/treatments-for-severe-illness.html.

59. Soleimanpour S, Yaghoubi A. COVID-19 vaccine: where are we now and where should we go? Expert Rev Vaccines. 2021;20(1):23-44. Epub 2021/01/14. doi: 10.1080/14760584.2021.1875824. PubMed PMID: 33435774; PubMed Central PMCID: PMCPMC7898300.

60. U.S. Food and Drug Admission (FDA). COVID-19 Vaccines 2024 [May 28, 2024]. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines.

61. Heinz FX, Stiasny K. Distinguishing features of current COVID-19 vaccines: knowns and unknowns of antigen presentation and modes of action. NPJ Vaccines. 2021;6(1):104. Epub 2021/08/18. doi: 10.1038/s41541-021-00369-6. PubMed PMID: 34400651; PubMed Central PMCID: PMCPMC8368295.

62. U.S. Food and Drug Admission (FDA). FDA Approves First COVID-19 Vaccine 2021 [May 28, 2024]. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine.

63. Meo SA, Bukhari IA, Akram J, Meo AS, Klonoff DC. COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines. Eur Rev Med Pharmacol Sci. 2021;25(3):1663-9. Epub 2021/02/26. doi: 10.26355/eurrev_202102_24877. PubMed PMID: 33629336.

64. U.S. Food and Drug Admission (FDA). Moderna COVID-19 Vaccine 2023 [May 28, 2024]. Available from: https://www.fda.gov/vaccines-blood-biologics/coronavirus-covid-19-cber-regulated-biologics/moderna-covid-19-vaccine#:~:text=On%20September%2011%2C%202023%2C%20the,of%20SARS%2DCoV%2D2.

65. Centers for Disease Control and Prevention (CDC). Overview of COVID-19 Vaccines 2024 [June 9, 2024]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/overview-COVID-19-vaccines.html.

66. Wong LAY, C.G.; Jahan, N.K.; Pillai, N. COVID-19 Vaccine: Review of the Mechanism of Action of Different Types of Vaccine. Open Access Library Journal. 2022;9. doi: https://doi.org/10.4236/oalib.1108624.

67. U.S. Food and Drug Admission (FDA). FDA Authorizes Updated Novavax COVID-19 Vaccine Formulated to Better Protect Against Currently Circulating Variants 2023 [May 28, 2024]. Available from: https://www.fda.gov/news-events/press-announcements/fda-authorizes-updated-novavax-covid-19-vaccine-formulated-better-protect-against-currently.

68. Alimoradi M. Comparison of COVID-19 vaccines 2022 [June 9, 2024]. Available from: https://myacare.com/blog/comparison-of-covid-19-vaccines.

69. Hadj Hassine I. Covid-19 vaccines and variants of concern: A review. Rev Med Virol. 2022;32(4):e2313. Epub 2021/11/11. doi: 10.1002/rmv.2313. PubMed PMID: 34755408; PubMed Central PMCID: PMCPMC8646685.

70. The World Health Organization (WHO). The Novavax vaccine against COVID-19: What you need to know 2022 [July 24, 2024]. Available from: https://www.who.int/news-room/feature-stories/detail/the-novavax-vaccine-against-covid-19-what-you-need-to-know.

71. online D. AstraZeneca COVID-19 Vaccine [July 24, 2024]. Available from: https://go.drugbank.com/drugs/DB15656.

72. Watch HP. Sputnik Vaccine Efficacy Data Published in Lancet Are 'Statistically Impossible' 2022 [July 24, 2024]. Available from: https://healthpolicy-watch.news/sputnik-vaccine-efficacy-data/#:~:text=More%20than%2070%20countries%20have,by%20The%20Lancet%20in%202021.

73. Centers for Disease Control and Prevention (CDC). ACIP Evidence to

Recommendations for Use of Janssen COVID-19 Vaccine under an Emergency Use Authorization [July 24, 2024]. Available from: https://www.cdc.gov/vaccines/acip/recs/grade/covid-19-janssen-etr.html.

74. The World Health Organization (WHO). The Sinovac-CoronaVac COVID-19 vaccine: What you need to know 2022 [July 24, 2024]. Available from: https://www.who.int/news-room/feature-stories/detail/the-sinovac-covid-19-vaccine-what-you-need-to-know.

75. The World Health Organization (WHO). The Sinopharm COVID-19 vaccine: What you need to know 2022 [July 24, 2024]. Available from: https://www.who.int/news-room/feature-stories/detail/the-sinopharm-covid-19-vaccine-what-you-need-to-know#:~:text=How%20efficacious%20is%20the%20vaccine,efficacy%20against%20hospitalization%20was%2079%25.

76. U.S. Food and Drug Admission (FDA). COVID-19 Vaccines for 2023-2024 2024 [July 9, 2024]. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines-2023-2024.

77. U.S. Food and Drug Admission (FDA). Updated COVID-19 Vaccines for Use in the United States Beginning in Fall 2024 2024 [cited July 9, 2024]. Available from: https://www.fda.gov/vaccines-blood-biologics/updated-covid-19-vaccines-use-united-states-beginning-fall-2024.

78. Gedes L. From Alpha to Omicron: Everything you need to know about SARS-CoV-2 variants of concern 2021 [June 9, 2024]. Available from: https://www.gavi.org/vaccineswork/alpha-omicron-everything-you-need-know-about-coronavirus-variants-concern.

79. Global Initiative on Sharing Avian Influenza Data (GISAID). hCoV-19 data sharing via GISAID 2024 [29/2/2024]. Available from: https://gisaid.org/.

80. GISAID. Clade and lineage nomenclature, March 2, 2021 2021 [April 28, 2024]. Available from: https://gisaid.org/resources/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/.

81. GISAID. 2022 [June 6, 2024]. Available from: https://www.epicov.org/epi3/frontend#294828.

82. Zoppi L. Viral Clades of SARS-CoV-2 2023 [April 29, 2024]. Available from: https://www.news-medical.net/health/Viral-Clades-of-SARS-CoV-2.aspx.

83. Sharif N, Alzahrani KJ, Ahmed SN, Khan A, Banjer HJ, Alzahrani FM, *et al.* Genomic surveillance, evolution and global transmission of SARS-CoV-2 during 2019-2022. PLoS One. 2022;17(8):e0271074. Epub 2022/08/02. doi: 10.1371/journal.pone.0271074. PubMed PMID: 35913920; PubMed Central PMCID: PMCPMC9342790.

84. Hodcroft EBH, J.; Neher, R. A.; Bedford, T. Year-letter Genetic Clade Naming for SARS-CoV-2 on Nextstrain.org 2020 [April 29,2024]. Available from: https://nextstrain.org/blog/2020-06-02-SARSCoV2-clade-naming.

85. Nextstrain team. Genomic epidemiology of SARS-CoV-2 with subsampling focused globally over the past 6 months 2024 [April 29, 2024]. Available from: https://nextstrain.org/ncov/gisaid/global/6m?branchLabel=clade&c=clade_membership.

86. Rambaut A, Holmes EC, O'Toole A, Hill V, McCrone JT, Ruis C, *et al.* A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol. 2020;5(11):1403-7. Epub 2020/07/17. doi: 10.1038/s41564-020-0770-5. PubMed PMID: 32669681; PubMed Central PMCID: PMCPMC7610519.

87. Pybus O. Pango Lineages Nomenclature: provisinal rules for naming recombinant lineages 2021 [June 3, 2024]. Available from: https://virological.org/t/pango-lineage-nomenclature-provisional-rules-for-naming-recombinant-lineages/657.

88. The World Health Organization (WHO). Updated working definitions and primary actions for SARS-CoV-2 variants 2023 [June 6, 2024]. Available from: https://www.who.int/publications/m/item/updated-working-definitions-and-primary-actions-for--sars-cov-2-variants.

89. Ashoor D, Marzouq M, Trabelsi K, Chlif S, Abotalib N, Khalaf NB, *et al.* How concerning is a SARS-CoV-2 variant of concern? Computational predictions and the variants labeling system. Front Cell Infect Microbiol. 2022;12:868205. Epub 2022/08/30. doi: 10.3389/fcimb.2022.868205. PubMed PMID: 36034694; PubMed Central PMCID: PMCPMC9399656.

90. Center of Disease Control and Prevetion (CDC). SARS-CoV-2 Variant Classifications and Definitions 2023 [updated 9/1/20239/17/2023]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html#anchor_1633452601089.

91. Zhu X, Mannar D, Srivastava SS, Berezuk AM, Demers JP, Saville JW, *et al.* Cryo-electron microscopy structures of the N501Y SARS-CoV-2 spike protein in complex with ACE2 and 2 potent neutralizing antibodies. PLoS Biol. 2021;19(4):e3001237. Epub 2021/04/30. doi: 10.1371/journal.pbio.3001237. PubMed PMID: 33914735; PubMed Central PMCID: PMCPMC8112707.

92. Farhud DD, Mojahed N. SARS-COV-2 Notable Mutations and Variants: A Review Article. Iran J Public Health. 2022;51(7):1494-501. Epub 2022/10/18. doi: 10.18502/ijph.v51i7.10083. PubMed PMID: 36248293; PubMed Central PMCID: PMCPMC9529736.

93. Becerra-Flores M, Cardozo T. SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate. Int J Clin Pract. 2020;74(8):e13525. Epub 2020/05/07. doi: 10.1111/ijcp.13525. PubMed PMID: 32374903; PubMed Central PMCID: PMCPMC7267315.

94. Focosi D. SARS-CoV-2 Spike Protein Convergent Evolution: Impact of Virus Variants on Efficacy of COVID-19 Therapeutics and Vaccines2021.

95. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingens AS, *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. Cell. 2020;182(5):1295-310 e20. Epub 2020/08/26. doi: 10.1016/j.cell.2020.08.012. PubMed PMID: 32841599; PubMed Central PMCID: PMCPMC7418704.

96. Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, *et al.* The biological and clinical significance of emerging SARS-CoV-2 variants. Nat Rev Genet. 2021;22(12):757-73. Epub 2021/09/19. doi: 10.1038/s41576-021-00408-x. PubMed PMID: 34535792; PubMed Central PMCID: PMCPMC8447121.

97. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, *et al.* Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 2020;182(4):812-27 e19. Epub 2020/07/23. doi: 10.1016/j.cell.2020.06.043. PubMed PMID: 32697968; PubMed Central PMCID: PMCPMC7332439.

98. Jackson CB, Zhang L, Farzan M, Choe H. Functional importance of the D614G mutation in the SARS-CoV-2 spike protein. Biochem Biophys Res Commun. 2021;538:108-15. Epub 2020/11/23. doi: 10.1016/j.bbrc.2020.11.026. PubMed PMID: 33220921; PubMed Central PMCID: PMCPMC7664360.

99. Tang JW, Toovey OTR, Harvey KN, Hui DDS. Introduction of the South African SARS-CoV-2 variant 501Y.V2 into the UK. J Infect. 2021;82(4):e8-e10. Epub 2021/01/21. doi: 10.1016/j.jinf.2021.01.007. PubMed PMID: 33472093; PubMed Central PMCID: PMCPMC7813514.

100. Liu Y, Liu J, Plante KS, Plante JA, Xie X, Zhang X, *et al.* The N501Y spike substitution enhances SARS-CoV-2 transmission. bioRxiv. 2021. Epub 2021/03/25. doi: 10.1101/2021.03.08.434499. PubMed PMID: 33758836; PubMed Central PMCID: PMCPMC7986995.

101. Tian F, Tong B, Sun L, Shi S, Zheng B, Wang Z, *et al.* N501Y mutation of spike protein in SARS-CoV-2 strengthens its binding to receptor ACE2. Elife. 2021;10. Epub 2021/08/21. doi: 10.7554/eLife.69091. PubMed PMID: 34414884; PubMed Central PMCID: PMCPMC8455130.

102. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, *et*

al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature. 2021;592(7854):438-43. Epub 2021/03/11. doi: 10.1038/s41586-021-03402-9. PubMed PMID: 33690265.

103. Yang WT, Huang WH, Liao TL, Hsiao TH, Chuang HN, Liu PY. SARS-CoV-2 E484K Mutation Narrative Review: Epidemiology, Immune Escape, Clinical Implications, and Future Considerations. Infect Drug Resist. 2022;15:373-85. Epub 2022/02/11. doi: 10.2147/IDR.S344099. PubMed PMID: 35140483; PubMed Central PMCID: PMCPMC8820839.

104. Ding C, He J, Zhang X, Jiang C, Sun Y, Zhang Y, *et al.* Crucial Mutations of Spike Protein on SARS-CoV-2 Evolved to Variant Strains Escaping Neutralization of Convalescent Plasmas and RBD-Specific Monoclonal Antibodies. Front Immunol. 2021;12:693775. Epub 2021/09/07. doi: 10.3389/fimmu.2021.693775. PubMed PMID: 34484190; PubMed Central PMCID: PMCPMC8416052.

105. Gangavarapu K, Latif AA, Mullen JL, Alkuzweny M, Hufbauer E, Tsueng G, *et al.* Outbreak.info genomic reports: scalable and dynamic surveillance of SARS-CoV-2 variants and mutations. Nat Methods. 2023;20(4):512-22. Epub 2023/02/25. doi: 10.1038/s41592-023-01769-3. PubMed PMID: 36823332; PubMed Central PMCID: PMCPMC10399614.

106. Duong D. Alpha, Beta, Delta, Gamma: What's important to know about SARS-CoV-2 variants of concern? CMAJ. 2021;193(27):E1059-E60. Epub 2021/07/14. doi: 10.1503/cmaj.1095949. PubMed PMID: 34253551; PubMed Central PMCID: PMCPMC8342008.

107. Khan T, Jamal SM. SARS-CoV-2 nomenclature: viruses, variants and vaccines need a standardized naming system. Future Virol. 2021. Epub 2021/11/16. doi: 10.2217/fvl-2021-0198. PubMed PMID: 34777556; PubMed Central PMCID: PMCPMC8577721.

108. The World Health Organization (WHO). WHO announces simple, easy-to-say labels for SARS-CoV-2 Variants of Interest and Concern 2021 [June 9, 2024]. Available from: https://www.who.int/news/item/31-05-2021-who-announces-simple-easy-to-say-labels-for-sars-cov-2-variants-of-interest-and-concern.

109. Buchan SA, Tibebu S, Daneman N, Whelan M, Vanniyasingam T, Murti M, *et al.* Increased Household Secondary Attacks Rates With Variant of Concern Severe Acute Respiratory Syndrome Coronavirus 2 Index Cases. Clin Infect Dis. 2022;74(4):703-6. Epub 2021/06/10. doi: 10.1093/cid/ciab496. PubMed PMID: 34105720; PubMed Central PMCID: PMCPMC8384411.

110. Sinha S, Tam B, Wang SM. RBD Double Mutations of SARS-CoV-2 Strains Increase Transmissibility through Enhanced Interaction between RBD and ACE2 Receptor.

Viruses. 2021;14(1). Epub 2022/01/23. doi: 10.3390/v14010001. PubMed PMID: 35062205; PubMed Central PMCID: PMCPMC8781274.

111. Campbell F, Archer B, Laurenson-Schafer H, Jinnai Y, Konings F, Batra N, *et al.* Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. Euro Surveill. 2021;26(24). Epub 2021/06/19. doi: 10.2807/1560-7917.ES.2021.26.24.2100509. PubMed PMID: 34142653; PubMed Central PMCID: PMCPMC8212592.

112. Dhar MS, Marwal R, Vs R, Ponnusamy K, Jolly B, Bhoyar RC, *et al.* Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. Science. 2021;374(6570):995-9. Epub 2021/10/15. doi: 10.1126/science.abj9932. PubMed PMID: 34648303; PubMed Central PMCID: PMCPMC7612010.

113. Grabowski F, Preibisch G, Gizinski S, Kochanczyk M, Lipniacki T. SARS-CoV-2 Variant of Concern 202012/01 Has about Twofold Replicative Advantage and Acquires Concerning Mutations. Viruses. 2021;13(3). Epub 2021/04/04. doi: 10.3390/v13030392. PubMed PMID: 33804556; PubMed Central PMCID: PMCPMC8000749.

114. Pearson CAB, Russell TW, Davies N, Kucharski AJ. Estimates of severity and transmissibility of novel SARS-CoV-2 variant 501Y.V2 in South Africa. Preprint at cmmid. 2021.

115. Curran J, Dol J, Boulos L, Somerville M, McCulloch H, MacDonald M, *et al.* Transmission characteristics of SARS-CoV-2 variants of concern Rapid Scoping Review. Preprint at medRxiv. 2021. doi: https://doi.org/10.1101/2021.04.23.21255515.

116. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, *et al.* Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science. 2021;372(6544):815-21. Epub 2021/04/16. doi: 10.1126/science.abh2644. PubMed PMID: 33853970; PubMed Central PMCID: PMCPMC8139423.

117. Andre M, Lau LS, Pokharel MD, Ramelow J, Owens F, Souchak J, *et al.* From Alpha to Omicron: How Different Variants of Concern of the SARS-Coronavirus-2 Impacted the World. Biology (Basel). 2023;12(9). Epub 2023/09/28. doi: 10.3390/biology12091267. PubMed PMID: 37759666; PubMed Central PMCID: PMCPMC10525159.

118. Choi JY, Smith DM. SARS-CoV-2 Variants of Concern. Yonsei Med J. 2021;62(11):961-8. Epub 2021/10/22. doi: 10.3349/ymj.2021.62.11.961. PubMed PMID: 34672129; PubMed Central PMCID: PMCPMC8542474.

119. (WHO) TWHO. Historical working definitions and primary actions for SARS-CoV-2 variants 2023 [June 6, 2024]. Available from: https://www.who.int/publications/m/item/historical-working-definitions-and-primary-actions-

for-sars-cov-2-variants.

120. Gomez CE, Perdiguero B, Esteban M. Emerging SARS-CoV-2 Variants and Impact in Global Vaccination Programs against SARS-CoV-2/COVID-19. Vaccines (Basel). 2021;9(3). Epub 2021/04/04. doi: 10.3390/vaccines9030243. PubMed PMID: 33799505; PubMed Central PMCID: PMCPMC7999234.

121. Guerrero-Preston R, Rivera-Amill V, Caraballo K, Rodriguez-Torres S, Purcell-Wiltz A, Garcia AA, *et al.* Precision health diagnostic and surveillance network uses S gene target failure (SGTF) combined with sequencing technologies to track emerging SARS-CoV-2 variants. Immun Inflamm Dis. 2022;10(6):e634. Epub 2022/06/01. doi: 10.1002/iid3.634. PubMed PMID: 35634961; PubMed Central PMCID: PMCPMC9092005.

122. Meng B, Kemp SA, Papa G, Datir R, Ferreira I, Marelli S, *et al.* Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. Cell Rep. 2021;35(13):109292. Epub 2021/06/25. doi: 10.1016/j.celrep.2021.109292. PubMed PMID: 34166617; PubMed Central PMCID: PMCPMC8185188.

123. Kemp SA, Collier DA, Datir RP, Ferreira I, Gayed S, Jahun A, *et al.* SARS-CoV-2 evolution during treatment of chronic infection. Nature. 2021;592(7853):277-82. Epub 2021/02/06. doi: 10.1038/s41586-021-03291-y. PubMed PMID: 33545711; PubMed Central PMCID: PMCPMC7610568.

124. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, *et al.* Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021;372(6538). Epub 2021/03/05. doi: 10.1126/science.abg3055. PubMed PMID: 33658326; PubMed Central PMCID: PMCPMC8128288.

125. Liu Y, Liu J, Plante KS, Plante JA, Xie X, Zhang X, *et al.* The N501Y spike substitution enhances SARS-CoV-2 infection and transmission. Nature. 2022;602(7896):294-9. Epub 2021/11/25. doi: 10.1038/s41586-021-04245-0. PubMed PMID: 34818667; PubMed Central PMCID: PMCPMC8900207.

126. Hoffmann M, Kleine-Weber H, Pohlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell. 2020;78(4):779-84 e5. Epub 2020/05/05. doi: 10.1016/j.molcel.2020.04.022. PubMed PMID: 32362314; PubMed Central PMCID: PMCPMC7194065.

127. England PH. Investigation of SARS-CoV-2 variants of concern in England 2021 [June 7, 2024]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/961299/Variants_of_Concern_VOC_Technical_Briefing_6_England-1.pdf.

128. European Centre for Disease Prevention and Control (ecdc). Risk assessment on

COVID-19 2021 [June 7, 2024]. Available from: https://www.ecdc.europa.eu/en/current-risk-assessment-novel-coronavirus-situation.

129. GOVUK. NERVTAG paper on COVID-19 variant of concern B.1.1.7 2021 [June 7, 2024]. Available from: https://www.gov.uk/government/publications/nervtag-paper-on-covid-19-variant-of-concern-b117.

130. Supasa P, Zhou D, Dejnirattisai W, Liu C, Mentzer AJ, Ginn HM, *et al.* Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell. 2021;184(8):2201-11 e7. Epub 2021/03/22. doi: 10.1016/j.cell.2021.02.033. PubMed PMID: 33743891; PubMed Central PMCID: PMCPMC7891044.

131. Muik A, Wallisch AK, Sanger B, Swanson KA, Muhl J, Chen W, *et al.* Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. Science. 2021;371(6534):1152-3. Epub 2021/01/31. doi: 10.1126/science.abg6105. PubMed PMID: 33514629; PubMed Central PMCID: PMCPMC7971771.

132. Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, *et al.* Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet. 2021;397(10282):1351-62. Epub 2021/04/03. doi: 10.1016/S0140-6736(21)00628-0. PubMed PMID: 33798499; PubMed Central PMCID: PMCPMC8009612.

133. Lumley SF, Rodger G, Constantinides B, Sanderson N, Chau KK, Street TL, *et al.* An Observational Cohort Study on the Incidence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection and B.1.1.7 Variant Infection in Healthcare Workers by Antibody and Vaccination Status. Clin Infect Dis. 2022;74(7):1208-19. Epub 2021/07/04. doi: 10.1093/cid/ciab608. PubMed PMID: 34216472; PubMed Central PMCID: PMCPMC8994591.

134. Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, *et al.* Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ. 2021;373:n1088. Epub 2021/05/15. doi: 10.1136/bmj.n1088. PubMed PMID: 33985964; PubMed Central PMCID: PMCPMC8116636.

135. Washington NL, Gangavarapu K, Zeller M, Bolze A, Cirulli ET, Schiabor Barrett KM, *et al.* Emergence and rapid transmission of SARS-CoV-2 B.1.1.7 in the United States. Cell. 2021;184(10):2587-94 e7. Epub 2021/04/17. doi: 10.1016/j.cell.2021.03.052. PubMed PMID: 33861950; PubMed Central PMCID: PMCPMC8009040.

136. Shen X, Tang H, McDanal C, Wagh K, Fischer W, Theiler J, *et al.* SARS-CoV-

2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral spike vaccines. Cell Host Microbe. 2021;29(4):529-39 e3. Epub 2021/03/12. doi: 10.1016/j.chom.2021.03.002. PubMed PMID: 33705729; PubMed Central PMCID: PMCPMC7934674.

137. Doi A, Iwata K, Nakamura T, Oh K, Isome K, Hasegawa K, *et al.* Clinical outcomes of COVID-19 caused by the Alpha variant compared with one by wild type in Kobe, Japan. A multi-center nested case-control study. J Infect Chemother. 2023;29(3):289-93. Epub 2022/12/10. doi: 10.1016/j.jiac.2022.11.014. PubMed PMID: 36494058; PubMed Central PMCID: PMCPMC9722619.

138. Grint DJ, Wing K, Houlihan C, Gibbs HP, Evans SJW, Williamson E, *et al.* Severity of Severe Acute Respiratory System Coronavirus 2 (SARS-CoV-2) Alpha Variant (B.1.1.7) in England. Clin Infect Dis. 2022;75(1):e1120-e7. Epub 2021/09/07. doi: 10.1093/cid/ciab754. PubMed PMID: 34487522; PubMed Central PMCID: PMCPMC8522415.

139. Curran JD, J.; Boulos, L.; Somerville, M.; McCulloch, H.; MacDonald, M. . Transmission characteristics of SARS-CoV-2 variants of concern Rapid Scoping Review. medRxiv. 2021. doi: https://doi.org/10.1101/2021.04.23.21255515.

140. Lin L, Liu Y, Tang X, He D. The Disease Severity and Clinical Outcomes of the SARS-CoV-2 Variants of Concern. Front Public Health. 2021;9:775224. Epub 2021/12/18. doi: 10.3389/fpubh.2021.775224. PubMed PMID: 34917580; PubMed Central PMCID: PMCPMC8669511.

141. Tegally HW, E.; Giovanetti, M.; Iranzadeh, A.; Fonseca, V.; Giandhari, J. . Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv. 2020. doi: 10.1101/2020.12.21.20248640.

142. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, Yassine HM, Benslimane FM, Al Khatib HA, *et al.* Severity, Criticality, and Fatality of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Beta Variant. Clin Infect Dis. 2022;75(1):e1188-e91. Epub 2021/10/18. doi: 10.1093/cid/ciab909. PubMed PMID: 34657152; PubMed Central PMCID: PMCPMC9402694.

143. Qassim SH, Hasan MR, Tang P, Chemaitelly H, Ayoub HH, Yassine HM, *et al.* Effects of SARS-CoV-2 Alpha, Beta, and Delta variants, age, vaccination, and prior infection on infectiousness of SARS-CoV-2 infections. Front Immunol. 2022;13:984784. Epub 2022/10/01. doi: 10.3389/fimmu.2022.984784. PubMed PMID: 36177014; PubMed Central PMCID: PMCPMC9513583.

144. Mohammad T, Choudhury A, Habib I, Asrani P, Mathur Y, Umair M, *et al.*

Genomic Variations in the Structural Proteins of SARS-CoV-2 and Their Deleterious Impact on Pathogenesis: A Comparative Genomics Approach. Front Cell Infect Microbiol. 2021;11:765039. Epub 2021/11/02. doi: 10.3389/fcimb.2021.765039. PubMed PMID: 34722346; PubMed Central PMCID: PMCPMC8548870.

145. Weisblum Y, Schmidt F, Zhang F, DaSilva J, Poston D, Lorenzi JC, *et al.* Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. Elife. 2020;9. Epub 2020/10/29. doi: 10.7554/eLife.61312. PubMed PMID: 33112236; PubMed Central PMCID: PMCPMC7723407.

146. Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, *et al.* SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat Med. 2021;27(4):622-5. Epub 2021/03/04. doi: 10.1038/s41591-021-01285-x. PubMed PMID: 33654292.

147. Wang P, Nair MS, Liu MSL, Iketani S, Luo Y, Guo Y, *et al.* Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7. Preprint at bioRxiv. 2021. doi: https://doi.org/10.1101/2021.01.25.428137.

148. Wang GL, Wang ZY, Duan LJ, Meng QC, Jiang MD, Cao J, *et al.* Susceptibility of Circulating SARS-CoV-2 Variants to Neutralization. N Engl J Med. 2021;384(24):2354-6. Epub 2021/04/07. doi: 10.1056/NEJMc2103022. PubMed PMID: 33822491; PubMed Central PMCID: PMCPMC8063885.

149. McCallum M, De Marco A, Lempp FA, Tortorici MA, Pinto D, Walls AC, *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell. 2021;184(9):2332-47 e16. Epub 2021/03/25. doi: 10.1016/j.cell.2021.03.028. PubMed PMID: 33761326; PubMed Central PMCID: PMCPMC7962585.

150. Yadav PD, Sarkale P, Razdan A, Gupta N, Nyayanit DA, Sahay RR, *et al.* Isolation and characterization of SARS-CoV-2 Beta variant from UAE travelers. J Infect Public Health. 2022;15(2):182-6. Epub 2022/01/03. doi: 10.1016/j.jiph.2021.12.011. PubMed PMID: 34974274; PubMed Central PMCID: PMCPMC8704724.

151. Han DP, Penn-Nicholson A, Cho MW. Identification of critical determinants on ACE2 for SARS-CoV entry and development of a potent entry inhibitor. Virology. 2006;350(1):15-25. Epub 2006/03/03. doi: 10.1016/j.virol.2006.01.029. PubMed PMID: 16510163; PubMed Central PMCID: PMCPMC7111894.

152. Barton MI, MacGowan SA, Kutuzov MA, Dushek O, Barton GJ, van der Merwe PA. Effects of common mutations in the SARS-CoV-2 Spike RBD and its ligand, the human ACE2 receptor on binding affinity and kinetics. Elife. 2021;10. Epub 2021/08/27. doi: 10.7554/eLife.70658. PubMed PMID: 34435953; PubMed Central PMCID:

PMCPMC8480977.

153. Banho CA, Sacchetto L, Campos GRF, Bittar C, Possebon FS, Ullmann LS, *et al.* Impact of SARS-CoV-2 Gamma lineage introduction and COVID-19 vaccination on the epidemiological landscape of a Brazilian city. Commun Med (Lond). 2022;2:41. Epub 2022/05/24. doi: 10.1038/s43856-022-00108-5. PubMed PMID: 35603276; PubMed Central PMCID: PMCPMC9053258.

154. Pathogesn Sweden. SARS-CoV-2 variants of concern (VoC) [May 20, 2024]. Available from: https://www.pathogens.se/pathogens/voc/.

155. The World Health Organization (WHO). Weekly epidemiological update on COVID-19 - 31 August 2021 2021 [May 17, 2024]. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---31-august-2021.

156. Giovanetti M, Fonseca V, Wilkinson E, Tegally H, San EJ, Althaus CL, *et al.* Replacement of the Gamma by the Delta variant in Brazil: Impact of lineage displacement on the ongoing pandemic. Virus Evol. 2022;8(1):veac024. Epub 2022/04/05. doi: 10.1093/ve/veac024. PubMed PMID: 35371559; PubMed Central PMCID: PMCPMC8971541.

157. Yepez Y, Marcano-Ruiz M, Bezerra RS, Fam B, Ximenez JP, Silva WA, Jr., *et al.* Evolutionary history of the SARS-CoV-2 Gamma variant of concern (P.1): a perfect storm. Genet Mol Biol. 2022;45(1):e20210309. Epub 2022/03/11. doi: 10.1590/1678-4685-GMB-2021-0309. PubMed PMID: 35266951; PubMed Central PMCID: PMCPMC8908351.

158. Thakur S, Sasi S, Pillai SG, Nag A, Shukla D, Singhal R, *et al.* SARS-CoV-2 Mutations and Their Impact on Diagnostics, Therapeutics and Vaccines. Front Med (Lausanne). 2022;9:815389. Epub 2022/03/12. doi: 10.3389/fmed.2022.815389. PubMed PMID: 35273977; PubMed Central PMCID: PMCPMC8902153.

159. Spira B. The Impact of the Highly Virulent SARS-CoV-2 Gamma Variant on Young Adults in the State of Sao Paulo: Was It Inevitable? Cureus. 2022;14(7):e26486. Epub 2022/08/04. doi: 10.7759/cureus.26486. PubMed PMID: 35919213; PubMed Central PMCID: PMCPMC9339207.

160. Alquraan L, Alzoubi KH, Rababa'h SY. Mutations of SARS-CoV-2 and their impact on disease diagnosis and severity. Inform Med Unlocked. 2023;39:101256. Epub 2023/05/03. doi: 10.1016/j.imu.2023.101256. PubMed PMID: 37131549; PubMed Central PMCID: PMCPMC10127666.

161. Naveca FG, Nascimento V, de Souza VC, Corado AL, Nascimento F, Silva G, *et al.* COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and

P.1 emergence. Nat Med. 2021;27(7):1230-8. Epub 2021/05/27. doi: 10.1038/s41591-021-01378-7. PubMed PMID: 34035535.

162. Perez-Gomez R. The Development of SARS-CoV-2 Variants: The Gene Makes the Disease. J Dev Biol. 2021;9(4). Epub 2021/12/24. doi: 10.3390/jdb9040058. PubMed PMID: 34940505; PubMed Central PMCID: PMCPMC8705434.

163. Wang Y, Chen R, Hu F, Lan Y, Yang Z, Zhan C, *et al.* Transmission, viral kinetics and clinical characteristics of the emergent SARS-CoV-2 Delta VOC in Guangzhou, China. EClinicalMedicine. 2021;40:101129. Epub 2021/09/21. doi: 10.1016/j.eclinm.2021.101129. PubMed PMID: 34541481; PubMed Central PMCID: PMCPMC8435265.

164. Luo CH, Morris CP, Sachithanandham J, Amadi A, Gaston D, Li M, *et al.* Infection with the SARS-CoV-2 Delta Variant is Associated with Higher Infectious Virus Loads Compared to the Alpha Variant in both Unvaccinated and Vaccinated Individuals. medRxiv. 2021. Epub 2021/09/01. doi: 10.1101/2021.08.15.21262077. PubMed PMID: 34462756; PubMed Central PMCID: PMCPMC8404894.

165. Tian D, Sun Y, Zhou J, Ye Q. The Global Epidemic of the SARS-CoV-2 Delta Variant, Key Spike Mutations and Immune Escape. Front Immunol. 2021;12:751778. Epub 2021/12/18. doi: 10.3389/fimmu.2021.751778. PubMed PMID: 34917076; PubMed Central PMCID: PMCPMC8669155.

166. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, *et al.* SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol. 2021;19(7):409-24. Epub 2021/06/03. doi: 10.1038/s41579-021-00573-0. PubMed PMID: 34075212; PubMed Central PMCID: PMCPMC8167834.

167. Di Giacomo S, Mercatelli D, Rakhimov A, Giorgi FM. Preliminary report on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Spike mutation T478K. J Med Virol. 2021;93(9):5638-43. Epub 2021/05/06. doi: 10.1002/jmv.27062. PubMed PMID: 33951211; PubMed Central PMCID: PMCPMC8242375.

168. Pascarella S, Ciccozzi M, Zella D, Bianchi M, Benedetti F, Benvenuto D, *et al.* SARS-CoV-2 B.1.617 Indian variants: Are electrostatic potential changes responsible for a higher transmission rate? J Med Virol. 2021;93(12):6551-6. Epub 2021/07/15. doi: 10.1002/jmv.27210. PubMed PMID: 34260088; PubMed Central PMCID: PMCPMC8426736.

169. Motozono C, Toyoda M, Zahradnik J, Saito A, Nasser H, Tan TS, *et al.* SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity. Cell Host Microbe. 2021;29(7):1124-36 e11. Epub 2021/06/26. doi: 10.1016/j.chom.2021.06.006. PubMed PMID: 34171266; PubMed Central PMCID: PMCPMC8205251.

170. Saito A, Irie T, Suzuki R, Maemura T, Nasser H, Uriu K, *et al.* Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature. 2022;602(7896):300-6. Epub 2021/11/26. doi: 10.1038/s41586-021-04266-9. PubMed PMID: 34823256; PubMed Central PMCID: PMCPMC8828475.

171. Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakhon A, *et al.* Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell. 2021;184(16):4220-36 e13. Epub 2021/07/10. doi: 10.1016/j.cell.2021.06.020. PubMed PMID: 34242578; PubMed Central PMCID: PMCPMC8218332.

172. Fisman DN, Tuite AR. Evaluation of the relative virulence of novel SARS-CoV-2 variants: a retrospective cohort study in Ontario, Canada. CMAJ. 2021;193(42):E1619-E25. Epub 2021/10/07. doi: 10.1503/cmaj.211248. PubMed PMID: 34610919; PubMed Central PMCID: PMCPMC8562985.

173. Sheikh A, McMenamin J, Taylor B, Robertson C, Public Health S, the EIIC. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. Lancet. 2021;397(10293):2461-2. Epub 2021/06/18. doi: 10.1016/S0140-6736(21)01358-1. PubMed PMID: 34139198; PubMed Central PMCID: PMCPMC8201647.

174. American Society for Microbiology. How Dangerous Is the Delta Variant (B.1.617.2)? 2021 [May 24, 2024]. Available from: https://asm.org/Articles/2021/July/How-Dangerous-is-the-Delta-Variant-B-1-617-2.

175. Arora P, Kempf A, Nehlmeier I, Graichen L, Sidarovich A, Winkler MS, *et al.* Delta variant (B.1.617.2) sublineages do not show increased neutralization resistance. Cell Mol Immunol. 2021;18(11):2557-9. Epub 2021/10/13. doi: 10.1038/s41423-021-00772-y. PubMed PMID: 34635807; PubMed Central PMCID: PMCPMC8503871.

176. Chavda VP, Apostolopoulos V. Global impact of delta plus variant and vaccination. Expert Rev Vaccines. 2022;21(5):597-600. Epub 2022/02/22. doi: 10.1080/14760584.2022.2044800. PubMed PMID: 35184660.

177. Eales O, Page AJ, de Oliveira Martins L, Wang H, Bodinier B, Haw D, *et al.* SARS-CoV-2 lineage dynamics in England from September to November 2021: high diversity of Delta sub-lineages and increased transmissibility of AY.4.2. BMC Infect Dis. 2022;22(1):647. Epub 2022/07/28. doi: 10.1186/s12879-022-07628-4. PubMed PMID: 35896970; PubMed Central PMCID: PMCPMC9326417.

178. Chatterjee S, Bhattacharya M, Nag S, Dhama K, Chakraborty C. A Detailed Overview of SARS-CoV-2 Omicron: Its Sub-Variants, Mutations and Pathophysiology, Clinical Characteristics, Immunological Landscape, Immune Escape, and Therapies. Viruses. 2023;15(1). Epub 2023/01/22. doi: 10.3390/v15010167. PubMed PMID: 36680207; PubMed

Central PMCID: PMCPMC9866114.

179. The World Health Organization (WHO). WHO Director-General's opening remarks at the media briefing on COVID-19 - 14 December 2021 2021 [May 7, 2024]. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---14-december-2021.

180. Ingraham NE, Ingbar DH. The omicron variant of SARS-CoV-2: Understanding the known and living with unknowns. Clin Transl Med. 2021;11(12):e685. Epub 2021/12/16. doi: 10.1002/ctm2.685. PubMed PMID: 34911167; PubMed Central PMCID: PMCPMC8673343.

181. Bhattacharya M, Sharma AR, Dhama K, Agoramoorthy G, Chakraborty C. Omicron variant (B.1.1.529) of SARS-CoV-2: understanding mutations in the genome, S-glycoprotein, and antibody-binding regions. Geroscience. 2022;44(2):619-37. Epub 2022/03/09. doi: 10.1007/s11357-022-00532-4. PubMed PMID: 35258772; PubMed Central PMCID: PMCPMC8902853.

182. Quarleri J, Galvan V, Delpino MV. Omicron variant of the SARS-CoV-2: a quest to define the consequences of its high mutational load. Geroscience. 2022;44(1):53-6. Epub 2021/12/19. doi: 10.1007/s11357-021-00500-4. PubMed PMID: 34921354; PubMed Central PMCID: PMCPMC8683309.

183. Kumar S, Thambiraja TS, Karuppanan K, Subramaniam G. Omicron and Delta variant of SARS-CoV-2: A comparative computational study of spike protein. J Med Virol. 2022;94(4):1641-9. Epub 2021/12/17. doi: 10.1002/jmv.27526. PubMed PMID: 34914115.

184. Ali F, Kasry A, Amin M. The new SARS-CoV-2 strain shows a stronger binding affinity to ACE2 due to N501Y mutant. Med Drug Discov. 2021;10:100086. Epub 2021/03/09. doi: 10.1016/j.medidd.2021.100086. PubMed PMID: 33681755; PubMed Central PMCID: PMCPMC7923861.

185. Chakraborty C, Saha A, Sharma AR, Bhattacharya M, Lee SS, Agoramoorthy G. D614G mutation eventuates in all VOI and VOC in SARS-CoV-2: Is it part of the positive selection pioneered by Darwin? Mol Ther Nucleic Acids. 2021;26:237-41. Epub 2021/09/07. doi: 10.1016/j.omtn.2021.07.011. PubMed PMID: 34484868; PubMed Central PMCID: PMCPMC8408019.

186. Kannan SR, Spratt AN, Sharma K, Chand HS, Byrareddy SN, Singh K. Omicron SARS-CoV-2 variant: Unique features and their impact on pre-existing antibodies. J Autoimmun. 2022;126:102779. Epub 2021/12/17. doi: 10.1016/j.jaut.2021.102779. PubMed PMID: 34915422; PubMed Central PMCID: PMCPMC8666303.

187. Bhattacharya M, Chatterjee S, Sharma AR, Agoramoorthy G, Chakraborty C.

D614G mutation and SARS-CoV-2: impact on S-protein structure, function, infectivity, and immunity. Appl Microbiol Biotechnol. 2021;105(24):9035-45. Epub 2021/11/11. doi: 10.1007/s00253-021-11676-2. PubMed PMID: 34755213; PubMed Central PMCID: PMCPMC8578012.

188. Gupta D, Sharma P, Singh M, Kumar M, Ethayathulla AS, Kaur P. Structural and functional insights into the spike protein mutations of emerging SARS-CoV-2 variants. Cell Mol Life Sci. 2021;78(24):7967-89. Epub 2021/11/04. doi: 10.1007/s00018-021-04008-0. PubMed PMID: 34731254; PubMed Central PMCID: PMCPMC11073194.

189. Liu Z, VanBlargan LA, Bloyet LM, Rothlauf PW, Chen RE, Stumpf S, *et al.* Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. Cell Host Microbe. 2021;29(3):477-88 e4. Epub 2021/02/04. doi: 10.1016/j.chom.2021.01.014. PubMed PMID: 33535027; PubMed Central PMCID: PMCPMC7839837.

190. Carreno JM, Alshammary H, Tcheou J, Singh G, Raskin AJ, Kawabata H, *et al.* Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. Nature. 2022;602(7898):682-8. Epub 2022/01/12. doi: 10.1038/s41586-022-04399-5. PubMed PMID: 35016197.

191. Centers for Disease Control and Prevention (CDC). SARS-CoV-2 Variant Classifications and Definitions 2020 [January 2, 2024]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html.

192. Sah R, Rais MA, Mohanty A, Chopra H, Chandran D, Bin Emran T, *et al.* Omicron (B.1.1.529) variant and its subvariants and lineages may lead to another COVID-19 wave in the world? -An overview of current evidence and counteracting strategies. Int J Surg Open. 2023;55:100625. Epub 2023/05/31. doi: 10.1016/j.ijso.2023.100625. PubMed PMID: 37255735; PubMed Central PMCID: PMCPMC10192062.

193. Shrestha LB, Foster C, Rawlinson W, Tedla N, Bull RA. Evolution of the SARS-CoV-2 omicron variants BA.1 to BA.5: Implications for immune escape and transmission. Rev Med Virol. 2022;32(5):e2381. Epub 2022/07/21. doi: 10.1002/rmv.2381. PubMed PMID: 35856385; PubMed Central PMCID: PMCPMC9349777.

194. National Institute for Communicable Disease. Tracking SARS-CoV-2 variants [May 28, 2024]. Available from: https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/sars-cov-2-genomic-surveillance-update/.

195. Subramoney K, Mtileni N, Bharuthram A, Davis A, Kalenga B, Rikhotso M, *et al.* Identification of SARS-CoV-2 Omicron variant using spike gene target failure and genotyping assays, Gauteng, South Africa, 2021. J Med Virol. 2022;94(8):3676-84. Epub

2022/04/21. doi: 10.1002/jmv.27797. PubMed PMID: 35441368; PubMed Central PMCID: PMCPMC9088381.

196. Hilti D, Wehrli F, Berchtold S, Bigler S, Bodmer T, Seth-Smith HMB, *et al.* S-Gene Target Failure as an Effective Tool for Tracking the Emergence of Dominant SARS-CoV-2 Variants in Switzerland and Liechtenstein, Including Alpha, Delta, and Omicron BA.1, BA.2, and BA.4/BA.5. Microorganisms. 2024;12(2). Epub 2024/02/24. doi: 10.3390/microorganisms12020321. PubMed PMID: 38399725; PubMed Central PMCID: PMCPMC10892681.

197. Chakraborty C, Bhattacharya M, Dhama K. Cases of BA.2.75 and recent BA.2.75.2 subvariant of Omicron are increasing in India: Is it alarming at the global level? Ann Med Surg (Lond). 2022;84:104963. Epub 2022/11/24. doi: 10.1016/j.amsu.2022.104963. PubMed PMID: 36415680; PubMed Central PMCID: PMCPMC9671875.

198. Hirotsu Y, Omata M. Detection of the Omicron BA.2.75 subvariant in Japan. J Infect. 2023;86(1):e5-e7. Epub 2022/09/11. doi: 10.1016/j.jinf.2022.08.038. PubMed PMID: 36087746; PubMed Central PMCID: PMCPMC9450472.

199. Callaway E. Will 'Centaurus' be the next global coronavirus variant? Indian cases offers clues. Nature. 2022;608(7923):462-3. Epub 2022/08/11. doi: 10.1038/d41586-022-02154-4. PubMed PMID: 35948681.

200. Markos M. BA.2.75: Boston Doctors on Whether We Should Worry About Competing Omicron Subvariant: NBC Boston; 2022 [May 16, 2024]. Available from: https://www.nbcboston.com/news/coronavirus/ba-2-75-boston-doctors-on-whether-we-should-worry-about-the-competing-omicron-subvariant/2772044/.

201. Chen J, Wang R, Hozumi Y, Liu G, Qiu Y, Wei X, *et al.* Emerging dominant SARS-CoV-2 variants. Preprint at arXiv. 2022.

202. Ao D, He X, Hong W, Wei X. The rapid rise of SARS-CoV-2 Omicron subvariants with immune evasion properties: XBB.1.5 and BQ.1.1 subvariants. MedComm (2020). 2023;4(2):e239. Epub 2023/03/21. doi: 10.1002/mco2.239. PubMed PMID: 36938325; PubMed Central PMCID: PMCPMC10015854.

203. The World Health Organization (WHO). Tracking SARS-CoV-2 variants [February 13, 2025]. Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants.

204. Outbreak.info. SARS-CoV-2 data explorer [May 16, 2024]. Available from: https://outbreak.info/.

205. The World Health Organization (WHO). TAG-VE statement on Omicron sublineages BQ.1 and XBB 2022 [July 4, 2024]. Available from:

https://www.who.int/news/item/27-10-2022-tag-ve-statement-on-omicron-sublineages-bq.1-and-xbb#:~:text=The%20two%20sublineages%20remain%20part,Member%20States%20and%20the%20public.&text=XBB*%20is%20a%20recombinant%20of%20BA.

206. Rhoden J, Hoffmann AT, Stein JF, da Silva MS, Gularte JS, Filippi M, *et al.* Diversity of Omicron sublineages and clinical characteristics in hospitalized patients in the southernmost state of Brazil. BMC Infect Dis. 2024;24(1):193. Epub 2024/02/14. doi: 10.1186/s12879-024-09089-3. PubMed PMID: 38350887; PubMed Central PMCID: PMCPMC10863127.

207. Sievers C, Zacher B, Ullrich A, Huska M, Fuchs S, Buda S, *et al.* SARS-CoV-2 Omicron variants BA.1 and BA.2 both show similarly reduced disease severity of COVID-19 compared to Delta, Germany, 2021 to 2022. Euro Surveill. 2022;27(22). Epub 2022/06/04. doi: 10.2807/1560-7917.ES.2022.27.22.2200396. PubMed PMID: 35656831; PubMed Central PMCID: PMCPMC9164675.

208. Mohapatra RK, Kandi V, Sarangi AK, Verma S, Tuli HS, Chakraborty S, *et al.* The recently emerged BA.4 and BA.5 lineages of Omicron and their global health concerns amid the ongoing wave of COVID-19 pandemic - Correspondence. Int J Surg. 2022;103:106698. Epub 2022/06/12. doi: 10.1016/j.ijsu.2022.106698. PubMed PMID: 35690362; PubMed Central PMCID: PMCPMC9176102.

209. Jhons Hopkins Medicine. COVID Variants: What You Should Know 2022 [May 16, 2024]. Available from: https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/a-new-strain-of-coronavirus-what-you-should-know.

210. Esper FP, Adhikari TM, Tu ZJ, Cheng YW, El-Haddad K, Farkas DH, *et al.* Alpha to Omicron: Disease Severity and Clinical Outcomes of Major SARS-CoV-2 Variants. J Infect Dis. 2023;227(3):344-52. Epub 2022/10/11. doi: 10.1093/infdis/jiac411. PubMed PMID: 36214810; PubMed Central PMCID: PMCPMC9619650.

211. Lewnard JA, Hong VX, Patel MM, Kahn R, Lipsitch M, Tartof SY. Clinical outcomes associated with SARS-CoV-2 Omicron (B.1.1.529) variant and BA.1/BA.1.1 or BA.2 subvariant infection in Southern California. Nat Med. 2022;28(9):1933-43. Epub 2022/06/09. doi: 10.1038/s41591-022-01887-z. PubMed PMID: 35675841; PubMed Central PMCID: PMCPMC10208005.

212. Menni C, Valdes AM, Polidori L, Antonelli M, Penamakuri S, Nogal A, *et al.* Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: a prospective observational study from the ZOE COVID Study. Lancet. 2022;399(10335):1618-24. Epub

2022/04/11. doi: 10.1016/S0140-6736(22)00327-0. PubMed PMID: 35397851; PubMed Central PMCID: PMCPMC8989396.

213. Azhar EI, Hui DSC, Memish ZA, Drosten C, Zumla A. The Middle East Respiratory Syndrome (MERS). Infect Dis Clin North Am. 2019;33(4):891-905. Epub 2019/11/02. doi: 10.1016/j.idc.2019.08.001. PubMed PMID: 31668197; PubMed Central PMCID: PMCPMC7127753.

214. Jassat W, Abdool Karim SS, Mudara C, Welch R, Ozougwu L, Groome MJ, *et al.* Clinical severity of COVID-19 in patients admitted to hospital during the omicron wave in South Africa: a retrospective observational study. Lancet Glob Health. 2022;10(7):e961-e9. Epub 2022/05/22. doi: 10.1016/S2214-109X(22)00114-0. PubMed PMID: 35597249; PubMed Central PMCID: PMCPMC9116895.

215. Wrenn JO, Pakala SB, Vestal G, Shilts MH, Brown HM, Bowen SM, *et al.* COVID-19 severity from Omicron and Delta SARS-CoV-2 variants. Influenza Other Respir Viruses. 2022;16(5):832-6. Epub 2022/04/14. doi: 10.1111/irv.12982. PubMed PMID: 35415869; PubMed Central PMCID: PMCPMC9111734.

216. Ulloa AC, Buchan SA, Daneman N, Brown KA. Estimates of SARS-CoV-2 Omicron Variant Severity in Ontario, Canada. JAMA. 2022;327(13):1286-8. Epub 2022/02/18. doi: 10.1001/jama.2022.2274. PubMed PMID: 35175280; PubMed Central PMCID: PMCPMC8855311.

217. Chen J, Wang R, Gilby NB, Wei GW. Omicron Variant (B.1.1.529): Infectivity, Vaccine Breakthrough, and Antibody Resistance. J Chem Inf Model. 2022;62(2):412-22. Epub 2022/01/07. doi: 10.1021/acs.jcim.1c01451. PubMed PMID: 34989238; PubMed Central PMCID: PMCPMC8751645.

218. Ito K, Piantham C, Nishiura H. Relative instantaneous reproduction number of Omicron SARS-CoV-2 variant with respect to the Delta variant in Denmark. J Med Virol. 2022;94(5):2265-8. Epub 2021/12/31. doi: 10.1002/jmv.27560. PubMed PMID: 34967453; PubMed Central PMCID: PMCPMC9015237.

219. Relan P, Motaze NV, Kothari K, Askie L, Le Polain O, Van Kerkhove MD, *et al.* Severity and outcomes of Omicron variant of SARS-CoV-2 compared to Delta variant and severity of Omicron sublineages: a systematic review and metanalysis. BMJ Glob Health. 2023;8(7). Epub 2023/07/08. doi: 10.1136/bmjgh-2023-012328. PubMed PMID: 37419502; PubMed Central PMCID: PMCPMC10347449.

220. The World Health Organization (WHO). Statement on the update of WHO's working definitions and tracking system for SARS-CoV-2 variants of concern and variants of interest 2023 [June 9, 2024]. Available from: https://www.who.int/news/item/16-03-2023-

statement-on-the-update-of-who-s-working-definitions-and-tracking-system-for-sars-cov-2-variants-of-concern-and-variants-of-interest.

221. IDSA. COVID-19 Variant Update [May 16, 2024]. Available from: https://www.idsociety.org/covid-19-real-time-learning-network/diagnostics/covid-19-variant-update/.

222. The World Health Organization (WHO). Statement on the update of WHO's working definitions and tracking system for SARS-CoV-2 variants of concern and variants of interest 2023 [June 6, 2024]. Available from: https://www.who.int/news/item/16-03-2023-statement-on-the-update-of-who-s-working-definitions-and-tracking-system-for-sars-cov-2-variants-of-concern-and-variants-of-interest.

223. The World Health Organization (WHO). Previously circulating VOIs [June 6, 2024]. Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants/previously-circulating-vois.

224. The World Health Organization (WHO). Weekly epidemiological update on COVID-19 - 23 March 2021 2021 [June 7, 2024]. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---23-march-2021.

225. Deng X, Garcia-Knight MA, Khalid MM, Servellita V, Wang C, Morris MK, *et al.* Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant. Cell. 2021;184(13):3426-37 e8. Epub 2021/05/16. doi: 10.1016/j.cell.2021.04.025. PubMed PMID: 33991487; PubMed Central PMCID: PMCPMC8057738.

226. Janik E, Niemcewicz M, Podogrocki M, Majsterek I, Bijak M. The Emerging Concern and Interest SARS-CoV-2 Variants. Pathogens. 2021;10(6). Epub 2021/06/03. doi: 10.3390/pathogens10060633. PubMed PMID: 34064143; PubMed Central PMCID: PMCPMC8224338.

227. Mohammadi M, Shayestehpour M, Mirzaei H. The impact of spike mutated variants of SARS-CoV2 [Alpha, Beta, Gamma, Delta, and Lambda] on the efficacy of subunit recombinant vaccines. Braz J Infect Dis. 2021;25(4):101606. Epub 2021/08/25. doi: 10.1016/j.bjid.2021.101606. PubMed PMID: 34428473; PubMed Central PMCID: PMCPMC8367756.

228. Chakraborty C, Bhattacharya M, Sharma AR. Present variants of concern and variants of interest of severe acute respiratory syndrome coronavirus 2: Their significant mutations in S-glycoprotein, infectivity, re-infectivity, immune escape and vaccines activity. Medical Virology. 2021;32(2). doi: https://doi.org/10.1002/rmv.2270.

229. Chen C, Shi Q, Dong X-P. SARS-CoV-2 Lambda Variant: Spatiotemporal

Distribution and Potential Public Health Impact. Zoonoses. 2021;1:1-7. doi: 10.15212/ZOONOSES-2021-0009.

230. Romero PE, Davila-Barclay A, Salvatierra G, Gonzalez L, Cuicapuza D, Solis L, *et al.* The Emergence of Sars-CoV-2 Variant Lambda (C.37) in South America. Microbiol Spectr. 2021;9(2):e0078921. Epub 2021/10/28. doi: 10.1128/Spectrum.00789-21. PubMed PMID: 34704780; PubMed Central PMCID: PMCPMC8549758.

231. Baj A, Novazzi F, Ferrante FD, Genoni A, Cassani G, Prestia M, *et al.* Introduction of SARS-COV-2 C.37 (WHO VOI lambda) from Peru to Italy. J Med Virol. 2021;93(12):6460-1. Epub 2021/07/28. doi: 10.1002/jmv.27235. PubMed PMID: 34314049; PubMed Central PMCID: PMCPMC8427114.

232. Pascarella S, Bianchi M, Giovanetti M, Narzi D, Cauda R, Cassone A, *et al.* The SARS-CoV-2 Mu variant should not be left aside: It warrants attention for its immuno-escaping ability. J Med Virol. 2022;94(6):2479-86. Epub 2022/02/18. doi: 10.1002/jmv.27663. PubMed PMID: 35174519; PubMed Central PMCID: PMCPMC9088528.

233. Uriu K, Kimura I, Shirakawa K, Takaori-Kondo A, Nakada TA, Kaneda A, *et al.* Neutralization of the SARS-CoV-2 Mu Variant by Convalescent and Vaccine Serum. N Engl J Med. 2021;385(25):2397-9. Epub 2021/11/04. doi: 10.1056/NEJMc2114706. PubMed PMID: 34731554; PubMed Central PMCID: PMCPMC8609602.

234. Laiton-Donato K, Franco-Munoz C, Alvarez-Diaz DA, Ruiz-Moreno HA, Usme-Ciro JA, Prada DA, *et al.* Characterization of the emerging B.1.621 variant of interest of SARS-CoV-2. Infect Genet Evol. 2021;95:105038. Epub 2021/08/18. doi: 10.1016/j.meegid.2021.105038. PubMed PMID: 34403832; PubMed Central PMCID: PMCPMC8364171.

235. Messali S, Bertelli A, Campisi G, Zani A, Ciccozzi M, Caruso A, *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;93(12):6468-70. Epub 2021/07/31. doi: 10.1002/jmv.27247. PubMed PMID: 34329486; PubMed Central PMCID: PMCPMC8426698.

Chapter 2: The role of Lebanon in the COVID-19 butterfly effect: the B.1.398 example.

Introduction

In light of the wide spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coupled with continuous mutations, genomic tracking of its different lineages is crucial to understanding the dynamic of the viral evolution, measuring the rate of genomic diversity, and detecting the transmission pathways of the virus. These are essential steps to implement more efficient containment and preventive measures of coronavirus disease 2019 (COVID-19), based on reliable scientific data. Whole genome sequencing (WGS) is the gold standard tool for detecting SARS-CoV-2's mutations and emerging lineages [1], since it provides a detailed genomic map of the sequence in question, presenting the rising mutations marking the phylogenic evolution. COVID-19 has propagated touching the larger part of the globe, and Lebanon wasn't an exception, registering its first case on February 21, 2020 [19]. Between January 2020 and February 2022, the country counted more than one million COVID-19 cases and 10,079 deaths [10,11], when only 1199 Lebanese sequences were available in the GISAID database, the world's largest repository for SARS-CoV-2 genome as assessed on February 4th, 2022 [12].

In this context, this chapter aims to get a clearer view of the circulating SARS-CoV-2 lineages in Lebanon, between July 2020 and February 2021. For this, the following article investigated SARS-CoV-2 lineages of 200 nasopharyngeal samples collected from different regions in Lebanon. We then studied the potential role of Lebanon as a dispersal center of the B.1.398 lineage.

References

1. Mintzer V, Moran-Gilad J, Simon-Tuval T. Operational models and criteria for incorporating microbial whole genome sequencing in hospital microbiology - A systematic literature review. Clin Microbiol Infect. 2019;25(9):1086-95. Epub 2019/05/01. doi: 10.1016/j.cmi.2019.04.019. PubMed PMID: 31039443.

2. The World Health Organization (WHO). WHO COVID-19 dashboard-Lebanon [June 25, 2024]. Available from: https://data.who.int/dashboards/covid19/deaths?m49=422&n=o.

Article

The role of Lebanon in COVID-19 butterfly effect: The B.1.398 example

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This paper is published in **"*viruses*"** journal on July 27th, 2022

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MDPI

Case Report

The Role of Lebanon in the COVID-19 Butterfly Effect: The B.1.398 Example

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Abstract: In the present study, we provide a retrospective genomic surveillance of the SARS-CoV-2 pandemic in Lebanon; we newly sequence the viral genomes of 200 nasopharyngeal samples collected between July 2020 and February 2021 from patients in different regions of Lebanon and from travelers crossing the Lebanese–Syrian border, and we also analyze the Lebanese genomic dataset available at GISAID. Our results show that SARS-CoV-2 infections in Lebanon during this period were shaped by the turnovers of four dominant SARS-CoV-2 lineages, with B.1.398 being the first to thoroughly dominate. Lebanon acted as a dispersal center of B.1.398 to other countries, with intercontinental transmissions being more common than within-continent. Within the country, the district of Tripoli, which was the source of 43% of the total B.1.398 sequences in our study, was identified as being an important source of dispersal in the country. In conclusion, our findings exemplify the butterfly effect, by which a lineage that emerges in a small area can be spread around the world, and highlight the potential role of developing countries in the emergence of new variants.

Keywords: SARS-CoV-2; lineages; B.1.398; Lebanon; dispersal

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Citation: Nour, D.; Rafei, R.; Lamarca, A.P.; de Almeida, L.G.P.; Osman, M.; Ismail, M.B.; Mallat, H.; Berry, A.; Burfin, G.; Semanas, Q.; et al. The Role of Lebanon in the COVID-19 Butterfly Effect: The B.1.398 Example. *Viruses* **2022**, *14*, 1640. https://doi.org/10.3390/v14081640

Academic Editors: Luis Martinez-Sobrido and Fernando Almazan Toral

Received: 7 July 2022
Accepted: 25 July 2022
Published: 27 July 2022

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1. Introduction

Since its first diagnosis in December 2019, COVID-19 has been an insurmountable worldwide pandemic, with the number of cases and deaths surpassing 485 million and 6.13 million, respectively, as of 30 March 2022 [1]. The fierce grasp of SARS-CoV-2, the virus that causes COVID-19, is sustained by its ongoing diversification into an enormous number of lineages (1700 recognized by PANGO as of 30 March 2022) [2] bristling with a myriad of new mutations. Some lineages, dubbed the variants of concern (VoCs) Alpha, Beta, Gamma, Delta, and Omicron by the WHO, have gained particular attention due to mutations causing elevated transmissibility, increased disease severity, and impacts on diagnostics and vaccine performance [3]. The resulting increase in the fitness of these VoCs has prompted consecutive lineage turnovers across the world [4,5] and triggered new surges in COVID-19 cases and deaths.

Most of the genomic surveillance of SARS-CoV-2 is conducted in a small number of countries located in North America and Europe. Developing countries originated only ~9.7% of the sequenced genomes available in GISAID (https://www.gisaid.org/ assessed on 9 May 2022) [6,7] despite being home to almost 49% of SARS-CoV-2 cases, as reported by the database on May 9th, 2022 [8]. This may be related to differences across the world in scientific funding, sequencing capacity, and the number of trained researchers [9,10]. The circulation of SARS-CoV-2 under the radar of researchers and public health authorities allows the unnoticed accumulation of worrisome mutations in lineages, resulting in the emergence of VoCs. It is not surprising, then, that most VoCs were first identified in developing countries.

The rapid release and analysis of whole viral genome sequences help researchers to understand the routes of SARS-CoV-2 transmission and paves the way for the implementation of appropriate measures for outbreak reduction and containment. In Lebanon, real-time genome sequencing is not implemented in the SARS-CoV-2 surveillance strategy due to many challenges, including the paucity of sequencing facilities and skilled human staff, the Lebanese economic collapse that exacerbates high sequencing costs, the lack of funding, and reagent delivery delays [11]. Although there were 1,066,840 cases and 10,079 deaths in Lebanon between January 2020 and February 2022 [12,13], only 1199 Lebanese sequences were available in GISAID, which is the world's largest repository for SARS-CoV-2 genomes as assessed on 4 February 2022 [14].

In our study, we aimed to describe the epidemiological dynamics of SARS-CoV-2 during the first two years of the pandemic in Lebanon. During this period, the average number of recorded Lebanese cases of COVID-19 varied between 5 and 33,605 cases per week [12,13]. We newly sequenced SARS-CoV-2 genomes from 200 nasopharyngeal samples collected between July 2020 and February 2021 from different regions in Lebanon as well as from travelers crossing the Lebanese–Syrian border. Among the lineages identified herein, we selected the first one to thoroughly dominate in Lebanon (B.1.398) to investigate how SARS-CoV-2 transmissions occurred both within Lebanon and between Lebanon and other countries. Finally, we discussed the potential emergence of new variants from developing countries.

2. Materials and Methods

2.1. Sampling

The study was approved by the ethical committee of the Lebanese University, under the number #CUER 23-2020, on 14 May 2020. The LMSE laboratory (Laboratoire Microbiologie Santé et Environnement) is accredited by the Ministry of Public Health in Lebanon to carry out the diagnosis of SARS-CoV-2 infections in North Lebanon. The diagnoses were carried out by RT-PCR on nasopharyngeal samples using the COVID-19 Genesig Real-time PCR assay (PrimerDesign, Eastleigh SO53 4DG, UK) targeting the RdRp gene; the GeneFinder COVID-19 Plus RealAmp kit (OSANG Healthcare, Dongan-gu, Anyang-si, Gyeonggi-do, Korea) targeting the N, E, and RdRp genes; and the Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR kit (Shanghai ZJ Bio-Tech Co.,Shanghai, China), which targets the N, E, and ORF1ab genes.

Our samples were collected between July 2020 and February 2021 from several sources: COVID-19 screening campaigns among Lebanese citizens launched by several municipalities in collaboration with the Lebanese Ministry of Public Health, several governmental hospitals in North Lebanon, as well as travelers crossing the Lebanese–Syrian border. All samples positive for SARS-CoV-2 were stored at −80 °C in the LMSE laboratory. The approach adopted for selecting samples for genomic sequencing was, briefly, as follows: all samples with a cycle threshold value (Ct) greater than 25 were excluded to avoid sequencing failure. Then, 200 samples (with Ct≤25) were chosen randomly, respecting the relative proportions of patients of each nationality (the patients were of three nationalities: Lebanese, Syrian, and Palestinian), as well as taking into account the differences between the number of samples in each of the target months. After setting the mentioned parameters and grouping the data in Excel files, we randomized our 200 nasopharyngeal samples taken from patients positive for SARS-CoV-2.

2.2. Collecting Information and Handling Samples

Phone calls were made to patients, and after they gave their consent to participate in the study, we filled out a questionnaire gathering socio-demographic information, health data, and data related to SARS-CoV-2 infection (i.e., the source of infection, travel during or before the period of infection, symptoms experienced, whether there was hospitalization or not, and whether or not medication was taken). After obtaining the requested number of participants (200), the corresponding samples underwent a new coding process. Then, total RNAs were extracted at LMSE with the AccuPrep Viral RNA Extraction Kit (BIONEER, Daejeon, Korea) and the QIAamp Viral RNA Kit (QIAGEN, Germantown, TN, USA), according to the recommendations of the suppliers. Finally, the RNA extracts were sent to the Laboratory of Virology, Institute of Infectious Agents, which is associated with the National Reference Center of Respiratory Infection Viruses, Hospice civil de Lyon in Lyon, France, for genomic sequencing by Illumina COVIDSeq (Illumina, San Diego, CA, USA). The amplification of extracted RNA was conducted with an automatized (SPTLabtech) COVIDSeq-Test™ protocol (Illumina) with an ARTIC nCoV-2019 Amplicon Panel v4 of primers. Libraries were sequenced with 100 base pair paired-end reads using the NovaSeq 6000 sequencing system with the SP Reagent Kit v1.0 200 cycles (Illumina) [15]. Reads were processed using the in-house bioinformatic pipeline seqmet, available at https://github.com/genepii/seqmet (accessed on 28 November 2021).

The generation of the consensus sequence and the identification of the SNPs of each genome were performed by aligning the reads with the reference genome (NC_045512.2) using bwa v0.7.17 [16] and post-processing with samtools v1.11, bcftools v1.10.2 [17], and bedtools v2.27.1 [18]. Lineages were identified with the Pangolin program v.3.1.20 [19]. The consensus genomes were added to the GISAID database, and accession IDs are available at https://doi.org/10.55876/gis8.220630un (accessed on 30 June 2022).

2.3. Evolutionary and Phylogeographic Analyses

In addition to the genomes sequenced in this study, we obtained the sequences of SARS-CoV-2 genomes collected in Lebanon that were available in the GISAID database as of 7 February 2022, listed at https://doi.org/10.55876/gis8.220630un (accessed on 30 June 2022). This dataset was filtered using the options "Complete", "Low coverage exclude", and "Collection date complete" available in GISAID. The final dataset containing the newly sequenced genomes and the GISAID sequences contained 1042 genomes. These sequences were aligned to the WH01 (EPI ISL 406798) genome from Wuhan, China using MAFFT [20]. The 3′ and 5′ ends were trimmed with the seqkit package [21] using the WH01 sequence as a reference. The maximum likelihood tree was then inferred using IQ-TREE [22], using the GTR+F+I+G4 model selected by the built-in algorithm ModelFinder [23] and 1000 ultrafast bootstrap replicates [24].

To evaluate how SARS-CoV-2 has spread across Lebanon, we used the dispersal of the B.1.398 lineage as a case study. To confirm the number of introductions of the lineage in

Lebanon, we obtained all sequences of B.1.398 available in GISAID on February 7th of 2022, listed at https://doi.org/10.55876/gis8.220630un (accessed on 30 June 2022), using the same filters described previously. Sequences obtained from samples collected before June of 2020 were removed because they had higher diversity than expected based on their sampling dates (Figure A1). Among the excluded sequences were genomes sampled in January 2020, as described in the GISAID database. We believe this date to be an error made during the submission of sequences, as the first confirmed COVID-19 cases in Lebanon occurred in February 2020. We aligned the new B.1.398 genomes and the sequences from GISAID to WH01 with MAFFT, removed the 3′ and 5′ ends with seqkit, and generated a maximum likelihood tree with IQ-TREE. The GTR+F+I substitution model was selected by ModelFinder, and branch support was calculated using 1000 ultrafast bootstrap replicates. The maximum likelihood tree of B.1.398 was used as input to TreeTime [25] to scale the tree branches to divergence dates, assuming the phylogenetic correlation of clock rates ("–covariation" option) and employing a coalescent prior with the skyline model ("–coalescent skyline" option). The ancestral locations of the samples were then inferred using the resulting tree with TreeTime, with the "mugration" model and the countries of origin as the samples' locations.

The dispersal of B.1.398 within Lebanon was reconstructed using BEAST [26]. Because the sequences from Lebanon available in GISAID did not have information about the district or province of sampling, in this analysis, we used only the sequences generated in this study. We employed the Relaxed Random Walk model with Cauchy's distribution on coordinates randomly selected within each sample's collection district; we employed the GTR substitution model with estimated base frequencies and the "Gamma + Invariant Sites" heterogeneity model with four Gamma categories. We also used a coalescent tree prior with the GMRF Bayesian Skyride model and a uniform prior for the clock rate. The MCMC ran through a chain of 100,000,000, with sampling every 10,000th and burn-in of 10% of the trees. Dispersal routes were extracted from the consensus tree using the seraphim package [27] and plotted using the ggplot2 package, both in R software. The base map used in the figures was obtained from https://gadm.org/index.html (accessed on 15 February 2022).

3. Results

In the present study, SARS-CoV-2 genomes were sequenced from 200 RNA samples from six of the eight Lebanese provinces (governorates), with the majority of samples being from North Lebanon (70%), followed by Akkar (16%) and Mount Lebanon (11%). The remaining samples were from Bekaa, Nabatieh, and South Lebanon. Among Lebanese districts, the district of Tripoli accounted for the most significant number of samples (36%). To summarize the data, the 200 patients participating in this study were of three nationalities: Lebanese (75.5%), Syrian (17.5%), and Palestinian (7.0%); 54% were male, and 46% were female. Regarding travel status, 10.5% of the patients traveled within 14 days of their infection with SARS-CoV-2: 19 patients to Syria, 1 patient to Italy and Switzerland, and 1 to Turkey. The mean age of the patients was 38.7 years. Regarding infection status, most of the patients (94%) developed symptoms with a mean duration of 10.2 days, and 6% of the patients went to the hospital after infection.

After sequencing, the SARS-CoV-2 genome assembly failed for seven samples, resulting in 193 sequences. We were able to attribute PANGO lineages to all but three of these sequences. In total, 21 PANGO lineages were identified, with B.1.398 predominant (52.5%) followed by the B.1.1.7 lineage (18.5%). With regard to nationalities, the lineages detected in non-Lebanese residents of Lebanon, including Syrians and Palestinians, were generally found in Lebanese citizens as well. Among the travelers to Syria, the lineages were mainly B.1 (36.8%), B.1.1 (21%), and B.1.36 (15.7%). The remaining lineages B.1.1.274, B.1.177, B.1.22, B.1.36.1, and B.1.398 each occurred in 5.3% of Syria travelers. Three of these lineages (B.1.1.274, B.1.177, and B.1.22) were not detected in the Lebanese population. B.1.1.44 was

identified in a traveler to Turkey and was not detected in the Lebanese sequences, while B.1.398 was recovered from a traveler from Europe.

Analyzing the whole Lebanese dataset in GISAID, including the new sequences, 40 different lineages were identified within Lebanon for the period ending December 2021. Despite the relatively high diversity in the country during this period, the pandemic in Lebanon can be defined by the dominance of four lineages (Figure 1A). From February 2020 to May 2020, the country repeated worldwide patterns and was dominated by the B.1 lineage. The lineage B.1.398 started to dominate in June of 2020, coinciding with a sharp increase in the number of COVID-19 cases in Lebanon (Figure 1B). The variant of concern Alpha (B.1.1.7 and the sublineage Q.1 in Lebanon) replaced B.1.398 as the predominant lineage in January of 2021, accompanied by a new surge in cases that was more intense than the previous one. Alpha dominated at least until May 2021, the last month of continuous genomic sequencing in Lebanon. The genomes available in GISAID show that by July of 2021, the variant of concern Delta (B.1.617 and the sublineages AY.10, AY.106, AY.120, AY.122, AY.127, AY.16, AY.33, and AY.39 in the country) had already completely substituted Alpha and was, in turn, completely replaced by Omicron (sublineages BA.1 and BA.1.1) by December 2021. Coincidently, the appearances of both lineages (Delta and Omicron) were followed by new increases in the number of cases in Lebanon.

To obtain a closer look at the B.1.398 lineage, we performed a double analysis to investigate how and where it was introduced at both the international and national scales. We determined that the lineage originated from B.1 in mid- to late-April 2020, either within Lebanon or in an unsampled country with immediate dispersal to Lebanon. The introduction to Lebanon was followed by a considerable diversification of the lineage, for which many subclades could be delimited. The extent of this heterogeneity was not observed for the Alpha variant, where few subclades could be distinguished, and the majority of sequences were present on a single subclade (Figure A2).

By plotting the connections between "origin" and "destination" countries, we see that Lebanon acted as a dispersal center to other countries, with intercontinental transmission being more common than within-continent transmission during the entire period in which the lineage circulated (Figure 2A and Figure A3). The country that received the highest number of importing events of the B.1.398 lineage from Lebanon between June 2020 and January 2021 was Denmark (n = 19) (Figure 2B). A high number of transmissions from Lebanon was also identified for the United Kingdom (n = 7) between October and December of 2020 and for Saudi Arabia (n = 2) in May of 2020. An increase in transmissions of B.1.398 from Lebanon to other countries can be observed beginning in July of 2020, concomitant to the reopening of international flights in the country. Lineage reintroductions into Lebanon from Denmark and Saudi Arabia occurred in January of 2021. These reintroductions did not cause any transmission within Lebanon, as far as the data available show.

Within the country, we identified that the district of Tripoli, which was the source of 43% of the total B.1.398 sequences, acted as an important source of dispersal in the country, especially as the origin of long-distance transmissions (Figure 3). The first long-distance dispersals happened between Tripoli and the district of Akkar, while in a later stage of the pandemic, dispersals were occurring between Tripoli and the southern and northern districts in similar proportions.

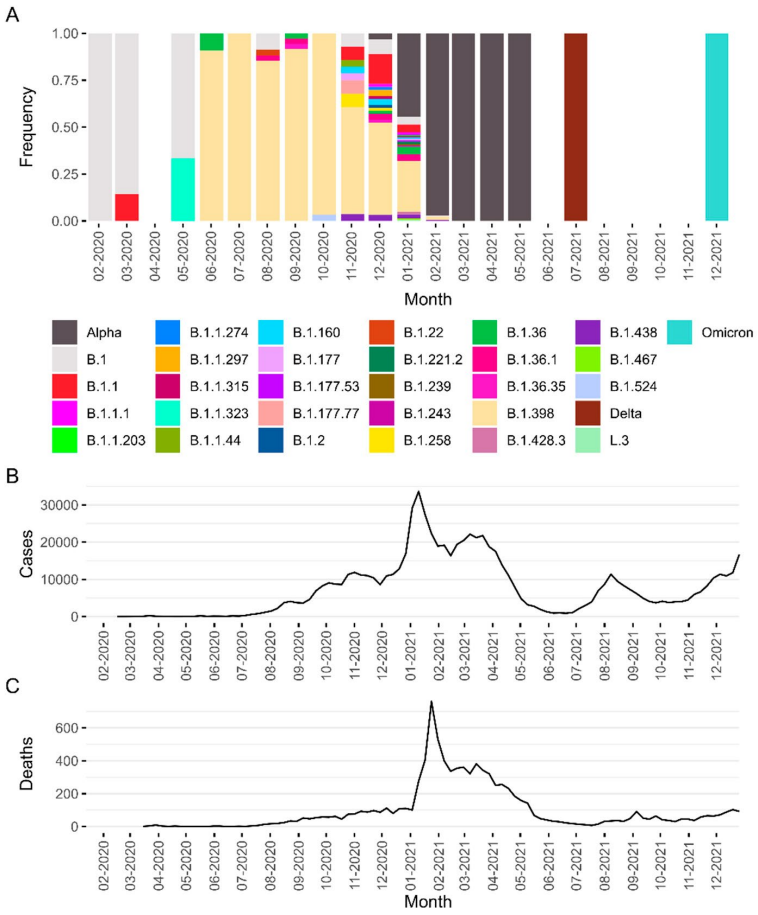

Figure 1. Epidemics of COVID-19 in the first two years of the pandemic in Lebanon. In (**A**), we see the relative frequency of SARS-CoV-2 lineages sampled in Lebanon between February 2020 and December 2021. Lineages belonging to the variants of concern Alpha, Delta, and Omicron are grouped under these labels. The numbers of COVID-19 cases and deaths are described in (**B**,**C**).

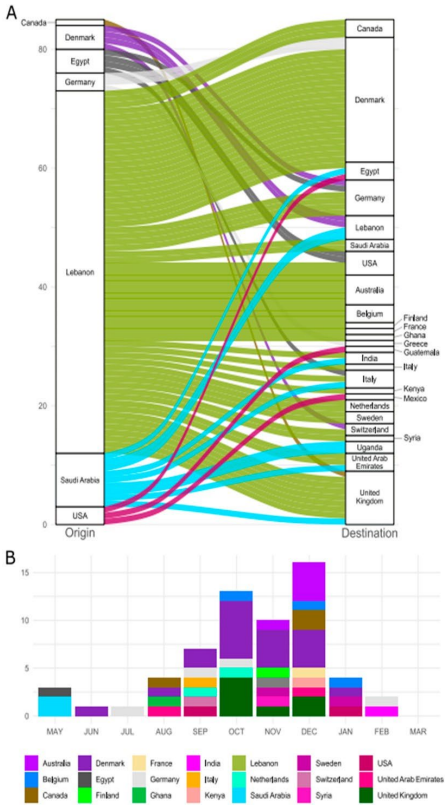

Figure 2. International dispersal of the B.1.398 lineage. In (**A**), all the international transmission routes are represented, with the left bar being the countries of origin of dispersal, the right bar their destinations, and the color of dispersal representing the originating country. In (**B**), the temporal distribution of countries receiving B.1.398 that originated in Lebanon is displayed. The legend for both graphs is located at the bottom.

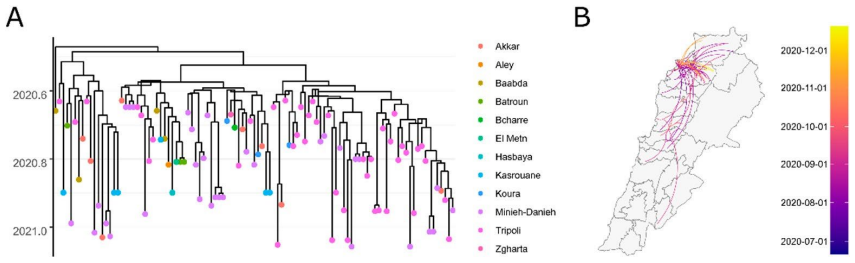

Figure 3. Phylodynamics of the B.1.398 lineage in Lebanon. In (**A**), we see the time tree of B.1.398, indicating divergence times between genomic sequences, with colors representing the districts in Lebanon. The Tripoli district acted as a dispersal hub in the country, as displayed in (**B**). Colors in (**B**) indicate the time at which dispersal started.

4. Discussion

In the present study, we provided a retrospective genomic surveillance of the main SARS-CoV-2 lineages circulating between July 2020 and February 2021 in Lebanon by sequencing a set of 200 samples and analyzing the Lebanese genomic dataset available

at GISAID as of 7 February 2022. We then focused our analysis on the B.1.398 lineage by reconstructing the transmission routes on both Lebanese and worldwide scales. The new genomes sequenced in this work more than doubled the number of sequences available for the first year of the pandemic in Lebanon. In particular, it greatly improved the sampling of the B.1.398 lineage in this period (Figure A2), which is critical for understanding how SARS-CoV-2 first spread across Lebanon and caused the first surge of COVID-19 cases in the country.

In comparison with previous studies [28–30], seven variants not available among GISAID's Lebanese sequences (B.1.1.203, B.1.1.274, B.1.1.44, B.1.177.77, B.1.22, B.1.428.3, and B.1.524), were detected in our study. Among them, B.1.1.44 was detected in a pa- tient returning from travel to Turkey, and B.1.1.274 and B.1.22 were detected in patients returning from Syria within 14 days of infection. On the other hand, unlike the study by Merhi et al. [30], our results showed that the Alpha variant was detected in Akkar province, with a percentage of 6.8% of our sequences.

Moreover, our findings revealed that the turnover of many SARS-CoV-2 lineages shaped the epidemic in Lebanon. The first officially confirmed COVID-19 case was signaled on 21 February 2020. Although the number of sequences is limited, there was apparently transmission of the B.1 lineage from February to May 2020, triggered by its first introduction from a patient returning from the city of Qom, Iran [29]. The B.1 lineage was an important European lineage originating from the Northern Italian outbreak that occurred early in 2020 [31]. During this period, the number of weekly confirmed Lebanese cases was low, reaching a maximum of 195 cases; this was associated with a strict nationwide lockdown [12,13]. In June 2020, although the international airport was closed, a new dominant lineage, namely B.1.398, was detected. This lineage was characterized by the mutation V1291I in the ORF1a gene, P314L in ORF1b, D614G and T95I in the spike gene, S194L in the nucleocapsid gene, and S84L in ORF8. The D614G mutation in the spike protein gene has been suggested as being responsible for an evolutionary advantage in comparison to the lineages that first circulated in Wuhan [32–34]. The presence of this lineage might be explained either by unnoticeable circulation during the first few months due to the low number of sequences available from the initial period, which is a factor we discussed herein, or by recent introduction (or emergence) from an unknown source. Unfortunately, there are no sequences from April in the Lebanese GISAID dataset to support this hypothesis. Although our samples are not equally distributed across the different Lebanese districts, and the district of Tripoli represented the biggest number of samples (66 without travelers), the dispersal analysis of the B.1.398 lineage uncovers the magnitude of transmissions occurring between the districts regardless of their geographical closeness. Community spread appeared to be promoted by many seeding events in which Tripoli may have played a central role. Indeed, Tripoli is the second-largest city in Lebanon, located 82 km from the capital Beirut, and it is the largest city in North Lebanon [35]. Additionally, in June, Lebanon witnessed many anti-government protests and the easing of the COVID-19 lockdown [36], which may explain the lineage's spread. Still, the number of cases remained low, with a maximum of 183 weekly confirmed cases [12,13]. We previously suggested that the emergence of the lineage might have been related to the airport opening in July 2020 (1148 weekly confirmed cases at maximum) and/or the massive Beirut blast on 4 August 2020 (4084 weekly confirmed cases at maximum during this month) [12,13]. However, the airport opening in July did not seem to introduce this lineage into Lebanon but appears to have played an important role in disseminating it to the world (Figure A3). Despite the absence of new sequences from Beirut, the massive blast that hit the city, with 6500 people injured, 220 deaths, and 300,000 displaced, had rippling consequences on the status of COVID-19 in Lebanon [37]. Indeed, the blast fragmented the healthcare sector, leading to severe damage to Beirut's major hospitals and overwhelming the remaining functional hospitals with blast casualties. Many injured individuals were transported to nearby regions, and many Lebanese across all provinces rushed to volunteer to clear the rub- ble, distribute food, etc. [38]. This intermingling served as a breeding ground for SARS-CoV-2 to disseminate, culminating in a new COVID-19 wave in which the chief lineage was presumably B.1.398, as emphasized by the BEAST time tree and dispersal route analysis. The preponderance

of this lineage over others remained discernible until December despite the imposition of a lockdown until November 2020 [39]. During its prevalence period (June to December), the number of weekly cases varied between 94 and 16,936, and weekly deaths varied between 0 and 109 [12,13]. Commensurate with the dynamic shifts in lineages observed worldwide [40], "Alpha variant was reported for the first time in Lebanon at the end of December 2020, and swept other lineages in the next following months. This quantum leap for the Alpha variant was potentially energized by the holiday months, specifically, December and January, which aligned with the removal of lockdown restrictions; January was the month with the highest daily morbidities and mortalities since the beginning of the pandemic [1,12,13]. Therefore, a full lockdown was set for 15 January 2021. Despite the reintroduction of the B.1.398 lineage in January from outside the country, its transmission faded and could not be sustained, suggesting thus a lower adaptive value than that of the Alpha variant. Such a disappearance could also be tied to the introduction of the vaccine to Lebanon on 14 February 2021 [41]; the B.1.398 lineage had potentially lower immune evasion than the Alpha variant and there was probably higher vaccination efficacy against it. If we compare cases and deaths in the period in which B.1.398 was prevalent (i.e., between June and December 2020: 189,865 cases, 1378 deaths) and the period in which the Alpha variant was prevalent (between January and May 2021: 355,015 cases, 6282 deaths), we observe the higher transmissibility and mortality of the Alpha variant [12,13].

To our knowledge, there are no sufficient data available in the literature regarding the B.1.398 lineage. According to the PANGO lineages website, this lineage appeared approximately in January 2020 and was denoted as a European lineage [2]. Before our study, there were 377 sequences of this lineage in the GISAID dataset, derived from 24 countries; almost all sequences were from Denmark 138 (37%), Lebanon 116 (31%), the United Kingdom 24 (6%), Germany 19 (5%), and Saudi Arabia 16 (4%) [14]. In our study, we added to GISAID 105 new B.1.398 sequences from Lebanon, of which one sequence is from a traveler crossing the Lebanese–Syrian border. Although this lineage did not encounter eminent success compared to the variant of concerns, we also demonstrated herein the ability of Lebanon to spread and seed B.1.398 to different continents worldwide. While Lebanon is a small country, with a population estimated at more than six million, it has a prodigious number of expatriates (between 4 and 13 million) scattered around the world; this fact explains the extent of connections observed here during holidays [42]. The geographic location of Lebanon also renders it a vivid hub for travel to and from all over the world. In addition to the Lebanese population, Lebanon provides shelter for many refugees, including Syrians and Palestinians, making Lebanon the tenth most populated country with respect to refugees; it also has the highest per-capita concentration of refugees in the world [43,44], allowing Lebanon to be an area of swift COVID-19 transmission and the emergence of potential new SARS-CoV-2 variants. This phenomenon may embody the butterfly effect, where a lineage that emerged in a small area can propagate around the world.

Moreover, Lebanon has witnessed many scourges that converged with and exacerbated the COVID-19 pandemic. Most important was the catastrophic economic and financial crisis that ranked among the world's three worst crises since the mid-1800s; it caused more than 80% of the Lebanese population to sink into poverty and created shortages of essential medicines, fuel, and electricity. Therefore, the government was not able to apply anti-COVID-19 measures with a tight rein, and for some Lebanese, COVID-19 was the least of their concerns [45,46].

It is worth mentioning that our conclusions may be limited by the absence of historical data both in Lebanon and in neighboring countries. In Lebanon, there is a substantial lack of sequences on both the temporal and spatial scales as many months and districts have no (or a limited number of) sequences. In Syria, there is a lack of genomic surveillance, and only 89 Syrian sequences obtained in Turkey from a short period (December 2021 and January/February 2022) are available in the GISAID database (6 April 2022); these samples correspond to 10 lineages, with BA.1.1 and AY.122 being the most prevalent. We demonstrated here a diverse pool of lineages harbored by the travelers crossing the

Lebanese–Syrian border, of which three lineages, B.1.1.274, B.1.177, and B.1.22, were not detected in the Lebanese population, hinting at the possible existence of a specific Syrian pool. With regard to the identified lineage B.1.398, our analysis showed that Lebanon spread this lineage to Syria. However, these conclusions could be altered if more samples from Syria were analyzed.

Furthermore, the current analysis cannot determine with certainty whether dispersal from Lebanon to other countries occurred directly or through stepping stones due to the sampling/sequencing bias caused by the inequality of genomic surveillance across the world. This inequality may also be the reason that the data show dispersal from Lebanon to European countries being more common than to close countries.

Therefore, fostering real-time genomic surveillance in developing countries is a cornerstone of monitoring and tracking the spread and evolution of the virus. These countries constitute potential hot spots for the emergence of new variants because they are regions where SARS-CoV-2 transmissibility is high, and there is an elevated likelihood of multi-variant coinfection and recombination, combined with low levels of surveillance, intervention, and sequencing capacities. Examining a series of retrospective sequences has allowed us, in this work, to bring into sharp focus the importance of small and interconnected countries such as Lebanon in sowing such variants as the B.1.398 lineage around the world. Therefore, the earlier genomic surveillance is implemented, the earlier the first seeding events will be unraveled, and the stronger the worldwide response against new variants will be.

Author Contributions: Conceptualization, A.T.R.V. and M.H.; data curation, D.N., R.R., A.P.L. and M.O.; formal analysis, D.N., R.R., A.P.L. and L.G.P.d.A.; funding acquisition A.T.R.V. and M.H.; investigation, D.N., R.R., A.P.L., L.G.P.d.A., M.O., and M.B.I.; methodology, D.N., R.R., A.P.L., A.T.R.V. and M.H.; project administration, H.M., A.B., G.B., Q.S., L.J., H.H., F.D., B.L., P.C., A.T.R.V. and M.H.; resources, D.N., R.R., A.P.L., L.G.P.d.A., M.O., M.B.I., H.M., A.B., G.B., Q.S., L.J., H.H., F.D., B.L., P.C., A.T.R.V. and M.H.; software, A.P.L. and L.G.P.d.A.; supervision, A.T.R.V. and M.H.; validation, D.N., R.R. and A.P.L.; visualization, A.P.L.; writing—original draft, D.N., R.R., A.P.L., L.G.P.d.A., M.O., M.B.I., H.M., A.B., G.B., Q.S., L.J., H.H., F.D., B.L., P.C., A.T.R.V. and M.H.; writing—review and editing, D.N., R.R., A.P.L., A.T.R.V. and M.H. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Lebanese University (COVID-19 project) and Fondation Mérieux. This work was developed within the frameworks of Corona-ômica-RJ (FAPERJ = E-26/210.179/2020 and E-26/211.107/2021) and NGS-BRICS (CNPq 440931/2020-7). A.T.R.V. is supported by CNPq (307145/2021-2) and FAPERJ (E-26/201.046/2022). D.N. is a Ph.D. student supported by a scholarship from the GABRIEL network and Fondation Mérieux. M.O. is supported by the Atkinson Postdoctoral Fellowship (Cornell University).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Lebanese University (protocol code #CUER 23-2020; 14 May 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All the accession numbers of the genomes used in this study are presented in https://doi.org/10.55876/gis8.220630un (accessed on 30 June 2022).

Acknowledgments: We acknowledge the support of the WHO in facilitating the sequencing of our SARS-CoV-2 strains at the Laboratory of Virology, Institute of Infectious Agents, which is associated with the National Reference Center of Respiratory Infection Viruses, Hospice civil de Lyon in Lyon, France; the National Laboratory for Scientific Computation (LNCC) in Brazil performed the bioinformatics analysis. We would like to thank all of the Lebanese Ministry of Public Health staff as well as the COVID-19 committee in North Lebanon, the municipalities, the Lebanese Red Cross, the Emergency and Relief Corps (ERC) of the Lebanese Succor Association (LSA), and all healthcare staff in hospitals and campaigns for their cooperation in this study. Additionally, we acknowledge the effort of the personnel and laboratories who generated and submitted the genomics sequences used in this work to the GISAID dataset, listed at https://doi.org/10.55876/gis8.220630un (accessed on 30 June 2022). The authors would also like to thank Nada Ghosn, the head of the Epidemiological Surveillance Program in the Ministry of Public Health, for her cooperation and support. They also

thank all the LMSE staff for their excellent technical assistance and the huge efforts made during the COVID-19 pandemic.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

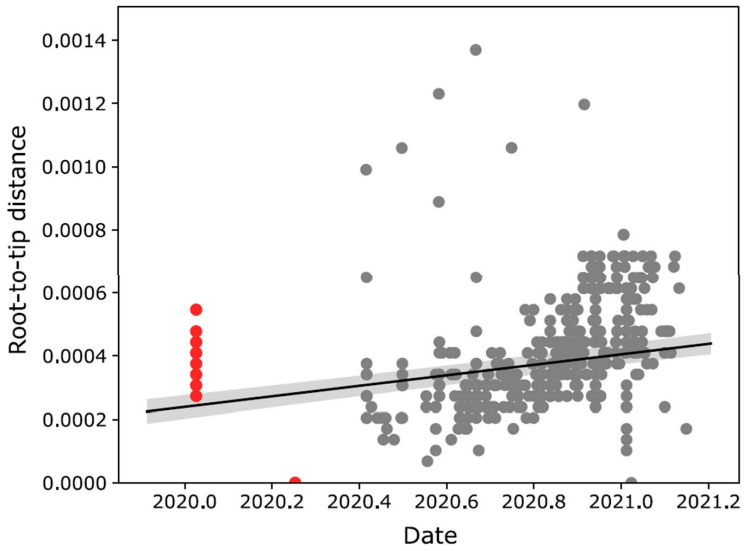

Figure A1. Correlation between sampling dates and the root-to–tip distance of the maximum likelihood tree of lineage B.1.398 when including sequences from GISAID prior to June, 2020 (red points). These sequences were removed for the final analysis.

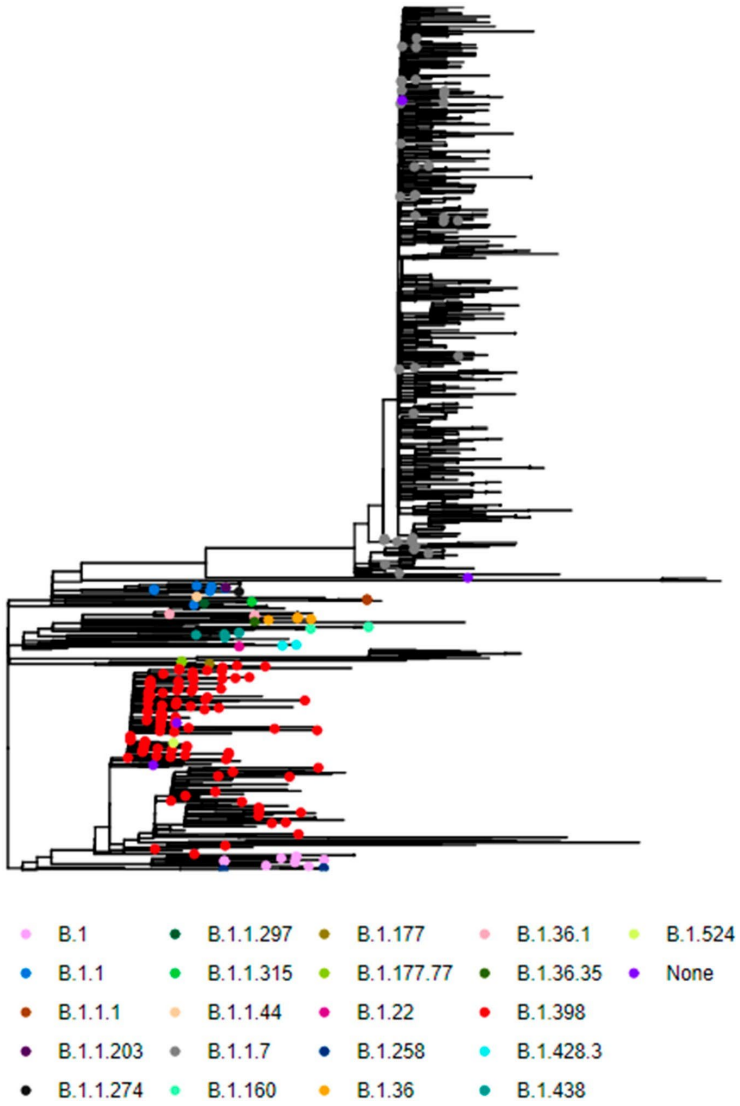

Figure A2. Maximum likelihood phylogeny of SARS-CoV-2 sequences sampled in Lebanon. Colored tip points show the newly-sequenced genomes and their attributed lineage.

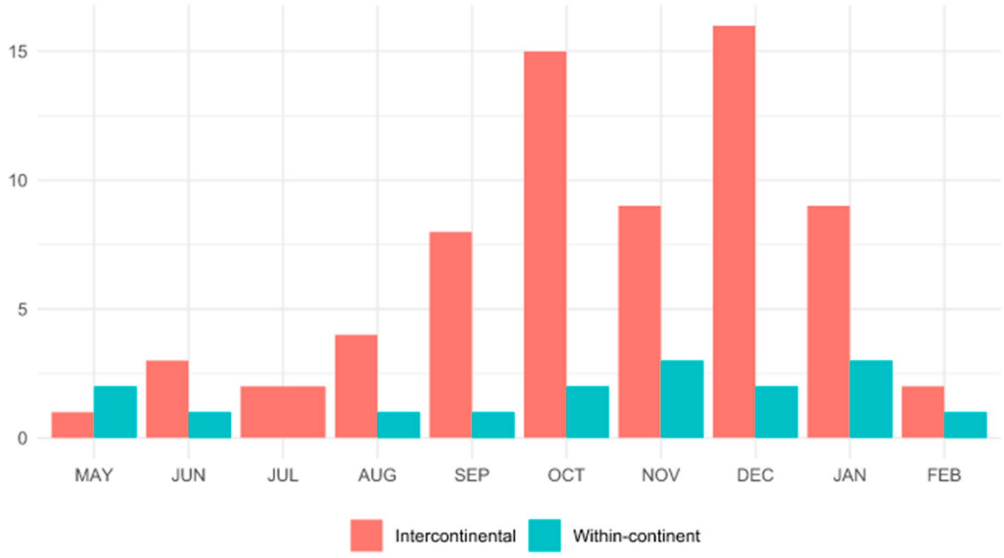

Figure A3. Temporal distribution of intercontinental (red) and within-continent (blue) dispersal of B.1.398 lineage.

References

1. Our World in Data. Available online: https://ourworldindata.org/explorers/coronavirus-data-explorer (accessed on 30 March 2022).
2. Pango Lineages: Latest Epidemiological Lineages of SARS-CoV-2. Available online: https://cov-lineages.org/ (accessed on 30 March 2022).
3. WHO: Tracking SARS-CoV-2 Variants. Available online: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/ (accessed on 30 March 2022).
4. Brussow, H. COVID-19: Emergence and mutational diversification of SARS-CoV-2. *Microb. Biotechnol.* **2021**, *14*, 756–768. [CrossRef]
5. Dubey, A.; Choudhary, S.; Kumar, P.; Tomar, S. Emerging SARS-CoV-2 Variants: Genetic Variability and Clinical Implications. *Curr. Microbiol.* **2021**, *79*, 20. [CrossRef] [PubMed]
6. World Economic Situation and Prospects. Country Classification. Available online: https://www.un.org/en/development/desa/policy/wesp/wesp_current/2014wesp_country_classification.pdf (accessed on 16 May 2022).
7. Elbe, S.; Buckland-Merrett, G. Data, disease and diplomacy: GISAID's innovative contribution to global health. *Glob. Chall.* **2017**, *1*, 33–46. [CrossRef] [PubMed]
8. GISAID. Available online: https://www.gisaid.org/submission-tracker-global/ (accessed on 16 May 2022).
9. Chen, Z.; Azman, A.S.; Chen, X.; Zou, J.; Tian, Y.; Sun, R.; Xu, X.; Wu, Y.; Lu, W.; Ge, S.; et al. Global landscape of SARS-CoV-2 genomic surveillance and data sharing. *Nat. Genet.* **2022**, *54*, 499–507. [CrossRef]
10. Brito, A.F.; Semenova, E.; Dudas, G.; Hassler, G.W.; Kalinich, C.C.; Kraemer, M.U.G.; Ho, J.; Tegally, H.; Githinji, G.; Agoti, C.N.; et al. Global disparities in SARS-CoV-2 genomic surveillance. *medRxiv* **2021**. [CrossRef]
11. Koweyes, J.; Salloum, T.; Haidar, S.; Merhi, G.; Tokajian, S. COVID-19 Pandemic in Lebanon: One Year Later, What Have We Learnt? *mSystems* **2021**, *6*, e00351-21. [CrossRef]
12. WHO Health Emergency Dashboard. Available online: https://covid19.who.int/region/emro/country/lb (accessed on 17 April 2022).
13. Republic of Lebanon Ministry of Public Health (MOPH). Epidemiological Surveillance Program. Official Updates Coronavirus-COVID-19 In Lebanon. Available online: https://www.moph.gov.lb/en/ (accessed on 17 April 2022).
14. GISAID. Available online: https://www.gisaid.org/ (accessed on 4 February 2022).
15. Bal, A.; Simon, B.; Destras, G.; Chalvignac, R.; Semanas, Q.; Oblette, A.; Queromes, G.; Fanget, R.; Regue, H.; Morfin, F.; et al. Detection and prevalence of SARS-CoV-2 co-infections during the Omicron variant circulation, France, December 2021—February 2022. *medRxiv* **2022**. [CrossRef]
16. Li, H.; Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **2009**, *25*, 1754–1760. [CrossRef]

17. Danecek, P.; Bonfield, J.K.; Liddle, J.; Marshall, J.; Ohan, V.; Pollard, M.O.; Whitwham, A.; Keane, T.; McCarthy, S.A.; Davies, R.M.; et al. Twelve years of SAMtools and BCFtools. *Gigascience* **2021**, *10*, giab008. [CrossRef]
18. Quinlan, A.R.; Hall, I.M. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **2010**, *26*, 841–842. [CrossRef]
19. Rambaut, A.; Holmes, E.C.; O'Toole, A.; Hill, V.; McCrone, J.T.; Ruis, C.; du Plessis, L.; Pybus, O.G. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat. Microbiol.* **2020**, *5*, 1403–1407. [CrossRef] [PubMed]
20. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef] [PubMed]
21. Shen, W.; Le, S.; Li, Y.; Hu, F. SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. *PLoS ONE* **2016**, *11*, e0163962. [CrossRef] [PubMed]
22. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; von Haeseler, A.; Lanfear, R. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **2020**, *37*, 1530–1534. [CrossRef] [PubMed]
23. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef] [PubMed]
24. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522. [CrossRef]
25. Sagulenko, P.; Puller, V.; Neher, R.A. TreeTime: Maximum-likelihood phylodynamic analysis. *Virus Evol.* **2018**, *4*, vex042. [CrossRef]
26. Suchard, M.A.; Lemey, P.; Baele, G.; Ayres, D.L.; Drummond, A.J.; Rambaut, A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* **2018**, *4*, vey016. [CrossRef]
27. Dellicour, S.; Rose, R.; Faria, N.R.; Lemey, P.; Pybus, O.G. SERAPHIM: Studying environmental rasters and phylogenetically informed movements. *Bioinformatics* **2016**, *32*, 3204–3206. [CrossRef]
28. Fayad, N.; Abi Habib, W.; Kandeil, A.; El-Shesheny, R.; Kamel, M.N.; Mourad, Y.; Mokhbat, J.; Kayali, G.; Goldstein, J.; Abdallah, J. SARS-CoV-2 Variants in Lebanon: Evolution and Current Situation. *Biology* **2021**, *10*, 531. [CrossRef]
29. Feghali, R.; Merhi, G.; Kwasiborski, A.; Hourdel, V.; Ghosn, N.; Tokajian, S. Genomic characterization and phylogenetic analysis of the first SARS-CoV-2 variants introduced in Lebanon. *PeerJ* **2021**, *9*, e11015. [CrossRef] [PubMed]
30. Merhi, G.T.A.; Martins, L.; Koweyes, J.; Le-Viet, T.; Abou Naja, H.; Al Buaini, M.; Prosolek, S.; Alikhan, N.F.; Lott, M.; Tohmeh, T.; et al. Replacement of the Alpha variant of SARS-CoV-2 by the Delta variant in Lebanon between April and June 2021. *MedRxiv* **2021**. [CrossRef] [PubMed]
31. Lineage List. Available online: https://cov-lineages.org/lineage_list.html (accessed on 17 April 2022).
32. Jackson, C.B.; Zhang, L.; Farzan, M.; Choe, H. Functional importance of the D614G mutation in the SARS-CoV-2 spike protein. *Biochem. Biophys Res. Commun.* **2021**, *538*, 108–115. [CrossRef] [PubMed]
33. Isabel, S.; Grana-Miraglia, L.; Gutierrez, J.M.; Bundalovic-Torma, C.; Groves, H.E.; Isabel, M.R.; Eshaghi, A.; Patel, S.N.; Gubbay, J.B.; Poutanen, T.; et al. Evolutionary and structural analyses of SARS-CoV-2 D614G spike protein mutation now documented worldwide. *Sci. Rep.* **2020**, *10*, 14031. [CrossRef] [PubMed]
34. Plante, J.A.; Liu, Y.; Liu, J.; Xia, H.; Johnson, B.A.; Lokugamage, K.G.; Zhang, X.; Muruato, A.E.; Zou, J.; Fontes-Garfias, C.R.; et al. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* **2021**, *592*, 116–121. [CrossRef] [PubMed]
35. Medcities. Available online: https://medcities.org/fr/member/tripoli/ (accessed on 10 April 2022).
36. Hubbard, B.; Saad, H. Lebanon's Currency Plunges, and Protesters Surge Into Streets. *The New York Times*, 2020. Available online: https://www.nytimes.com/2020/06/11/world/middleeast/lebanon-protests.html (accessed on 10 April 2022).
37. Cheaito, M.A.; Al-Hajj, S. A Brief Report on the Beirut Port Explosion. *Mediterr. J. Emerg. Med. Acute Care* **2020**, *1*. [CrossRef]
38. Cheeseman, A. After Beirut explosion, Lebanese vOlunteers Flock to Help Clean up. *NBC News*. 2020. Available online: https://www.nbcnews.com/news/world/after-beirut-explosion-lebanese-volunteers-flock-help-clean-n1236403 (accessed on 17 April 2022).
39. Aboud, C. Lebanon: A New General Quarantine Begins to Counter the Rise in Corona Virus Infections (Available in Arabic Version). *France24* **2020**. Available online: https://www.france24.com/ar/ (accessed on 17 April 2022).
40. Gomez, C.E.; Perdiguero, B.; Esteban, M. Emerging SARS-CoV-2 Variants and Impact in Global Vaccination Programs against SARS-CoV-2/COVID-19. *Vaccines* **2021**, *9*, 243. [CrossRef]
41. Mumtaz, G.R.; El-Jardali, F.; Jabbour, M.; Harb, A.; Abu-Raddad, L.J.; Makhoul, M. Modeling the Impact of COVID-19 Vaccination in Lebanon: A Call to Speed-Up Vaccine Roll Out. *Vaccines* **2021**, *9*, 697. [CrossRef] [PubMed]
42. Tabar, P.; Denison, A. IMISCOE Research Series. Diaspora Policies, Consular Services and Social Protection for Lebanese Citizens Abroad. *Migr. Soc. Prot. Eur. Beyond* **2020**, *3*,199–215.
43. World Population Review. Available online: https://worldpopulationreview.com/countries/lebanon-population (accessed on 6 April 2022).
44. International Labor Organization. Available online: https://www.ilo.org/global/programmes-and-projects/prospects/countries/lebanon/lang--en/index.htm (accessed on 17 April 2022).

45. Unated Nations ESCWA. Available online: https://www.unescwa.org/publications/multidimensional-poverty-lebanon-2019-2021 (accessed on 6 April 2022).
46. Hubbard, B. As Lebanon's Crisis Deepens, Lines for Fuel Grow, and Food and Medicine Are Scarce. *New York Times*. 2021. Available online: https://www.nytimes.com/2021/07/05/world/middleeast/lebanon-economic-crisis.html (accessed on 6 April 2022).

Chapter 3: Unveiling the identity of the circulating variants of concern (VOCs) and Omicron subvariants in Lebanon during the first three years of the COVID-19 pandemic using a variant-specific PCR

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is susceptible to a large mutational rate, of approximately 2.5 nucleotides per month [1]. Thus, continuous investigation of SARS-CoV-2's genome is essential to track any mutation apparition and detect the emerging viral lineages after phylogenetic evaluation. Whole genome sequencing (WGS) constitutes the gold standard tool for valuable genotypic information on viral evolution [2]. However, the high cost and the requirement for compliant personnel and sophisticated facilities, make this technique difficult to be implemented, especially in low-income countries [2]. On the other hand, another approach, such as quantitative reverse transcriptase polymerization chain reaction (RT-qPCR), can constitute a less expensive and more rapid and accurate screening tool [3]. Several commercial kits were developed for this purpose, targeting mutations specific to a particular SARS-CoV-2 lineage, and have shown a complete concordance with WGS results [3].

Since October 2019, Lebanon has suffered from a rising economic and financial crisis among the worst in the world [4]. Subsequently, the country was attacked by COVID-19 on February 21, 2020, and the number of documented cases has reached more than one million as of June 9, 2024 [5, 6], while the number of sequences present in the global initiative on sharing avian influenza data (GISAID), as of June 30, 2024, is small (3,289, not exceeding 0.26% of the total notified SARS-CoV-2 cases in Lebanon) and perhaps does not tell the story of the dynamics of coronavirus disease 2019 (COVID-19) lineages in Lebanon. This shed light on the need to use an accurate tool for SARS-CoV-2's lineage investigations. A previous Lebanese study tested SYBER Green-based RT-qPCR, targeting the 69-70del in the S gene and the 106-108del in the nsp6 gene, to screen for the Alpha variant, and has shown that the samples developing an S gene failure (identifying the Alpha variant), were all carrying these mutations [7].

Based on all that has been mentioned, in this chapter, consisting of an article paper, we used an RT-qPCR approach to detect the variants of concern Alpha and Delta, as well as the Omicron subvariants BA.1, BA.2, BA.4, and BA.5, in a collection of 597 nasopharyngeal samples, collected during the first three years of the pandemic in Lebanon. We then compared our results to the profile of the SARS-CoV-2 sequences available in GISAID database.

References

1. Duchene S, Featherstone L, Haritopoulou-Sinanidou M, Rambaut A, Lemey P, Baele G. Temporal signal and the phylodynamic threshold of SARS-CoV-2. Virus Evol. 2020;6(2):veaa061. Epub 2020/11/26. doi: 10.1093/ve/veaa061. PubMed PMID: 33235813; PubMed Central PMCID: PMCPMC7454936.

2. Mintzer V, Moran-Gilad J, Simon-Tuval T. Operational models and criteria for incorporating microbial whole genome sequencing in hospital microbiology - A systematic literature review. Clin Microbiol Infect. 2019;25(9):1086-95. Epub 2019/05/01. doi: 10.1016/j.cmi.2019.04.019. PubMed PMID: 31039443.

3. Munoz-Gallego I, Melendez Carmona MA, Martin Higuera C, Viedma E, Delgado R, Folgueira MD. Rapid screening of SARS-CoV-2 variants, a key tool for pandemic surveillance. Sci Rep. 2023;13(1):11094. Epub 2023/07/09. doi: 10.1038/s41598-023-37866-8. PubMed PMID: 37422474; PubMed Central PMCID: PMCPMC10329708.

4. Lebanon TWBI. Overview 2022 [July 4, 2024]. Available from: https://www.worldbank.org/en/country/lebanon/overview.

5. Health LMoP. Novel Coronavirus 2019-Lebanon [June 25, 2024]. Available from: https://www.moph.gov.lb/en/Pages/2/24870/novel-coronavirus-2019.

6. The World Health Organization (WHO). WHO COVID-19 dashboard-Lebanon [June 25, 2024]. Available from: https://data.who.int/dashboards/covid19/deaths?m49=422&n=o.

7. Abdel Sater F, Younes M, Nassar H, Nguewa P, Hamze K. A rapid and low-cost protocol for the detection of B.1.1.7 lineage of SARS-CoV-2 by using SYBR Green-based RT-qPCR. Mol Biol Rep. 2021;48(11):7243-9. Epub 2021/10/07. doi: 10.1007/s11033-021-06717-y. PubMed PMID: 34613565; PubMed Central PMCID: PMCPMC8493944.

Article

Unveiling the identity of the circulating variants of concern (VOCs) and Omicron subvariants in Lebanon during the first three years of the COVID-19 pandemic using a variant-specific PCR.

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Abstract

Originated in Wuhan, China, the coronavirus disease 2019 (COVID-19) has spread across the globe, due to its highly-mutated causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulting thereby in a large number of viral lineages, of which, variants of concern (VOCs), were of particular importance. Screening for these variants constitutes an important step to follow the evolution of the viral pathways and mutations. Lebanon, a developing country, faces a financial crisis ranking among the worst globally. In this context, this was the first study aiming to detect SARS-CoV-2's VOCs and Omicron subvariants, using an affordable technique, variant-specific quantitative *reverse transcription polymerase chain reaction (RT-qPCR*) detecting the Alpha and Delta variants, Delta 21J/AY.4 subvariant, as well as the Omicron BA.1, BA.2, BA.4 and BA.5 subvariants. A total of 597 samples, collected from seven Lebanese governorates were analyzed in this investigation. The results were compared to previous Lebanese SARS-CoV-2 sequences available in the global initiative on sharing avian influenza data (GISAID) database. Our findings showed a concordant image to that observed in the previously available sequences, revealing that Lebanon was dominated gradually by all of the tested variants. This may present the variant-specific RT-qPCR as a promising reliable substitution tool for SARS-CoV-2 screening variants in low-income countries.

Keywords: SARS-CoV-2, variants of concern, screening, variant-specific RT-qPCR, Lebanon

1. Introduction

Stemming from China in 2019, the coronavirus disease 20219 (COVID-19) has rapidly spread worldwide to become a global pandemic, amounting to more than 775 million cases and 7 million deaths globally, as of June 24, 2024 [1, 2]. The causative agent of this disease was the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped virus with a single-stranded positive-sense RNA genome of approximately 29.8 kb long [3]. The genome is composed of 14 open reading frames (ORF), encoding 27 proteins [3]. ORF1a and ORF1b occupy two-thirds of the genome and encode sixteen non-structural proteins (Nsp) [4]. Nsp12 constitutes the RNA-dependent RNA polymerase (RdRp), an essential viral enzyme for generating new viral RNA [4]. The last one-third of the viral genome encodes four structural proteins namely: spike (S), envelope (E), membrane (M), nucleocapsid (N) as well as ten accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b, ORF9b, ORF9c, ORF10) [4]. SARS-CoV-2 infection can be revealed with reverse transcription polymerization chain reaction (RT-PCR) targeting specific viral genes, and constituting the gold standard diagnosis test for SARS-CoV-2 [5].

Enormous genetic diversity, leading to the emergence of various viral lineages, has been observed within SARS-CoV-2, because of its high-frequency mutation rate, as is the case in RNA viruses [6]. Five particular variants were marked by their impact on viral characteristics such as increasing virulence, transmissibility, immune escape, and decreasing the effectiveness of therapies, diagnostic tests, and vaccinations. These variants were identified by the world health organization (WHO) as variants of concern (VOC) and were named using Greek letters [7]. The Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1 or B.1.1.28.1), and Delta (B.1.617.2) variants were first detected in 2020, in September, May, November and October respectively [8]. Regarding the last VOC, Omicron (B.1.1.529), it has been isolated for the first time in November 2021 [8].

Whole genome sequencing (WGS) is the cornerstone to identifying circulating SARS-CoV-2 lineages, tracking the evolution of the virus, and developing adequate diagnostic, vaccines, and therapeutic tools [9]. Low-income countries face many challenges in sequencing implementation due to the high cost of facilities, the lack of skilled personnel, and the limited access to necessary tools and internet resources [10]. Using RT-PCR to detect VOCs or mutations from this point can be a rapid and more accurate substitution [11]. Many kits have been manufactured in this field, including TaqPath COVID-19 assay (Thermo Fisher Scientific, Waltham, MA, USA) targeting H69-V70del; VirSNiP SARS-CoV-2 Spike N501Y, del H69/V70, E484K (TIB Molbiol, Berlin, Germany) targeting H69-V70del and N501Y, E484K mutations; VirSNiP SARS-CoV-2 Spike L452R (TIB Molbiol, Berlin, Germany) targeting

L452R; Allplex SARS-CoV-2 Variants I Assay (Seegene, Seoul, South Korea) targeting H69-V70del and N501Y, E484K; Allplex SARS-CoV-2 Variants II Assay (Seegene, Seoul, South Korea) targeting K417N, K417T, L452R, and W152C. A previous study conducted in Spain has tested the effectiveness of RT-PCR as a rapid, less expansive, and time-saving screening tool to detect SARS-CoV-2 variants, and it has shown complete concordance with WGS results [12]. On the other hand, another rapid, low-cost technique that has also been employed to detect SARS-CoV-2's clades (G, GH, GR, V, and S) and Q57H mutation, the amplification refractory mutation system (ARMS), based on point-mutation PCR [13, 14], was previously used to detect clades or subtypes of other respiratory viruses [15-17]. In Lebanon, a published study screened for B.1.1.7 lineage through SYBER Green-based quantitative RT-PCR (RT-qPCR), targeting the 69-70del in the S gene and 106-108del in the nsp6 gene, and revealed that all the COVID-19 positive samples manifesting an S gene target failure (SGTF), characterizing the Alpha variant, were harboring the mutations in question [18]. COVID-19 was first detected in Lebanon on February 21, 2020, in a woman returning from Iran, tested positive [19]. Subsequently, an increasing number of cases has been registered nationally, reaching 1,239,904 cases and 10,947 deaths, as of June 9, 2024 [20]. The first study investigating the circulating SARS-CoV-2 lineages in Lebanon targeted only the first month of the pandemic, between February and March 2020 [21]. Subsequently, another national survey has studied the period between March and December 2020 [22]. The last studies have targeted more extended periods, reaching two years of follow-up [23-25].

To our knowledge, no previous national studies have investigated the circulating SARS-CoV-2 VOCs, using variant-specific RT-qPCR, during the whole period of the first three years of the pandemic. In this context, this study aims to retrospectively identify the circulating VOCs and Omicron subvariants in a batch of samples collected over three years, using an affordable and feasible method, especially during the economic crises occurring in the country since October 2019. These identified variants herein will then be compared to SARS-CoV-2's lineages available in Global Initiative on Sharing Avian Influenza Data (GISAID), obtained by WGS of viral sequences originating from Lebanon.

2. Material and methods

2.1. Sampling

This study was approved by the ethical committee of the Lebanese University, under the number #CUER 23-2020, on 14 May 2020. The analyzed samples were provided from the LMSE laboratory (Laboratoire de Microbiologie, Santé et Environnement), accredited by the Ministry of Public Health in Lebanon to carry out the diagnosis of SARS-CoV-2 infection in

North Lebanon, by RT-qPCR on nasopharyngeal samples. The LMSE team has used five different SARS-CoV-2 scanning PCR kits to diagnose SARS-CoV-2 during the first three years of the pandemic: the COVID-19 Genesig Real-time PCR assay (PrimerDesign, United Kingdom) targeting RdRp gene; the GeneFinder COVID-19 Plus Real*Amp* kit (OSANG Healthcare, Korea) targeting N, E, and RdRp genes; the Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR kit (Shanghai ZJ Bio-Tech Co, China) targeting N, E and ORF1ab genes; TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA) targeting N, S and ORF1ab. All SARS-CoV-2 PCR-positive samples were stored in the LMSE at -80°C. In order to investigate the circulating VOCs in Lebanon, a total of 597 samples were randomly selected from the date of the implementation of the first diagnostic tests in July 2020, until February 2023. These samples were derived from various sources: several governmental hospitals in North Lebanon, Nini Hospital in Tripoli, COVID-19 screening campaigns among Lebanese citizens organized by several municipalities in collaboration with the Lebanese Ministry of Public Health in different regions in the country, in addition to travelers crossing the Lebanese-Syrian borders. Although the selection was random, we fixed the number of selected patients from each month to be between 25-30, in a scattered mode during the month if applicable, to cover evenly the whole study period. Besides, the selected samples should have a cycle threshold (Ct) value lower than 25 in at least one of the genes tested by RT-qPCR, to avoid any failure in the specific VOCs and Omicron subvariants RT-qPCR detection. Subsequently, total RNAs were extracted from samples at the LMSE using the AccuPrep Viral RNA Extraction Kit (BIONEER, Korea), QIAamp Viral RNA Kit (QIAGEN, USA), and Viral DNA/RNA kit (CWBIO, China), according to the manufacturer's instructions.

2.2. VOCs and Omicron subvariants-specific RT-qPCR

To main this part of the study, we have used specific in-house RT-qPCR developed at "Institut hospitalo-universitaire en maladies infectieuses de Marseille (IHU MI)", France, detecting SARS-CoV-2's VOCs and Omicron subvariants. Seven detection systems were in question, concerning the Alpha variant, the parental Delta variant (all Delta variants with P681R mutation, targeted gene: S), Delta 21J/AY.4 variant (targeted mutation: A11201G in the nsp6 gene (corresponding to amino acid substitution T77A)), as well as 21K/BA.1 (targeted mutations: A23040G, G23048A, A23055G in the spike gene (Q493R, G496S, Q498R)), 21L/BA.2 (targeted gene S), 22A/BA.4 (targeted gene ORF7a) and 22B/BA.5 Omicron (targeted gene M) subvariants. Positive controls have also been used. The details of the PCR tests, including target sequences and primers are presented in Table 1.

Table 2: Targeted genes, primers, and probe sequences for the seven variants of RT-qPCR detection systems.

Detected variant	Targeted gene	Primers (in 5'-3' orientation)	Probes (in 5'-3' orientation)
Alpha	N	(F): CATGACGTTCGTGTTGTTTTAGA (R): CTGAGGGTCCACCAAACGTA	6VIC- GTCTCTAAATGGACCCCAAAATCA
Delta (P681R)	S	(F): TGACATACCCATTGGTGCAG (R): GGCAATGATGGATTGACTAGC	6FAM- ATTCTCGTCGGCGGGCA
Delta 21J/AY.4 (T77A)	nsp6	(F): CTGCTTTTGCAATGATGTTTGT (R): TACGCATCACCCAACTAGCA	6FAM- CTTGCCGCTGTAGCTTATTTTAAT
Omicron BA.1	S	(F): CCTTGTAATGGTGTTGAAGGTTTT (R): CTGGTGCATGTAGAAGTTCAAAAG	6VIC- TTTACGATCATATAGTTTCCGACCC
Omicron BA.2	S	(F): TCTGCTTTACTAATGTCTATGCAGATTC (R): CGCAGCCTGTAAAATCATCTG	6FAM-AGAGGTAATGAAGTCAGCCAAATCGC
Omicron BA.4	ORF7a	(F): TCAAAAGAAAGACAGAATGATTGAAC (R): CTTGCAGTTCAAGTGAGAACCA	6FAM-CTATTTTTGCTTTTTAGCCTTTCTGC
Omicron BA.5	M	(F): CCTGATCTTCTGGTCTAAACGAA (R): TTCCATTGTTCAAGGAGCTTTT	6FAM-ATGGCAAATTCCAACGGTACTATT

N: nucleocapsid targeted gene, S: spike glycoprotein targeted gene, nsp6: non-structural protein 6 targeted gene, ORF7a: open reading frame 7a targeted gene, F: forward, R: reverse.

In order to detect the variants among our samples, we proceed as follows, respecting the VOCs' first apparition dates worldwide. Firstly, the first samples were tested by the Alpha screening system. If a sample showed a positive RT-qPCR result, it was considered an Alpha variant. In the case of a negative result, we tried the Delta detection system. If we obtained a positive outcome, we continued to test the Delta 21J/AY.4 variant, while in the other case, no attributed VOC was associated with the relevant sample. This was applicable until late January 2021, when we added the Omicron BA.1 detection system to the screening, i.e. the samples tested negative for Alpha and Delta variant were tested by the BA.1 system. BA.2 was then added to the investigation since mid-September 2021 to test the samples BA.1 negative. Afterward, as long as a sample showed negative RT-qPCR for BA.1 and BA.2, it underwent testing by BA.4 and BA.5 detection systems successively. The scanning procedure is summarized in Figure 1.

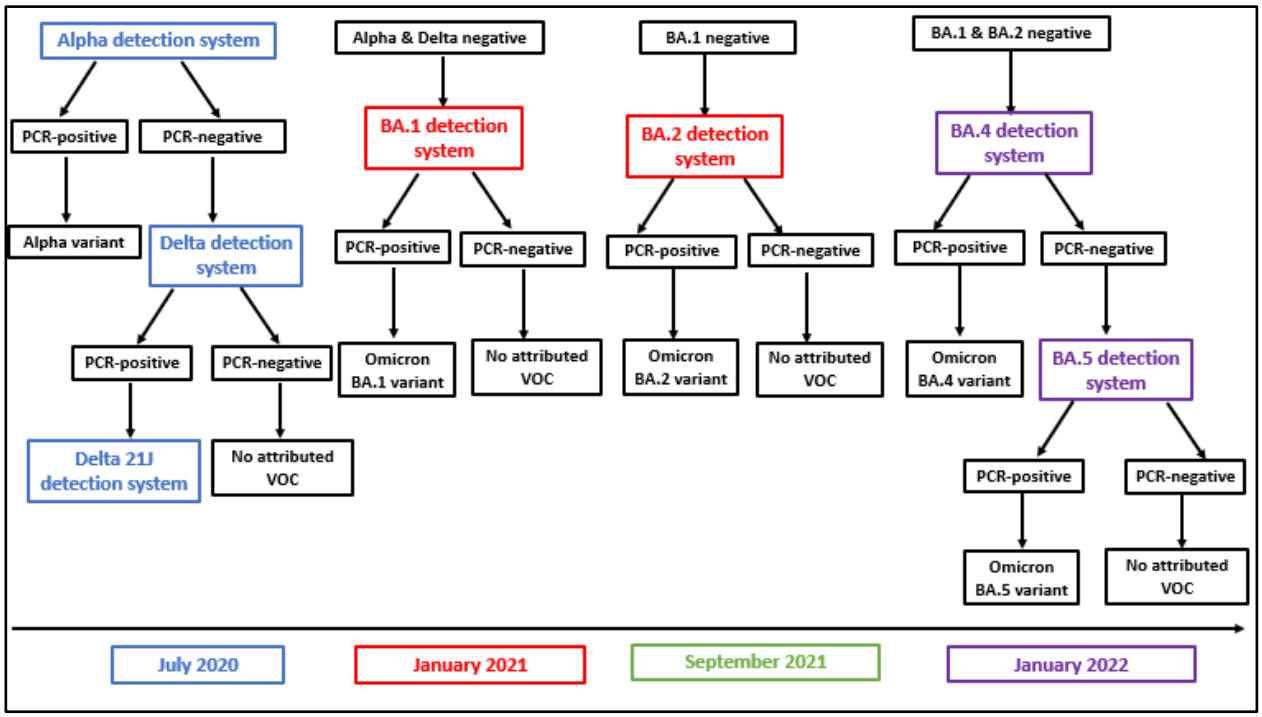

Figure 4: Flowchart summarizing the followed procedure to detect VOCs among 597 RNA samples recovered from different regions in Lebanon. PCR means quantitative reverse transcription PCR (RT-qPCR).

2.3. GISAID data collection

In order to compare our findings to previous Lebanese SARS-CoV-2 genomes, we downloaded the metadata of the viral sequences collected in Lebanon from GISAID, as accessed on June 30, 2024, after filtration using the options "Complete", "Low coverage exclude", and "Collection date complete" available in the database. After excluding seven sequences manifesting "Unassigned" lineage results and 25 sequences from samples isolated in January 2020 (because their uploaded date may have an error during the submission, since the first Lebanese COVID-19 case was registered in February 2020), the total obtained number of GISAID Lebanese sequences with assigned lineage by GISAID was 2186 sequences.

3. Results

3.1. Data summary

The mean age of the participants was 42.2 years, with 45.2% being male and 54.8% female patients. Most patients were Lebanese (87.6%), 9.7% were Syrian, and the rest were from different nationalities (including Palestinian, Philippian, Ethiopian, Australian, American, and French). 72.5% of the samples were derived from the North Lebanon governorate, with the district of Tripoli being the source of 250 samples, corresponding to 41.9% of the total number of samples, and 15.9% of the samples were from Akkar. The distribution of the samples within the national governorates is presented in Figure 2.

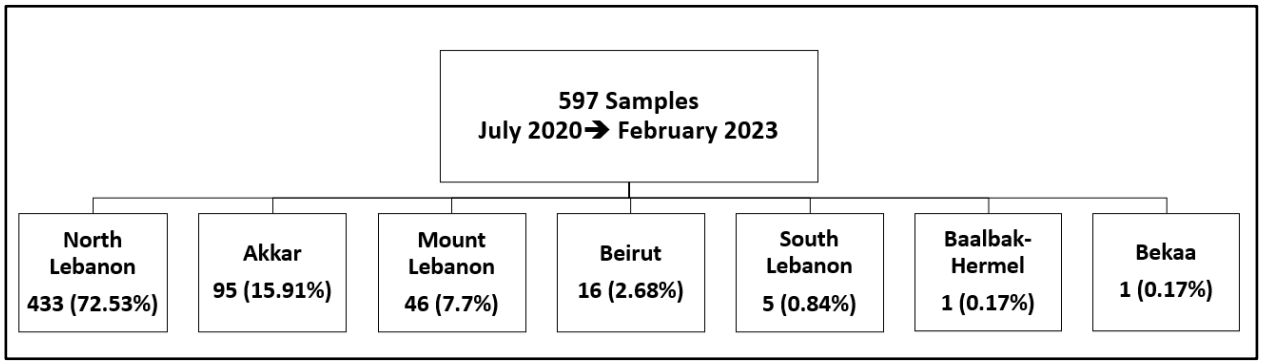

Figure 5: Flowchart showing the distribution of the 597 samples within seven governorates of Lebanon (North Lebanon, Akkar, Mount Lebanon, Beirut, South Lebanon, Baalbak-Hermel, and Bekaa).

3.2. VOCs and Omicron subvariants-specific RT-qPCR results

Since the beginning of our sampling on July 27, 2020, and till January 27, 2021, no VOCs have been detected with the specific RT-qPCR screening, among 155 tested samples representing 26% of the total samples. Delta variant was the first VOC to be detected in our samples. It was the predominant variant over one year, between January 29, 2021, and January 3, 2022, with 191 (31.99% of the total samples) Delta-positive samples (Figure 3).

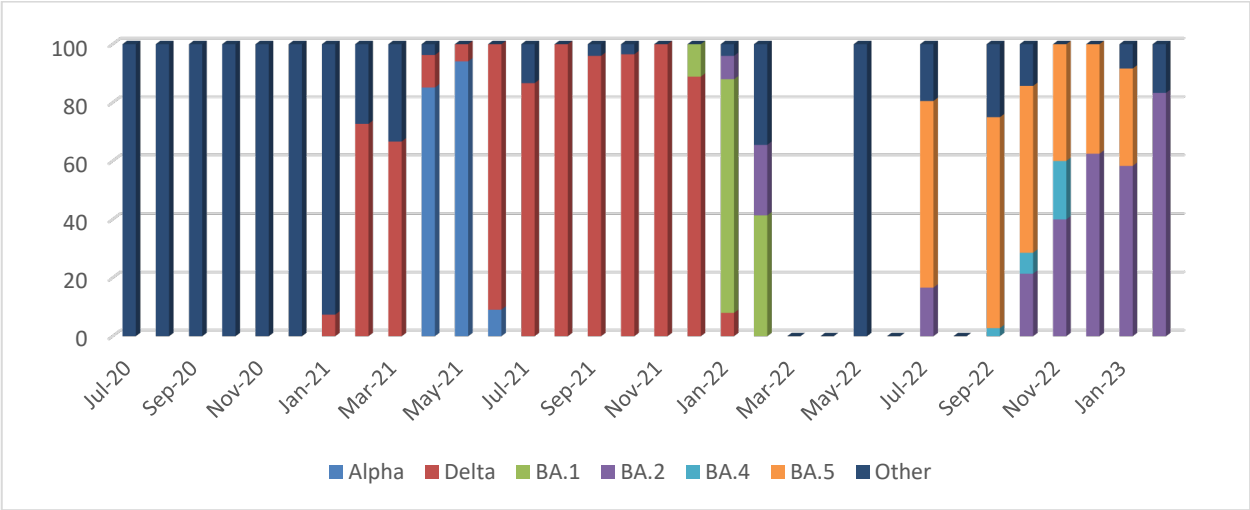

Figure 6 Epidemics of COVID-19 in the first three years of the pandemic in Lebanon, among 597 samples. The percentage of the variants of concern (VOC) Alpha and Delta and the Omicron subvariants BA.1, BA.2, BA.3, and BA.4 are presented. The figure is created with Microsoft Excel software.

These samples were all tested with Delta 21J/AY.4, except nine of them, due to logistical reasons. We have started detecting 21J since June 8, 2021. Out of 182 tested Delta samples, 128 were Delta 21J (corresponding to 70.33% of the tested Delta samples). On April 2, 2021, the first Alpha-positive sample was identified, and till June 9, 2021, the total number has reached 40 (6.7%) samples. Regarding the Omicron variant, 35 (5.86%) BA.1-positive samples were detected between December 27, 2021 and May 23, 2022. A total of 49 BA.2-positive samples (8.2%) were found over one year, between January 24, 2022, and February 27, 2023, the last date of our sampling. Only 3 samples (0.5%) tested positive for the BA.4 Omicron variant,

between June 9, 2022, and November 8, 2022. As for the BA.5 Omicron variant, we have detected 70 (11.72%) positive samples, between July 18, 2022, and January 28, 2023. Figure 4 presents the count of each variant detected.

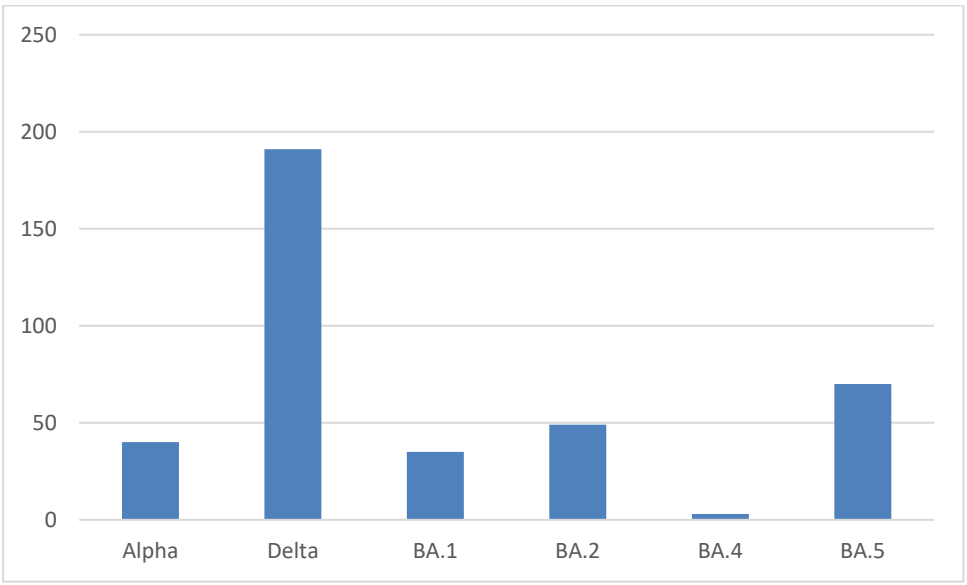

Figure 7: Bar chart presenting the count of the VOCs Alpha and Delta and the Omicron subvariant detected from a total of 597 samples. The figure is created with Microsoft Excel software.

3.3.Comparison with GISAID sequences:

The analysis of 2,186 sequences obtained from GISAID, with collection dates between February 2020 and December 2023, has shown that the first four months of the pandemic in Lebanon were dominated by B.1 (65%), B.1.1 (22.5%), and B.4 (7.5%) lineages, among a total of 40 sequences recovered in this period (Figure 5).

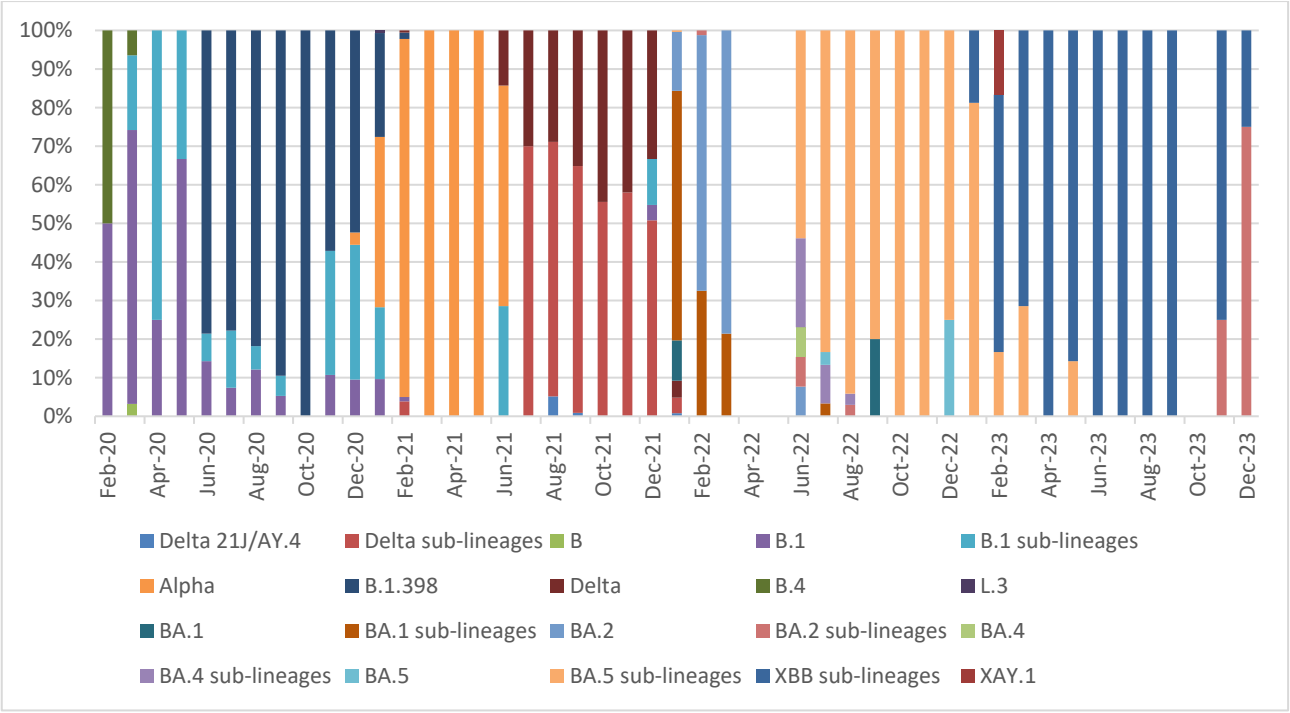

Figure 8: Epidemics of COVID-19 from February 2020 to December 2023 in Lebanon, among 2,186 available sequences in the GISAID database. B.1 sub-lineages correspond to B1.1, B.1.1.1, B.1.1.297, B.1.1.315, B.1.1.318,

B.1.1.323, B.1.160, B.1.177, B.1.15, B.1.17, B.1.18, B.1.177.53, B.1.177.77, B.1.2, B.1.22, B.1.243, B.1.221.2, B.1.258, B.1.36, B.1.36.1, B.1.36.35, B.1.441, B.1.428.3 and C.36.3 lineages, that were available on GISAID From February 2020 to December 2021. Delta sub-lineages correspond to AY.4, AY.5, AY.7.1, AY.16, AY.23, AY.33, AY.34, AY.34.1, AY.38, AY.39, AY.39.1, AY.43, AY.44, AY.46.4, AY.73, AY.98.1, AY.103, AY.106, AY.112, AY.116, AY.120, AY.121, AY.122, AY.126 and AY.127. BA.1 sub-lineages correspond to BA.1.1, BA.1.14, BA.1.15, BA.1.15.1, BA.1.17, BA.1.17.2, BA.1.18, BA.1.1.1, BA.1.1.10, BA.1.1.14 and BA.1.1.15. BA.2 lineages correspond to BA.2.12, BA.2.56, BA.2.74, BA.2.86.3, BN.1, BN.1.3.5, BN.1.4.2, JN.1 and JN.1.60. BA.4 sub-lineages correspond to BA.4.1 and BA.4.6. BA.5 sub-lineages correspond to BA.5.1, BA.5.1.1, BA.5.1.17, BA.5.1.23, BA.5.2, BA.5.2.1, BA.5.2.20, BA.5.2.2, BA.5.2.34, BA.5.2.47, BA.5.5, BE.1, BE.1.1, BF.5, BF.7.21, BF.10, BQ.1.1, BQ.1.1.18, BQ.1.1.32, BQ.1.5, BQ.1.25, BQ.1.1.29, CL.1 and CL.1.3. XBB sub-lineages correspond to XBB.1.16, XBB.1.16.19, XBB.1.16.2, XBB.1.16.8, XBB.1.16.17, XBB.1.17.1, XBB.1.42.1, XBB.1.5, XBB.1.5.20, XBB.1.5.98, XBB.1.9.1, XBB.2.3.11, EG.4, EG.4.3, EG.5.1.4, EL.1, FL.2, FL.10, FL.13, FL.36, FY.5, GW.5, and JD.1.1.1. The figure is created with Microsoft Excel software.

Subsequently, the lineage B.1.398 has dominated till the end of the year (corresponding to 174 B.1.938 sequences among a total of 235 sequences recuperated between June and December 2020). The Alpha variant began to appear in December 2020 and became prevalent from January to June 2021 (718/815 sequences available in this period). The Delta variant appeared first in February 2021, but the number of Delta sequences remained relatively low till June 2021 (only 2 sequences), to dominate along with its subvariants, till the end of the year (547/567 sequences available in this period). The subvariant Delta 21J/AY.4 appeared in August 2021, with only eight available sequences till January 2022. BA.1 and its sub-lineages presented 75.1% of the available sequences in January 2022, while 15.26% of the sequences belonged to the BA.2 Omicron subvariant. BA.1 sub-lineages were also detected in February and March 2022, with a percentage of 32.53% and 21.43% respectively. Meanwhile, BA.2 was the prevalent lineage in these two months, with a proportion of 67.47% and 78.57% respectively. Notably, no viral sequences have been detected in April and May 2022 in GISAID. The number of the total BA.4 sequences among the Lebanese dataset was only eight, recuperated in June, July, and August 2022. BA.5 and its sub-lineages were the prevalent lineages in this period, till January 2023 with a percentage of 85.84%, while BA.2 and its sub-lineages were still detected till August 2022. The recombinant BA.2 lineage XBB (recombinant lineage of BJ.1 (BA.2.10.1.1) and BM.1.1.1 (BA.2.75.3.1.1.1)) appeared at the beginning of 2023 and by February and became the prevalent variant till September 2023 (57/62 of the sequences corresponding to 91.93%). No available sequences were registered in October 2023. BA.2 sub-lineages and XBB were the only available lineages in the last two months of the year.

4. Discussion

This investigation screens, using a specific RT-qPCR technique, the circulating SARS-CoV-2 variants during the first three years of the COVID-19 pandemic in Lebanon. It has shown that all of the tested variants namely Alpha, Delta, Delta 21J/AY.4, and Omicron subvariants (BA.1, BA.2, BA.4, and BA.5), were observed gradually among our samples, drawing out the

story events of the SARS-CoV-2's epidemiology within the country.

Our study has shown that no VOC was detected between July 2020 and January 2021, the period that was dominated by B.1.398 lineage as mentioned in our previous investigation [23]. This may suggest that our samples between July and December 2020, were dominated by this same lineage that was associated with an increase in the number of COVID-19 in June 2020 [23, 26]. A second sharp increase in the number of cases was observed in January 2021 and was associated with the dominance of the Alpha variant, replacing the B.1.398 lineage [23, 26]. In July 2021, a new increasing wave in the number of cases was associated with the replacement of the Alpha variant with the Delta variant and its subvariants [23, 26]. In contrast to the previous analysis and GISAID data, our study has shown that the Delta variant began to be detected earlier, in January 2021, and dominated from February to March 2021, when one single Delta sequence was registered in GISAID among a total of 180 sequences available in these two months. This period (February-March 2021) was associated with a surge of new cases, but less intense than the previous one in January [26]. Notably, all of the Delta-positive samples in this period were negative when tested with Delta 21J/AY.4, reflecting that the prevalent variant was the parental Delta variant with P681R S mutation. The amino acid mutation T77A seen in Delta 21J/AY.4 is located in the transmembrane (TM) domain of the Nsp6. Mutations in this protein are predicted to enhance viral infectivity, due to its role in inducing autophagy [27].

Subsequently, our study shows that the Alpha variant had the highest count among tested variants in April and May 2021, before Delta redominated from June 2021, till the end of the year, replacing the Alpha variant. This was concordant to our analysis of the Lebanese sequences. It is worth mentioning that in our study, the subvariant Delta 21J/AY.4 began to be detected in June 2021, and was predominant till early January 2022. The mentioned variant (Delta 21J/AY.4) was detected among previous GISAID sequences in August and September 2021 and January 2022, with a total of 8 sequences only. Our study has added 128 new Delta21J/AY.4 cases between June 2021 and January 2022.

GISAID Lebanese sequences revealed that, by December 2021, a substitution of the Delta variant by the Omicron variant was observed; BA.1 and its sub-lineages dominated in January 2022, and still in February and March 2022. Similarly to our study, Omicron BA.1 subvariant was observed for the first time in December 2021 [23, 26]. It has the highest count in the first two months of 2022. Meanwhile, Lebanon witnessed a huge increase in the infected population, reaching a peak of 8,257 seven-day average of new cases in February 2022 [26], which may be related to a new wave of SARS-CoV-2 infection with the BA.1 variant. Meanwhile, Omicron subvariants BA.1, BA.2, and BA.3 were first identified in November 2021 in South Africa [28]. Interestingly, all of the BA.1-positive samples tested previously with

TaqPath COVID-19 CE-IVD RT-PCR Kit targeting N, S, and ORF1ab, have shown an SGTF, or S dropout related to the 69–70del mutation in the S gene associated with the Omicron BA.1 subvariant [29]. This Omicron subvariant was also related to atypical increased infectivity and an immune evasion capability, due to the accumulation of a large number of mutations in its genome, especially those occurring in the receptor binding domain (RBD) of the S protein, essential for viral attachment to the host cell and infection [30].

Soon after, BA.2 was detected by RT-qPCR in late January and February 2022 but the number of cases was still relatively low, while this subvariant prevailed among the Lebanese sequences in February and March 2022. Later on, BA.2 predominated in the last three months of our investigation, namely, December 2022, January, and February 2023, in parallel with a small increase in the number of daily cases, reaching 219.43 seven-day rolling average of daily new cases on January 21, 2023. During these three months, XBB subvariants counted 26.92% of the GISAID sequences (7/26), while BA.5 and its sub-lineages dominated (69.23%). This may suggest that the BA.2 subvariant detected with the specific RT-qPCR, could refer to the recombinant BA.2 lineage XBB, as GISAID data shows the presence of this lineage instead of the parental BA.2 during the concerned period. Indeed, XBB was prevalent in India and Singapore in December 2022 and has spread to many countries [31]. XBB and its sub-lineages were related to an increased transmissibility as well as an improvement in the immune escape [32]. WHO has classified XBB as an Omicron subvariant under monitoring as of October 28, 2022 [33]. Contrary to BA.1, BA.2-positive samples didn't show the characteristic of SGTF as this Omicron subvariant does not exhibit the 69-70del mutation in its genome [34]. The spike gene of BA.2 has 32 mutations in common with BA.1 and 28 unique ones. It was associated with a significant increase in transmissibility and vaccine breakthrough compared to the BA.1 subvariant [30].

Notably, no positive COVID-19 cases were detected in our samples in June 2022, when the number of national cases was relatively low (80.14-619.71 seven-day average new cases) [26]. Besides, a limited number of BA.4 (3 cases) were found by RT-qPCR in September, October, and November 2022. Also in the Lebanese GISAID sequences, there were only eight BA.4 sequences recuperated in June, July, and August 2022. On the other side, the BA.5 Omicron subvariant was associated with the highest headcount among the tested Omicron subvariants (70 BA.5-positive samples) and dominated in July, September, and October 2022. An increase in COVID-19 cases was coincidently documented in July, reaching a peak of 2,372.71 seven-day rolling average of daily new cases on the 31st of the month [26]. Consequently, the number of cases decreased in August, then increased by January 2023, the period of the BA.2 variant predomination. BA.4 and BA.5 subvariants were detected for the

first time in South Africa in early 2022 [35]. These two subvariants accumulate six mutations in the S protein, differing them from the BA.2 subvariant [36]. They have shown more transmissibility compared with BA.2 [36].

Our findings were similar to those observed in a previous investigation of SARS-CoV-2 circulating variants in the flu season, between September 2022 and August 2023 [25]. It has shown that BA.5 sub-lineages (BA.5.2, BA.5.2.20, BA.5.2.25, and BQ.1.1.5) prevailed in September, October, and November 2022 [25]. BA.2.75.2, a sub-lineage of the BA.2 variant, was also observed during October, concordantly with our findings [25]. Moreover, December 2022 and January 2023 were dominated by BA.2 sub-lineages (BA.2.75.5, BN.1, BN.1.4, and CH.1.1, CM.4) and BA.5 sub-lineages (BQ.1, BQ.1.1, BQ.1.1.23); BA.2 recombinant variant XBK was detected as well. As of February 2023, recombinant BA.2 lineage XBB and its sub-lineages began to dominate (XBB.1, XBB.1.5, XBB.1.5.2, XBB.1.5.3, XBB.1.9, XBB.1.9.1, XBB.1.9.2, XBB.1.16, XBB.1.22 and XBB.1.42) [25].

Overall, our findings reflect a concordant image to that observed with the WGS results of the circulating SARS-CoV-2 lineages in Lebanon. Even though WGS remained the gold standard tool for detecting and screening SARS-CoV-2's mutations and variants, it is a high-cost and time-consuming technique, requiring a compliant staff and specific material [37]. RT-qPCR targeting specific variants could be a more affordable and practical substitutional technique, notably in developing countries such as Lebanon, which is living now an economic and financial crisis ranking among the worst globally since the mid-nineteenth century [38]. In addition, it can be used as a rapid screening technique in the periods of the apparition of new viral waves.

Finally, our investigation may be limited by not covering all the months targeted in our study period, due to the absence of suitable samples in some of them. Besides, specific PCR targeting a specific mutation in the SARS-CoV-2 genome can detect the potential VOC in question but does not reveal the possible presence of sub-lineage or recombinant lineage. Also, the mutations used to define the lineages may be shared with other lineages, which can limit its wide implementation as a surrogate of WGS. Although a concordant figure with GISAID sequences was obtained, we haven't tested each sample with all the variants targeting primers due to a shortage of reagents and time; restricting thus our conclusions.

5. Conclusion:

This study has investigated the circulating SARS-CoV-2 VOCs in the first three years of the pandemic in Lebanon, using specific VOCs and Omicron subvariants-defining RT-qPCR, and shown concordant results with GISAID Lebanese sequences. Our findings shed light on the

importance of such an approach, being a reliable and useful tool, particularly in low-income countries. Nonetheless, WGS would be an important next step to validate our results and detect the exact subvariants and the recombinant ones. Moreover, screening of newly collected samples is needed to unravel the identity of currently circulating SARS-CoV-2 lineages in the country.

References

1. The World Health Organization (WHO). WHO COVID-19 dashboard [June 24, 2024]. Available from: https://data.who.int/dashboards/covid19/.

2. The World Health Organization (WHO). Coronavirus disease (COVID-19) pandemic [July 7, 2024]. Available from: https://www.who.int/europe/emergencies/situations/covid-19#:~:text=Cases%20of%20novel%20coronavirus%20(nCoV,pandemic%20on%2011%20March%202020.

3. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, *et al*. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9. Epub 2020/02/06. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508; PubMed Central PMCID: PMCPMC7094943.

4. Ellis P, Somogyvari F, Virok DP, Noseda M, McLean GR. Decoding Covid-19 with the SARS-CoV-2 Genome. Curr Genet Med Rep. 2021;9(1):1-12. Epub 2021/01/19. doi: 10.1007/s40142-020-00197-5. PubMed PMID: 33457109; PubMed Central PMCID: PMCPMC7794078.

5. Yuce M, Filiztekin E, Ozkaya KG. COVID-19 diagnosis -A review of current methods. Biosens Bioelectron. 2021;172:112752. Epub 2020/10/31. doi: 10.1016/j.bios.2020.112752. PubMed PMID: 33126180; PubMed Central PMCID: PMCPMC7584564.

6. Eskier D, Karakulah G, Suner A, Oktay Y. RdRp mutations are associated with SARS-CoV-2 genome evolution. PeerJ. 2020;8:e9587. Epub 2020/08/04. doi: 10.7717/peerj.9587. PubMed PMID: 32742818; PubMed Central PMCID: PMCPMC7380272.

7. The World Health Organization (WHO). Updated working definitions and primary actions for SARS-CoV-2 variants 2023 [June 6, 2024]. Available from: https://www.who.int/publications/m/item/updated-working-definitions-and-primary-actions-for--sars-cov-2-variants.

8. (WHO) TWHO. Historical working definitions and primary actions for SARS-CoV-2 variants 2023 [June 6, 2024]. Available from: https://www.who.int/publications/m/item/historical-working-definitions-and-primary-actions-for-sars-cov-2-variants.

9. The World Health Organization (WHO). Genomic sequencing of SARS-CoV-2 2021 [July 7, 2024]. Available from: https://www.who.int/publications/i/item/9789240018440.

10. Helmy M, Awad M, Mosa KA. Limited resources of genome sequencing in developing countries: Challenges and solutions. Appl Transl Genom. 2016;9:15-9. Epub 2016/06/30. doi: 10.1016/j.atg.2016.03.003. PubMed PMID: 27354935; PubMed Central PMCID: PMCPMC4911431.

11. Matic N, Lowe CF, Ritchie G, Stefanovic A, Lawson T, Jang W, *et al*. Rapid Detection of SARS-CoV-2 Variants of Concern, Including B.1.1.28/P.1, British Columbia, Canada. Emerg Infect Dis. 2021;27(6):1673-6. Epub 2021/03/31. doi: 10.3201/eid2706.210532. PubMed PMID: 33784237; PubMed Central PMCID: PMCPMC8153894.

12. Munoz-Gallego I, Melendez Carmona MA, Martin Higuera C, Viedma E, Delgado R, Folgueira MD. Rapid screening of SARS-CoV-2 variants, a key tool for pandemic surveillance. Sci Rep. 2023;13(1):11094. Epub 2023/07/09. doi: 10.1038/s41598-023-37866-8. PubMed PMID: 37422474;

PubMed Central PMCID: PMCPMC10329708.

13. Islam MT, Alam ARU, Sakib N, Hasan MS, Chakrovarty T, Tawyabur M, *et al*. A rapid and cost-effective multiplex ARMS-PCR method for the simultaneous genotyping of the circulating SARS-CoV-2 phylogenetic clades. J Med Virol. 2021;93(5):2962-70. Epub 2021/01/26. doi: 10.1002/jmv.26818. PubMed PMID: 33491822; PubMed Central PMCID: PMCPMC8014803.

14. Nawab M, Riaz SK, Ismail E, Ahamed A, Tariq A, Malik MFA, *et al*. Integrated approach for detection of SARS-CoV-2 and its variant by utilizing LAMP and ARMS-PCR. Ann Clin Microbiol Antimicrob. 2024;23(1):11. Epub 2024/02/02. doi: 10.1186/s12941-023-00665-0. PubMed PMID: 38303011; PubMed Central PMCID: PMCPMC10836012.

15. Lee E, Kim EJ, Shin YK, Song JY. Design and testing of multiplex RT-PCR primers for the rapid detection of influenza A virus genomic segments: Application to equine influenza virus. J Virol Methods. 2016;228:114-22. Epub 2015/12/15. doi: 10.1016/j.jviromet.2015.11.012. PubMed PMID: 26655588.

16. Wang W, Ren P, Mardi S, Hou L, Tsai C, Chan KH, *et al*. design of multiplexed detection assays for identification of avian influenza a virus subtypes pathogenic to humans by SmartCycler real-time reverse transcription-PCR. J Clin Microbiol. 2009;47(1):86-92. Epub 2008/10/31. doi: 10.1128/JCM.01090-08. PubMed PMID: 18971359; PubMed Central PMCID: PMCPMC2620859.

17. Brister H, Barnum SM, Reedy S, Chambers TM, Pusterla N. Validation of two multiplex real-time PCR assays based on single nucleotide polymorphisms of the HA1 gene of equine influenza A virus in order to differentiate between clade 1 and clade 2 Florida sublineage isolates. J Vet Diagn Invest. 2019;31(1):137-41. Epub 2019/02/26. doi: 10.1177/1040638718822693. PubMed PMID: 30803412; PubMed Central PMCID: PMCPMC6505758.

18. Abdel Sater F, Younes M, Nassar H, Nguewa P, Hamze K. A rapid and low-cost protocol for the detection of B.1.1.7 lineage of SARS-CoV-2 by using SYBR Green-based RT-qPCR. Mol Biol Rep. 2021;48(11):7243-9. Epub 2021/10/07. doi: 10.1007/s11033-021-06717-y. PubMed PMID: 34613565; PubMed Central PMCID: PMCPMC8493944.

19. Health LMoP. Novel Coronavirus 2019-Lebanon [June 25, 2024]. Available from: https://www.moph.gov.lb/en/Pages/2/24870/novel-coronavirus-2019.

20. The World Health Organization (WHO). WHO COVID-19 dashboard-Lebanon [June 25, 2024]. Available from: https://data.who.int/dashboards/covid19/deaths?m49=422&n=o.

21. Feghali R, Merhi G, Kwasiborski A, Hourdel V, Ghosn N, Tokajian S. Genomic characterization and phylogenetic analysis of the first SARS-CoV-2 variants introduced in Lebanon. PeerJ. 2021;9:e11015. Epub 2021/10/07. doi: 10.7717/peerj.11015. PubMed PMID: 34611501; PubMed Central PMCID: PMCPMC8447710.

22. Fayad N, Abi Habib W, Kandeil A, El-Shesheny R, Kamel MN, Mourad Y, *et al*. SARS-CoV-2 Variants in Lebanon: Evolution and Current Situation. Biology (Basel). 2021;10(6). Epub 2021/07/03. doi: 10.3390/biology10060531. PubMed PMID: 34198622; PubMed Central PMCID: PMCPMC8232177.

23. Nour D, Rafei R, Lamarca AP, de Almeida LGP, Osman M, Ismail MB, *et al*. The Role of

Lebanon in the COVID-19 Butterfly Effect: The B.1.398 Example. Viruses. 2022;14(8). Epub 2022/08/27. doi: 10.3390/v14081640. PubMed PMID: 36016262; PubMed Central PMCID: PMCPMC9412248.

24. Merhi G, Trotter AJ, de Oliveira Martins L, Koweyes J, Le-Viet T, Abou Naja H, *et al*. Replacement of the Alpha variant of SARS-CoV-2 by the Delta variant in Lebanon between April and June 2021. Microb Genom. 2022;8(7). Epub 2022/07/26. doi: 10.1099/mgen.0.000838. PubMed PMID: 35876490; PubMed Central PMCID: PMCPMC9455693.

25. Kodsi IA, Rayes DE, Koweyes J, Khoury CA, Rahy K, Thoumi S, *et al*. Tracking SARS-CoV-2 variants during the 2023 flu season and beyond in Lebanon. Virus Res. 2024;339:199289. Epub 2023/12/01. doi: 10.1016/j.virusres.2023.199289. PubMed PMID: 38036064; PubMed Central PMCID: PMCPMC10704499.

26. Our World in Data. Lebanon: Coronavirus Pandemic Country Profile [June 29, 2024]. Available from: https://ourworldindata.org/coronavirus/country/lebanon#what-is-the-daily-number-of-confirmed-cases.

27. fooladinezhad H, Shahidi M, Mahmanzar M, Mahdavi B, Tokhanbigli S, Moradi B, *et al*. SARS-CoV-2 NSP3, NSP4 and NSP6 mutations and Epistasis during the pandemic in the world: Evolutionary Trends and Natural Selections in Six Continents,. Preprint at medRXiv. 2022. doi: https://doi.org/10.1101/2022.05.22.22275422.

28. Desingu PA, Nagarajan K, Dhama K. Emergence of Omicron third lineage BA.3 and its importance. J Med Virol. 2022;94(5):1808-10. Epub 2022/01/20. doi: 10.1002/jmv.27601. PubMed PMID: 35043399; PubMed Central PMCID: PMCPMC9015590.

29. Eggink D, Andeweg SP, Vennema H, van Maarseveen N, Vermaas K, Vlaemynck B, *et al*. Increased risk of infection with SARS-CoV-2 Omicron BA.1 compared with Delta in vaccinated and previously infected individuals, the Netherlands, 22 November 2021 to 19 January 2022. Euro Surveill. 2022;27(4). Epub 2022/01/29. doi: 10.2807/1560-7917.ES.2022.27.4.2101196. PubMed PMID: 35086609; PubMed Central PMCID: PMCPMC8796294.

30. Chen J, Wei GW. Omicron BA.2 (B.1.1.529.2): High Potential for Becoming the Next Dominant Variant. J Phys Chem Lett. 2022;13(17):3840-9. Epub 2022/04/26. doi: 10.1021/acs.jpclett.2c00469. PubMed PMID: 35467344; PubMed Central PMCID: PMCPMC9063109.

31. The World Health Organization (WHO). TAG-VE statement on Omicron sublineages BQ.1 and XBB 2022 [July 4, 2024]. Available from: https://www.who.int/news/item/27-10-2022-tag-ve-statement-on-omicron-sublineages-bq.1-and-xbb#:~:text=The%20two%20sublineages%20remain%20part,Member%20States%20and%20the%20public.&text=XBB*%20is%20a%20recombinant%20of%20BA.

32. Zhang QE, Lindenberger J, Parsons R, Thakur B, Parks R, Park CS, *et al*. SARS-CoV-2 Omicron XBB lineage spike structures, conformations, antigenicity, and receptor recognition. bioRxiv. 2024. Epub 2024/02/26. doi: 10.1101/2024.02.12.580004. PubMed PMID: 38405707; PubMed Central PMCID: PMCPMC10888797.

33. The World Health Organization (WHO). Tracking SARS-CoV-2 variants [July 4, 2024].

Available from: https://www.who.int/activities/tracking-SARS-CoV-2-variants.

34. Subramoney K, Mtileni N, Bharuthram A, Davis A, Kalenga B, Rikhotso M, *et al*. Identification of SARS-CoV-2 Omicron variant using spike gene target failure and genotyping assays, Gauteng, South Africa, 2021. J Med Virol. 2022;94(8):3676-84. Epub 2022/04/21. doi: 10.1002/jmv.27797. PubMed PMID: 35441368; PubMed Central PMCID: PMCPMC9088381.

35. Parums DV. Editorial: World Health Organization (WHO) Variants of Concern Lineages Under Monitoring (VOC-LUM) in Response to the Global Spread of Lineages and Sublineages of Omicron, or B.1.1.529, SARS-CoV-2. Med Sci Monit. 2022;28:e937676. Epub 2022/07/02. doi: 10.12659/MSM.937676. PubMed PMID: 35775166; PubMed Central PMCID: PMCPMC9254723.

36. Tallei TE, Alhumaid S, AlMusa Z, Fatimawali, Kusumawaty D, Alynbiawi A, *et al*. Update on the omicron subvariants BA.4 and BA.5. Rev Med Virol. 2023;33(1):e2391. Epub 2022/08/27. doi: 10.1002/rmv.2391. PubMed PMID: 36017597; PubMed Central PMCID: PMCPMC9539252.

37. Mintzer V, Moran-Gilad J, Simon-Tuval T. Operational models and criteria for incorporating microbial whole genome sequencing in hospital microbiology - A systematic literature review. Clin Microbiol Infect. 2019;25(9):1086-95. Epub 2019/05/01. doi: 10.1016/j.cmi.2019.04.019. PubMed PMID: 31039443.

38. Lebanon TWBI. Overview 2022 [July 4, 2024]. Available from: https://www.worldbank.org/en/country/lebanon/overview.

Chapter 4: Evaluation of SARS-CoV-2 anti-Spike antibody levels and breakthrough infection risk among vaccinated adults in North Lebanon

Introduction

Several varieties of vaccines have been designed to prevent the new coronavirus disease COVID-19 from spreading and infecting more people, including mRNA-based vaccines, viral-vector-based vaccines, viral protein subunits, live attenuated viruses, inactivated viruses, and DNA vaccines [1].

On February 14, 2021, the Lebanese Ministry of Public Health began a vaccination campaign in Lebanon, using firstly Comirnaty® (BNT162b2, Pfizer/BioNTech) vaccine [2]. Subsequently, Vaxzevria® (AZD1222) -AstraZeneca/University of Oxford, Sputnik V® (Gam-COVID-Vax), and BBIBP-CorV® (Wuhan Sinopharm/Chinese Academy of Science) vaccines were introduced as well [2]. BNT162b2 is an m-RNA vaccine, containing mRNA encoding the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) antigen [4]. While AZD1222 and Gam-COVID-Vax belong to the adenovirus vector vaccines, implicating a modified adenoviral DNA producing SARS-CoV-2 S proteins in the recipient cells [4]. As of November 26, 2023, 35% of the Lebanese population was vaccinated with a complete primary series of a COVID-19 vaccine [3]. A previous Lebanese investigation has shown that BTN162b2 was more effective in protecting against the Delta variant infection than Gam-COVID-Vax. Meanwhile, a lower protection level was procured by BTN162b2 against the Omicron variant, and Gam-COVID-Vax didn't show any protection [30]. Nonetheless, the characteristics of the immune response provided by vaccination against SARS-CoV-2 in Lebanon remain ambiguous.

For this, in the present chapter, we present a published article paper, in which we evaluated the progression of anti-spike SARS-CoV-2 antibody titers at two-time points (T1: shortly after the second vaccination dose, T2: six months later), in a population of 201 vaccinated adults in North Lebanon, using the Elecsys-Anti-SARS-CoV-2 S assay (Roche Diagnostics, Switzerland). The three vaccines involved in this study were BNT162b2, AZD1222, and Gam-COVID-Vax vaccines. We then predicted determinants of the decrease in the immune response. Breakthrough infection was also investigated against participants to compare the vaccine's effectiveness.

References

1. Soleimanpour S, Yaghoubi A. COVID-19 vaccine: where are we now and where should we go? Expert Rev Vaccines. 2021;20(1):23-44. Epub 2021/01/14. doi: 10.1080/14760584.2021.1875824. PubMed PMID: 33435774; PubMed Central PMCID: PMCPMC7898300.

2. France 24. Lebanon starts Covid-19 vaccination campaign 2021 [cited 2023 18/8/2023]. Available from: https://www.france24.com/en/middle-east/20210214-lebanon-starts-covid-19-vaccination-campaign.

3. The World Health Organization (WHO). WHO COVID-19 dashboard-Lebanon [June 25, 2024]. Available from: https://data.who.int/dashboards/covid19/deaths?m49=422&n=o.

4. Heinz FX, Stiasny K. Distinguishing features of current COVID-19 vaccines: knowns and unknowns of antigen presentation and modes of action. NPJ Vaccines. 2021;6(1):104. Epub 2021/08/18. doi: 10.1038/s41541-021-00369-6. PubMed PMID: 34400651; PubMed Central PMCID: PMCPMC8368295.

Article

Evaluation of SARS-CoV-2 anti-Spike antibody levels and breakthrough infection risk among vaccinated adults in North Lebanon

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This paper is published in ***"PLoS ONE"* journal** on May 9th, 2024

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RESEARCH ARTICLE

Evaluation of SARS-CoV-2 anti-Spike antibody levels and breakthrough infection risk among vaccinated adults in North Lebanon

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Citation: Nour D, Ismail MB, Osman M, Rafei R, Kasir D, Dabboussi F, et al. (2024) Evaluation of SARS-CoV-2 anti-Spike antibody levels and breakthrough infection risk among vaccinated adults in North Lebanon. PLoS ONE 19(5): e0302579. https://doi.org/10.1371/journal.pone.0302579

Editor: Ashraful Hoque, Sheikh Hasina National Institute of Burn & Plastic Surgery, BANGLADESH

Received: December 9, 2023

Accepted: April 4, 2024

Published: May 9, 2024

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Data Availability Statement: The raw data R codes necessary to replicate the analysis are publicly available (DOI: 10.5281/zenodo.8266879).

Funding: This research was funded by the Lebanese University, (COVID-19 project), Sanabel Nour associa-tion, and Fondation Me´rieux. D.N. is a Ph.D. student supported by a scholarship from the GABRIEL network and Fondation Me´rieux. The funders had no role in study design, data collection

Abstract

Since March 2020, the COVID-19 pandemic has swiftly propagated, triggering a competitive race among medical firms to forge vaccines that thwart the infection. Lebanon initiated its vaccination campaign on February 14, 2021. Despite numerous studies conducted to elucidate the characteristics of immune responses elicited by vaccination, the topic remains unclear. Here, we aimed to track the progression of anti-spike SARS-CoV-2 antibody titers at two-time points (T1: shortly after the second vaccination dose, T2: six months later) within a cohort of 201 adults who received Pfizer-BioNTech (BNT162b2), AstraZeneca, or Sputnik V vaccines in North Lebanon. Blood specimens were obtained from participants, and antibody titers against SARS-CoV-2 were quantified through the Elecsys-Anti-SARS-CoV-2 S assay (Roche Diagnostics, Switzerland). We used univariate analysis and multivariable logistic regression models to predict determinants influencing the decline in immune response and the occurrence of breakthrough infections among vaccinated patients. Among the 201 participants, 141 exhibited unchanging levels of antibody titers between the two sample collections, 55 displayed waning antibody titers, and only five participants demonstrated heightened antibody levels. Notably, age emerged as the sole variable significantly linked to the waning immune response. Moreover, the BNT162b2 vaccine exhibited significantly higher efficacy concerning the occurrence of breakthrough infections when compared with the AstraZeneca vaccine. Overall, our study reflected the immune status of a sample of vaccinated adults in North Lebanon. Further studies on a larger scale are needed at the national level to follow the immune response after vaccination, especially after the addition of the third vaccination dose.

and analysis, decision to publish, or preparation of the manuscript.

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

Introduction

In December 2019, an outbreak of atypical pneumonia was reported in Wuhan City, China [1]. Its rapid spread across the country and around the world led the World Health Organization (WHO), in March 2020, to identify this new infectious disease of Corona Virus (COVID-19), a global pandemic [2]. As of 22 November 2023, the Coronavirus disease (COVID-19), caused by the Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), has attacked more than 772 million people and its-related cumulative deaths have exceeded 6.9 million around the world [3]. The genome codes for 4 structural proteins: Spike protein (S), Nucleocapsid (N), Membrane (M), and Envelope (E), and 16 non-structural proteins noted (nsp1-16) [4]. This virus enters the target cell after binding its Receptor Binding domain (RBD), present on the S1 subunit of S glycoprotein, with Angiotensin-Converting Enzyme 2 (ACE 2) receptor present on the surface of epithelial cells of the nasal cavity, to begin its viral replication. SARS-CoV-2 triggers the production of host antibodies that begins early against the N protein, while it takes 4–8 days after symptom onset to release antibodies against the S protein [5, 6].

Different types of COVID-19 vaccines have been designed. The first vaccine that has been FDA-approved for people above 18 years was Pfizer-BioNTech (BNT162b2) (now called Comirnaty). It is an mRNA-based vaccine surrounded by lipid nanoparticles and coding for the full-length spike protein of SARS-CoV-2, stimulating the immune response of the body against the virus [7, 8]. AstraZeneca-vaccine (ChAdOx1 nCoV-19), known by Vaxzevria and Covishield as trademarks, is a viral vector vaccine coding for a full-length S-Protein of SARS-CoV-2 [9, 10]. It is given also to individuals above 18 years old [11]. Sputnik V (Gam-COVID-Vac) vaccine is another example of a viral vector vaccine that encodes a full-length S-protein of SARS-CoV2 [12, 13]. As of 18 November 2023, more than 13,5 billion COVID-19 vaccines have been administered around the world according to the WHO [3].

The antibody levels developed against SARS-CoV-2 after infection or after vaccine intake can be measured by serological tests, based on the specific binding between antigen and antibodies [14]. Different methods can be used to detect anti-SARS-CoV-2 antibodies including the chemiluminescence immunoassay (CLIA) which represents a high-throughput assay that can test a large number of samples at the same time with accurate results and low signal-to-noise ratio [15, 16]. Elecsys[1] Anti-SARS-CoV-2 S developed by Roche, is one of the CLIA immunoassays that detects and quantifies total antibody titers of high affinity to SARS-CoV-2 S protein RBD with high sensitivity and specificity [17].

Lebanon started its vaccination campaign against COVID-19 on Sunday, February 14, 2021, using the BNT162b2 vaccine [18]. However, a notable lack of data prevails regarding SARS-CoV-2 antibody dynamics and the comparative efficacy of distinct vaccine types in Lebanon. To address this knowledge gap, this study aimed to monitor the level of anti-Spike SARS-CoV-2 antibody titers and breakthrough infections among a demographically diverse adult vaccinated population at two time points (referred to as T1 and T2), after the second booster vaccination dose. Notably, the detection of anti-Spike SARS-CoV-2 antibody titers was performed through the Elecsys-anti-SARS-CoV-2 S assay.

Materials and methods

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board (IRB) of the Nini Hospital (IRB-F01-29.3.2021; March 29, 2021). Written informed consent was obtained from all subjects involved in the study.

Study design

Initially, BNT162b2 emerged as Lebanon's only available COVID-19 vaccine, with a primary focus on safeguarding the elderly (>75 years) and healthcare professionals [19]. In this context, our recruitment efforts started with these two pivotal groups from various regions in North Lebanon, each of whom had received the BNT162b2 vaccination. Additional vaccine options were later available, including AstraZeneca and Sputnik V. Subsequently, our study population expanded to include a broader demographic, encompassing individuals of all ages above 18 from the same geographical area. The inclusion criteria for participation in the study include: Being aged 18 or above, having completed the full regimen of a singular COVID-19 vaccine type, with the second dose administered from 14 days to 3 months before the collection of blood samples for the study. Moreover, individuals were required to have no prior history of COVID-19 infection or any indicative symptoms.

Sampling and data collection

After a signed consent for the participation in the study, demographic, socio-economic, health, and vaccine-related data were collected from each eligible participant, using an online questionnaire. Afterwards, 2 blood samples were collected: the first one was collected from 14 to 30 days after taking the second dose of the vaccine (T1) and the second one was taken 6 months after (T2). The sampling was performed between April 1, 2021, and March 31, 2022. In total, we have recruited 216 participants: 140 have received the BNT162b2 vaccine, 54 have received the AstraZeneca vaccine, and 22 have received the Sputnik V vaccine. However, 15 cases were lost of follow-up, and thus, second blood samples were obtained from 201 participants. The number of participants enrolled in the study is shown in the following flowchart, based on the type of vaccine taken (Fig 1).

Elecsys® Anti-SARS-CoV-2 S assay

Anti-spike antibody titers were assessed at T1 and T2 using the Roche Elecsys® Anti-SARS-CoV-2 S test on the cobas™ e801 (Roche Diagnostics, Mannheim, Germany). The assay and

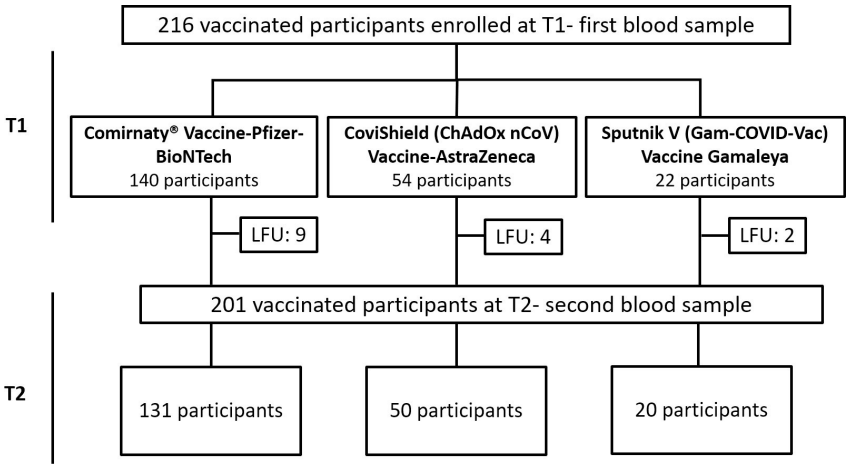

Fig 1. Flowchart showing the number of participants enrolled in the study based on the type of vaccine taken (Comirnaty® vaccine-BNT162b2, CoviShield (ChAdOx1 nCoV) vaccine-AstraZeneca, Sputnik V(Gam-COVID-Vac) vaccine-Gamaleya). LFU: Loss to follow-up.

https://doi.org/10.1371/journal.pone.0302579.g001

results interpretation were conducted according to the manufacturer's instructions: results <0.80 U/ml were considered negative for anti-SARS-CoV-2-S while those = or > 0.80 U/ml were considered positive. Results that exceed a value of 250 U/ml were recorded as > 250 U/ml. To note, the unit "U/ml" obtained in Elecsys® anti-SARS-CoV-2 S assay results is equivalent to WHO BAU/ml [17]. Comparative analysis was then done using Microsoft Excel software to determine the evolution of anti-Spike SARS-CoV-2 antibody titers between T1 and T2 in each participant.

Measuring breakthrough infections

After taking the second blood sample, participants were asked if they had developed COVID-19 symptoms and whether they had done a PCR test confirming an infection with SARS-CoV-2 in the period between the first (T1) and the second (T2) sampling, searching for any breakthrough infection after they have completed their vaccine regimen.

Statistical analysis

Data recorded in the questionnaire and the laboratory analysis were evaluated for completeness and consistency before analysis using the R software (R Core Team, version 4.2.2; R Studio, version 2023.06.1+524 "Mountain Hydrangea"). The dataset was imported for cleaning, variable coding, and subsequent analysis. Descriptive analysis was performed on all variables using several packages (e.g., dplyr, stringr, prettyR, summarytools). Data were presented as mean [standard deviation (SD); min-max] for continuous variables and as frequency distributions for categorical variables. To predict the determinants of a decrease in COVID-19 immunity among vaccinated patients over 6 months at the univariate level, we compared the differences across groups using the t-test and the Pearson chi-squared test for continuous and categorical variables, respectively. The decrease in COVID-19 immunity (yes/no) and the occurrence of a breakthrough infection among vaccinated patients (yes/no) were the outcomes and sociodemographic, behavioral, and clinical data were the explanatory variables. Subsequently, multivariable logistic regression analysis was done, including variables with a P-value (P) < 0.40 obtained at the univariate level. We also performed a backward stepwise model to identify and confirm the associations of covariates with the decrease in COVID-19 immunity. Statistical tests were two-sided, with a type I error set at $\alpha = 0.05$.

Results

Characteristics of the population

After excluding 15 individuals who were lost of follow-up from the rest of the statistical analysis, the total number of the population studied was 201 individuals. 131 were vaccinated with BNT162b2, 50 with AstraZeneca, and 20 with Sputnik V vaccines. As a data summary, 48.8% of the participants were women, and 51.2% were men. The mean age of the participants was 51.7 years (standard deviation = 16.6; min-max = [21–91]). Regarding smoking and alcoholism status, 39.8% were smokers and 13.4% were alcohol consumers. Data regarding body Mass Index (BMI), blood type, the presence of any chronic disease, and the symptoms developed after vaccine intake were also collected from participants. Anti-Spike SARS-CoV-2 antibody titers were stratified as follows: high (>249 U/ml), moderate (100–249 U/ml) and low (<100 U/ml). This stratification was based on previous studies having used Elecsys® Anti-SARS-CoV-2 S assay [20–23] (Table 1). Although we monitored the antibody levels (Fig 2), we assessed changes in the immune response between the initial and subsequent samplings by comparing them to the predefined levels of immune response (Table 1). To clarify, if the

Table 1. Sociodemographic and clinical characteristics of the study population, stratified by the temporal trend of COVID-19 antibodies.

	Total n = 201		Decrease in antibodies' trend n = 55		Stability in antibodies' trend n = 141		Increase in antibodies' trend n = 5	
	n	%	n	%	n	%	n	%
Antibodies' level (U/ml) (T_1¶)								
High (>249)	**176**	87.6	48	87.3	128	90.8	0	0.0
Moderate (100–249)	**10**	5.0	7	12.7	2	1.4	1	20.0
vLow (<100)	**15**	7.5	0	0.0	11	7.8	4	80.0
Antibodies' level (U/ml) (T_2 = 6 months)								
High (>249)	**133**	66.2	0	0.0	128	90.8	5	100.0
Moderate (100–249)	**35**	17.4	33	60.0	2	1.4	0	0.0
Low (<100)	**33**	16.4	22	40.0	11	7.8	0	0.0
Sex								
Female	**98**	48.8	27	49.1	68	48.2	3	60.0
Male	**103**	51.2	28	50.9	73	51.8	2	40.0
Age (years)								
<50	**99**	49.2	21	38.2	75	53.2	3	60.0
[50–75]	**82**	40.8	26	47.3	54	38.3	2	40.0
≥75	**20**	10	8	14.5	12	8.5	0	0.0
Smoking								
No	**121**	60.2	35	63.6	85	60.3	1	20.0
Yes	**80**	39.8	20	36.4	56	39.7	4	80.0
Alcohol consumption								
No	**174**	86.6	45	81.8	124	87.9	5	100.0
Yes	**27**	13.4	10	18.2	17	12.1	0	0.0
Body Mass Index (kg/m^2) (Mean [SD; min-max])	27.1 [5.8; 17.6–67.6]		27.3 [5.3; 19.1–46.9]		26.9 [6; 17.6–67.6]		29.2 [5.7; 21.5–35.5]	
Blood type & Rhesus factor								
Unknown	**15**	7.5	4	7.3	10	7.1	1	20.0
A^-	**7**	3.5	1	1.8	6	4.3	0	0.00
A^+	**57**	28.4	13	23.6	42	29.8	2	40.0
AB^+	**10**	5.0	4	7.3	6	4.3	0	0.0
B^-	**6**	3.0	2	3.6	4	2.8	0	0.0
B^+	**29**	14.4	6	10.9	22	15.6	1	20.0
O^-	**9**	4.5	3	5.5	5	3.5	1	20.0
O^+	**68**	33.8	22	40.0	46	32.6	0	0.0
Chronic disease								
No	**112**	55.7	29	52.7	82	58.2	1	20.0
Yes	**89**	44.3	26	47.3	59	41.8	4	80.0
Vaccine name								
Pfizer-BioNTech (BNT162b2)	**131**	65.2	37	67.3	93	66.0	1	20.0
AstraZeneca	**50**	24.9	10	18.2	36	25.5	4	80.0
Sputnik V	**20**	10.0	8	14.5	12	8.5	0	0.0
Side effects after the first shot								
No	**57**	28.4	18	32.7	39	27.7	0	0.0
Yes	**144**	71.6	37	67.3	102	72.3	5	100.0
Side effect or symptoms after second shot								

(*Continued*)

Table 1. (Continued)

	Total n = 201		Decrease in antibodies' trend n = 55		Stability in antibodies' trend n = 141		Increase in antibodies' trend n = 5	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
No	**62**	30.8	21	38.2	40	28.4	1	20.0
Yes	**139**	69.2	34	61.8	101	71.6	4	80.0
Breakthrough cases*								
No	**193**	96	54	98.2	135	95.7	4	80.0
Yes	**8**	4	1	1.8	6	4.3	1	20.0

*During the study period. ¶T1 ranges between 2 weeks and 2 months after receiving the COVID-19 booster dose; T2 represents a 6-month duration after the initial collection.

https://doi.org/10.1371/journal.pone.0302579.t001

antibody titer transitions from the high to the moderate level, it indicates a decrease in the immune response. Additionally, maintaining the same level of antibody titer (i.e., high, moderate, low) regardless of the antibody titer value between the first and second samplings is considered indicative of stability.

Evolution of SARS-CoV-2 anti-S antibody titers

Our results show that 55 of the participants (27.4%) confronted a decrease in the antibody titer between the first and second sampling, among them, 37 (67.3%) were vaccinated with BNT162b2, 10 have taken AstraZeneca (18.2%) and 8 were vaccinated with Sputnik V (14.6%). Only 5 participants have shown an increase in the antibody titers, among them 4 were vaccinated with AstraZeneca and 1 with BNT162b2. Stability in the antibody titers was detected in the remaining 141 participants (70.1%), with 90.8% among them maintaining a high antibody level. Notably, no negative results (i.e., SARS-CoV-2 anti-S antibody titer < 0.8 U/ml) have been detected neither in T1 nor in T2 results. The levels of anti-Spike SARS-CoV-2 antibody at both T1 and T2 and their evolution between these two-time points are shown in Fig 2.

Age was the only variable significantly associated with the decrease in immune levels (Table 2). The mean age of people who showed a significant decrease in SARS-CoV-2 anti-S antibody titers was 56.3 years (standard deviation = 16.0; min-max = [24–87]).

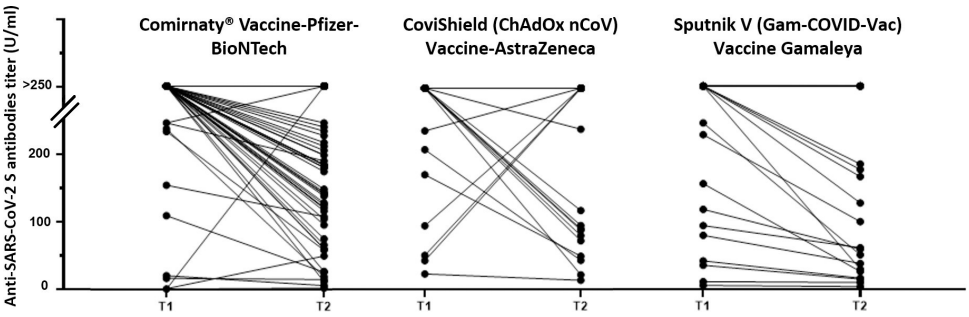

Fig 2. Levels of Anti-SARS-CoV-2 S antibody titers (U/ml) measured during two time points (T1 and T2). T1 ranges between 2 weeks and 2 months after receiving the COVID-19 booster dose. T2 represents a 6-month duration after the initial collection. These measurements were taken from participants who had received the Pfizer-BioNTech (BNT162b2), AstraZeneca, or Sputnik V vaccine. //The upper values (>200 U/ml) do not follow the same scale as the lower ones (≤ 200 U/ml).

https://doi.org/10.1371/journal.pone.0302579.g002

Table 2. Determinants of decrease in COVID-19 immunity among vaccinated patients over a 6-month period using univariate analysis and multivariable logistic regression models.

	Univariate analysis		Multivariable logistic regression models					
			Model 1[i]			Model 2[ii]		
Categorical variables	%	***P***	**adj. OR**	**95%CI**	***P***	**adj. OR**	**95%CI**	***P***
Sex								
Female[1]	28.4							
Male	27.8	1.00						
Smoking								
No[1]	29.2							
Yes	26.3	0.79						
Alcohol consumption								
No[1]	26.7							
Yes	37.0	0.37	1.67	0.67–3.99	0.26			
Blood type & Rhesus factor								
A^{-1}	28.6							
A^{+}	14.3							
AB^{+}	40.0							
B^{-}	33.3							
B^{+}	21.4							
O^{-}	37.5							
O^{+}	32.4	0.82						
Chronic disease								
No[1]	26.1							
Yes	30.6	0.60						
Vaccine type								
Pfizer-BioNTech (BNT162b2)[1]	28.5							
AstraZeneca	21.7		0.69	0.29–1.54	0.38			
Sputnik V	40.0	0.31	1.95	0.69–5.32	0.19			
Side effects after the first dose								
No[1]	31.6							
Yes	26.6	0.60						
Side effects after the booster dose								
No[1]	34.4							
Yes	25.2	0.25	0.75	0.38–1.53	0.42			
Breakthrough cases*								
No[1]	28.6							
Yes	14.3	0.68						
Continuous variables	*Decrease in antibodies' level*	*No decrease in antibodies' level*	***P***	**adj. OR**	**95%CI**	***P***	**adj. OR**	**95%CI**	***P***
Age	**56.3**	**49.9**	**0.02**	**1.02**	**1.00–1.04**	**0.04**	**1.02**	**1.00–1.04**	**0.02**
Body Mass Index (BMI)	27.3	26.9	0.62						

[¶]Determinants of decrease in COVID-19 immunity were predicted using univariate (t-test and the chi-squared test for continuous and categorical variables, respectively) and multivariable analysis (logistic regression models). [i]The variables tested by univariate analysis that had a P-value (P) < 0.40 were included in Model 1 (multivariable logistic regression analysis). [ii]In Model 2, a backward logistic regression model was created including only complete cases. [1]Reference group. *During the study period.

https://doi.org/10.1371/journal.pone.0302579.t002

Determinants of breakthrough infection

In total, among the 201 participants vaccinated with 2 doses, 29 reported COVID-19 symptoms between the first and second sampling time, and 16 among them underwent PCR leading to the confirmation of 8 breakthrough infections. Results are shown in Table 3 according to the 3 types of vaccine. Overall, eight individuals developed COVID-19-like symptoms over the study period and received a positive PCR result. The mean age of these participants was 51.4 years, with six and two being women and men, respectively. Moreover, five of them suffer from clinical comorbidities. Notably, although five of these cases suffered from comorbidities making them at risk for severe COVID-19, symptoms were moderate, and nobody developed severe disease.

After predicting the determinants of breakthrough infection with SARS-CoV-2 among the participants over the 6 months, the type of vaccine AstraZeneca was the only variable associated significantly with the appearance of breakthrough infections (Table 4). This means that the BNT162b2 vaccine has shown more effectiveness than the AstraZeneca vaccine about this parameter. Individuals who were vaccinated with Sputnik V were removed from this analysis because of their low total number.

Discussion

This study enrolled 201 individuals who were 2-dose vaccinated with one of these 3 types of vaccines: BNT162b2, AstraZeneca, or Sputnik V, in North Lebanon. Enrolled participants were not previously infected by SARS-CoV-2, meaning that their immune response is mainly induced by vaccination intake. SARS-CoV-2 antibody titers against Spike protein were evaluated in 2 different time points (T1 shortly after the second vaccine dose and T2, 6 months after), by Elecsys-Anti-SARS-CoV- 2 S assay, an accurate, highly specific, and sensitive CLIA assay. The 3 levels of stratification, high (>249 U/ml), moderate (100–249 U/ml), and low (<100 U/ml), for the anti-Spike SARS-CoV-2 antibody titers in our study were based on previous studies [20–23].

In our study, the majority (70.14%) of the participants have shown stability in the antibody titers between the first and second sampling, while a decrease in the immune response was observed in 27.4% of the participants. An increase in antibody titers was observed in 5 participants. In all of those 3 cases, antibody presence was still detectable after 6 months after the booster dose intake of the vaccines. Regarding the correlation between variables and the decrease in immune responses, our study shows that only age was significantly associated with this decrease.

This result was unsurprising because age is associated with the decline and dysregulation of many immune mechanisms, resulting in a deficit in both innate and adaptive immune responses [24]. A validation prospective, longitudinal cohort study was carried out by Lustig *et al*., who followed the immune responses of 2607 healthcare workers who tested negative for

Table 3. Number of participants developing COVID-19 symptoms between T1 and T2 and whether this was confirmed or not by a PCR test.

Vaccine type	Participants with COVID-19 symptoms	Symptomatic participants tested by PCR	Unconfirmed infection (negative or indeterminate PCR)	Confirmed infection (positive PCR)
Pfizer-BioNTech (BNT162b2)	14	9	6	3
AstraZeneca	12	6	1	5
Sputnik	3	1	1	-
Total	29	16	8	8

https://doi.org/10.1371/journal.pone.0302579.t003

Table 4. Determinants of breakthrough infections among vaccinated patients over a 6-month period using univariate analysis and multivariable logistic regression models.

	Univariate analysis		Multivariable logistic regression models					
			Model 1[i]			Model 2[ii]		
Categorical variables	%	***P***	**adj. OR**	**95%CI**	***P***	**adj. OR**	**95%CI**	***P***
Sex								
Female[1]	6.81							
Male	2.15	0.16	0.35	0.05–1.63	0.21	0.30	0.04–1.37	0.15
Smoking								
No[1]	3.57							
Yes	5.80	0.48						
Alcohol consumption								
No[1]	5.09							
Yes	0.00	0.60						
Blood type & Rhesus factor								
A^{-1}	0.00							
A^{+}	3.92							
AB^{+}	10.0							
B^{-}	0.00							
B^{+}	11.5							
O^{-}	0.00							
O^{+}	3.33	0.59						
Chronic disease								
No[1]	2.91							
Yes	6.41	0.29	2.48	0.55–13.1	0.25			
Vaccine type[¶]								
Pfizer-BioNTech (BNT162b2)[1]	**2.29**							
AstraZeneca	**10.0**	**0.04**	**5.35**	**1.22–27.9**	**0.03**	**4.75**	**1.11–24.2**	**0.04**
Side effects after the first shot								
No[1]	4.00							
Yes	4.58	1.00						
Continuous variables	*Breakthrough infection*	*No breakthrough infection*	***P***	**adj. OR**	**95%CI**	***P***	**adj. OR**	**95%CI**	***P***
Age (years)	51.4	52.2	0.86						
Body Mass Index (kg/m²)	28.1	27.1	0.74						

[¶]Determinants of breakthrough infections were predicted using univariate (t-test and the chi-squared test for continuous and categorical variables, respectively) and multivariable analysis (logistic regression models). [i]The variables tested by univariate analysis that had a P value (P) < 0.40 were included in Model 1 (multivariable logistic regression analysis). [ii]In Model 2, a backward logistic regression model was created including only complete cases. [1]Reference group. *During the study period. [¶]Individuals who had received a Sputnik V vaccination were removed from the analysis because of their low total number.

https://doi.org/10.1371/journal.pone.0302579.t004

anti-SARS-CoV-2 IgG before receiving the first dose of BNT162b2 vaccine. The follow-up was done 3 times: at 1–2 weeks after the first vaccine dose, at the time of the second dose administration, and at 1–2 weeks after the second dose. Results of this study showed that male sex, age, and comorbidities were associated with lower antibody levels [25]. Another retrospective cohort in the United States including a large number of individuals above 12 years (approximately 3.5 million people), followed the effectiveness of the BNT162b2 vaccine over time for up to 6 months after taking the vaccination. A decline in the vaccine effectiveness against SARS-CoV-2 infection has been recorded between the first month after full vaccination (88% of effectiveness (95% CI: 86–89)) and after 5 months (47% of effectiveness (95% CI:43–51)).

Vaccine effectiveness in individuals older than 65 years declined from 80% (95% CI: 73–58; first month) to 43% (95%CI: 30–54) [26]. In the same context, IgG antibody levels were followed up in a prospective longitudinal study 4 times after BNT162b2 vaccination: S1: 3 weeks after the first vaccine dose, S2: 1 month after the second dose, S3: 3 months after the second dose and S4: 6 months after the second dose [27]. Participants in this study had or did not have a pre-vaccination history of COVID-19 infection. A significant difference in antibody titers was detected between age groups; participants aged between 18 and 30 showed the highest antibody levels at all of the sampling times, while the lowest antibody titers were detected in participants older than 60 years at S1, S2, and S4, which is in concordance with our study [27]. However, these differences seem to decrease with time, were more accentuated at S1 sampling time, and lowered significantly after 6 months of the second booster dose. Nonetheless, this analysis did not include participants older than 66 years, who may develop lower antibody titers after the first and possibly the booster dose as well [27]. With regards to COVID-19 pre-vaccination status, a moderate negative correlation between age and antibody levels was detected at S1, and this correlation decreased with time for naïve COVID-19 participants. Individuals, who were previously infected with COVID-19, developed higher antibody levels at S1, S3, and S4 regardless of their age [27]. Moreover, a cohort study by Müller *et al*. comparing the immune response after the first and second dose of the BNT162b2 vaccine found that antibody titers were significantly higher in the group of younger participants (below 60 years) compared to elderly ones (over 80 years) [28].

On the other hand, a similar study conducted in Poland investigated the levels of anti-SARS-CoV-2 S antibodies in a population being infected or not with SARS-CoV-2, 8 months after taking the second dose of the BNT162b2 vaccine [29]. The results showed that convalescent participants developed a significant increase in the immune response that was associated with age, severity of COVID-19 symptoms, and their work type. In contrast to our results, the authors found that participants older than 50 years showed also higher levels of anti-SARS-CoV-2 S antibodies after the BNT162b2 vaccine intake in the group of participants non-previously infected with COVID-19, but this result was not statistically significant [29]. Furthermore, Subbarao *et al*. studied a population of adults aged 70 years and above vaccinated with the BNT162b2 vaccine, showing that anti-S SARS-CoV-2 antibody levels after 2 doses of the vaccine were very robust, significantly higher than levels of antibodies in convalescent participants previously infected with COVID-19 [30]. While, in the study of Tretyn *et al*. on individuals vaccinated by the BNT162b2 vaccine, no significant association was observed between age and anti-SARS-CoV-2 IgG antibody concentrations [31].

Further, our study has investigated the determinants of breakthrough infection in participants after 6 months of the booster vaccination dose. While Sputnik V vaccinated individuals were excluded from this analysis because of their low headcount, our data showed that the AstraZeneca vaccine was more associated with breakthrough infection than the BNT162b2 vaccine. Notably, our study was conducted from April 2021 to March 2022, a time during which Lebanon experienced successive domination by the Alpha, Delta, and Omicron variants [32]. We observed four breakthrough infection cases in November 2021, when the Delta variant was the predominant strain. The remaining four breakthrough infection cases occurred between December 2021 and January 2022, coinciding with the period dominated by the Omicron variant [32]. This observation may provide additional insights into the potential association between breakthrough infections and the Delta and Omicron variants. Indeed, although some data revealed that 2 doses of the BNT162b2 or AstraZeneca vaccines have the same effectiveness against SARS-CoV-2 Alpha and Delta variants [33], other studies reported that individuals vaccinated with BNT162b2 are more protected than those vaccinated with AstraZeneca. In this context, an observational study, conducted in the United Kingdom,

examined the effectiveness of 2-dose vaccines of either BNT162b2 or AstraZeneca, against infection with Alpha or Delta variants of SARS-CoV-2. Results showed that even though the effectiveness was reduced in Delta variant infection for both vaccines, the BNT162b2 vaccine has shown higher effectiveness for the 2 variants: (93.7% (95%CI: 91.6–95.3) for Alpha, and 88% (95%CI: 85.3–90.1) for Delta variant. However, the effectiveness of the AstraZeneca vaccine was 74.5% (95% CI: 68.4–79.4) for the Alpha variant and 67% (95%CI: 61.3–71.8) for Delta [34]. Besides, Stouten *et al*. showed that the 2 viral-vector vaccines JNJ-78436735 (Ad26.COV2.S) and AstraZeneca vaccine (ChAdOx1 nCoV-19) were associated with a higher risk of breakthrough infection compared to BNT162b2 [35]. This is concordant with a study conducted in Iraq, showing that the AstraZeneca vaccine was more associated with breakthrough infections in comparison with mixed vaccines or BNT162b2 vaccines [36]. A study in the United Kingdom including a group of vaccinated index patients and unvaccinated ones, compared the probability of infection of people in contact with these 2 groups. It showed that people vaccinated with BNT162b2 were at a lower probability of infecting their contacts than AstraZeneca vaccinated index patients, making BNT162b2 more protective than AstraZeneca [37, 38]. 192 individuals having received 2 doses of either BTN162b2 or AstraZeneca vaccine and a booster third dose of BTN162b2 vaccine were followed up in an investigation conducted in Poland, using Elecsys-anti-SARS-CoV-2 S to measure antibody levels. Total antibody titers were significantly higher in participants who have received 3 doses of BTN162b2 than those who have received 2 doses of AstraZeneca and a booster dose of BTN162b2 [39].

Moreover, previous studies have shown a significant increase in breakthrough infection with the apparition of a new variant of concern compared with prior strains circulating [40]. Indeed, the study of Kustin *et al*. in people vaccinated with BNT162b2 revealed more breakthrough infection cases with the apparition of the Alpha variant compared with previous strains [41]. Equally, a study in the United States investigated the effectiveness of BNT162b2, mRNA-1273, and Janssen Ad26.COV2.S vaccines, showing that more breakthrough infections were documented with the emergence of the Delta variant [42]. Furthermore, a study conducted in Germany investigated SARS-CoV-2 breakthrough infection in healthcare workers fully vaccinated (3 vaccine doses) with one of these types of vaccines (BNT162b2, Vaxzevria (viral vector vaccine), Spikevax (mRNA vaccine) or Jcovden (viral vector vaccine)), have noticed that the number of breakthrough infections has increased rapidly subsequently with the regional spread of Delta and Omicron variants [43]. Additionally, an investigation in South Africa describing breakthrough infections after vaccination in the period of domination of Delta and Omicron variants has shown that these infections were more than 3 times higher when Omicron was dominating than at the peak of the Delta variant domination [44]. A recent case-control analysis on breakthrough infections in the periods of the emergence of Delta and Omicron variants in New York has shown that the Omicron variant was associated with a higher level of breakthrough in fully vaccinated and boosted participants. The vaccines that were taken in this study were Moderna, BNT162b2, and Janssen [40]. On the other hand, Regenhardt *et al*. found that Delta and Omicron breakthrough infections were associated with higher viral load than breakthrough infections reported with the Alpha variant, this may be associated with the immune evasive characteristics of these variants [43, 45]. This is concordant with our study, where all the breakthrough infection cases recorded an antibody titer higher than 250 U/ml at the second sampling and were all observed in the period of the domination of the Delta and Omicron variants in Lebanon.

Finally, it should be mentioned that like any epidemiological survey, our study is not without limitations, including mainly the low number of participants. Besides, we were not able to do dilutions for samples having an antibody titer >250 U/ml and remeasure anti-Spike antibody titers in order to get the exact concentration. Indeed, during the period of this study,

Lebanon faced a catastrophic economic crisis and the currency has lost more than 95 percent of its pre-crisis value, thus limiting our ability to purchase additional assay kits. This step would be important to compare the vaccine effectiveness regarding the level of the immune response. Finally, the Sputnik V vaccine was not included in the further analysis in reason to its low headcount.

Conclusions

This study investigated SARS-CoV-2 anti-S antibody titers in a population of 201 individuals vaccinated with two doses against SARS-CoV-2 in North Lebanon. The measurement was accomplished 2 times (T1, 14 to 30 days after the booster dose, and T2, 6 months after). The 3 vaccines examined in the study were: BNT162b2, AstraZeneca, and Sputnik V. The results show that 70.14% of the participants maintained their immune response between the 2 sampling, 27.4% manifested a decrease in the antibody titers, while only 2.5% showed an increase in the antibody levels. Age was the only variable significantly associated with the decrease in the immune response. On the other hand, participants vaccinated with BNT162b2 manifested fewer breakthrough infections than those vaccinated with AstraZeneca.

Overall, our study has provided an overview of the evolution of the immune response after COVID-19 vaccination in a small sample of demographically diverse vaccinated adults in North Lebanon. Notably, after the addition of the third vaccination dose, many Lebanese citizens have undergone this booster dose. In this context, further national studies are needed to follow the immune response after 3 vaccination doses over a longer period and to evaluate the vaccine's effectiveness over time. The comparison of the immune response generated after previous COVID-19 infection and that generated by vaccination is also needed to make a clearer view of the immune status of the population in Lebanon.

Acknowledgments

We would like to thank all the individuals who accepted to participate in this study. We also acknowledge all the staff of the Laboratoire Microbiologie, Santé et Environnement (LMSE) for their excellent technical assistance and the huge efforts made during the COVID-19 pandemic in Lebanon. The technical assistance of Mr. Abdel Rahman Majdalani is also gratefully acknowledged.

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References

1. Kim JY, Choe PG, Oh Y, Oh KJ, Kim J, Park SJ, et al. The First Case of 2019 Novel Coronavirus Pneumonia Imported into Korea from Wuhan, China: Implication for Infection Prevention and Control Measures. *J Korean Med Sci*. 2020; 35(5):e61. Epub 2020/02/08. https://doi.org/10.3346/jkms.2020.35.e61 PMID: 32030925; PubMed Central PMCID: PMC7008073.
2. World Health Organization. Coronavirus disease (COVID-19) pandemic 2023. Available from: https://www.who.int/europe/emergencies/situations/covid-19. Accessed August 8, 2023.
3. World Health Organization. WHO Coronavirus (COVID-19) Dashboard 2023. Available from: https://covid19.who.int/. Accessed August 8, 2023.
4. Shah VK, Firmal P, Alam A, Ganguly D, Chattopadhyay S. Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. *Front Immunol*. 2020; 11:1949. Epub 2020/08/28. https://doi.org/10.3389/fimmu.2020.01949 PMID: 32849654; PubMed Central PMCID: PMC7426442.
5. Tan YJ, Goh PY, Fielding BC, Shen S, Chou CF, Fu JL, et al. Profiles of antibody responses against severe acute respiratory syndrome coronavirus recombinant proteins and their potential use as diagnostic markers. *Clin Diagn Lab Immunol*. 2004; 11(2):362–71. Epub 2004/03/12. https://doi.org/10.1128/cdli.11.2.362-371.2004 PMID: 15013989; PubMed Central PMCID: PMC371215.
6. Wu HS, Hsieh YC, Su IJ, Lin TH, Chiu SC, Hsu YF, et al. Early detection of antibodies against various structural proteins of the SARS-associated coronavirus in SARS patients. *J Biomed Sci*. 2004; 11 (1):117–26. Epub 2004/01/20. https://doi.org/10.1007/BF02256554 PMID: 14730215; PubMed Central PMCID: PMC7089234.
7. Walsh EE, Frenck RW Jr., Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N Engl J Med*. 2020; 383(25):2439–50. Epub 2020/10/15. https://doi.org/10.1056/NEJMoa2027906 PMID: 33053279; PubMed Central PMCID: PMC7583697.
8. Coppeta L, Balbi O, Grattagliano Z, Mina GG, Pietroiusti A, Magrini A, et al. First Dose of the BNT162b2 mRNA COVID-19 Vaccine Reduces Symptom Duration and Viral Clearance in Healthcare Workers. *Vaccines (Basel)*. 2021; 9(6). Epub 2021/07/03. https://doi.org/10.3390/vaccines9060659 PMID: 34204252; PubMed Central PMCID: PMC8234325.
9. van Doremalen N, Lambe T, Spencer A, Belij-Rammerstorfer S, Purushotham JN, Port JR, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature*. 2020; 586 (7830):578–82. Epub 2020/07/31. https://doi.org/10.1038/s41586-020-2608-y PMID: 32731258; PubMed Central PMCID: PMC8436420.
10. Kaur U, Ojha B, Pathak BK, Singh A, Giri KR, Singh A, et al. A prospective observational safety study on ChAdOx1 nCoV-19 corona virus vaccine (recombinant) use in healthcare workers- first results from India. *EClinicalMedicine*. 2021; 38:101038. Epub 2021/09/11. https://doi.org/10.1016/j.eclinm.2021.101038 PMID: 34505032; PubMed Central PMCID: PMC8413251.
11. Sabitha S, Shobana N, Prakash P, Padmanaban S, Sathiyashree M, Saigeetha S, et al. A Review of Different Vaccines and Strategies to Combat COVID-19. *Vaccines (Basel)*. 2022; 10(5). Epub 2022/05/29. https://doi.org/10.3390/vaccines10050737 PMID: 35632493; PubMed Central PMCID: PMC9145217.
12. Rab S, Afjal, Javaid M, Haleem A, Vaishya R. An update on the global vaccine development for coronavirus. *Diabetes Metab Syndr*. 2020; 14(6):2053–5. Epub 2020/10/29. https://doi.org/10.1016/j.dsx.2020.10.023 PMID: 33113472; PubMed Central PMCID: PMC7582051.

13. Logunov DY, Dolzhikova IV, Shcheblyakov DV, Tukhvatulin AI, Zubkova OV, Dzharullaeva AS, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet*. 2021; 397(10275):671–81. Epub 2021/02/06. https://doi.org/10.1016/S0140-6736(21)00234-8 PMID: 33545094; PubMed Central PMCID: PMC7852454.

14. Gong F, Wei HX, Li Q, Liu L, Li B. Evaluation and Comparison of Serological Methods for COVID-19 Diagnosis. *Front Mol Biosci*. 2021; 8:682405. Epub 2021/08/10. https://doi.org/10.3389/fmolb.2021.682405 PMID: 34368226; PubMed Central PMCID: PMC8343015.

15. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. *Clin Chem Lab Med*. 2020; 58(7):1081–8. Epub 2020/04/18. https://doi.org/10.1515/cclm-2020-0443 PMID: 32301749.

16. Tan Y, Pang W, Jia X, Zhao MH. Comparison of the performance of a chemiluminescence assay and an ELISA for detection of anti-GBM antibodies. *Ren Fail*. 2020; 42(1):48–53. Epub 2019/12/31. https://doi.org/10.1080/0886022X.2019.1702056 PMID: 31885301; PubMed Central PMCID: PMC6968565.

17. Diagnostics R. Elecsys® Anti-SARS-CoV-2 S 2020. Available from: https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2-s.html. Accessed August 18, 2323.

18. France 24. Lebanon starts Covid-19 vaccination campaign 2021. Available from: https://www.france24.com/en/middle-east/20210214-lebanon-starts-covid-19-vaccination-campaign. Accessed August 18, 2023.

19. France 24. Lebanon receives first Covid vaccines 2021. Available from: https://www.france24.com/en/live-news/20210213-lebanon-receives-first-covid-vaccines. Accessed August 18, 2023.

20. Beerlage A, Leuzinger K, Valore L, Mathew R, Junker T, Drexler B, et al. Antibody response to mRNA SARS-CoV-2 vaccination in 182 patients after allogeneic hematopoietic cell transplantation. *Transpl Infect Dis*. 2022; 24(3):e13828. Epub 2022/03/25. https://doi.org/10.1111/tid.13828 PMID: 35324045; PubMed Central PMCID: PMC9115355.

21. Ruether DF, Schaub GM, Duengelhoef PM, Haag F, Brehm TT, Fathi A, et al. SARS-CoV2-specific Humoral and T-cell Immune Response After Second Vaccination in Liver Cirrhosis and Transplant Patients. *Clin Gastroenterol Hepatol*. 2022; 20(1):162–72 e9. Epub 2021/09/13. https://doi.org/10.1016/j.cgh.2021.09.003 PMID: 34509643; PubMed Central PMCID: PMC8427908.

22. Resman Rus K, Korva M, Knap N, Avsic Zupanc T, Poljak M. Performance of the rapid high-throughput automated electrochemiluminescence immunoassay targeting total antibodies to the SARS-CoV-2 spike protein receptor binding domain in comparison to the neutralization assay. *J Clin Virol*. 2021; 139:104820. Epub 2021/04/18. https://doi.org/10.1016/j.jcv.2021.104820 PMID: 33865031; PubMed Central PMCID: PMC8035809.

23. Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized Trial of a Third Dose of mRNA-1273 Vaccine in Transplant Recipients. *N Engl J Med*. 2021; 385(13):1244–6. Epub 2021/08/12. https://doi.org/10.1056/NEJMc2111462 PMID: 34379917; PubMed Central PMCID: PMC8385563.

24. Weinberger B. Vaccines and Vaccination against SARS-CoV-2: Considerations for the Older Population. Vaccines (Basel). 2021; 9(12). Epub 2021/12/29. https://doi.org/10.3390/vaccines9121435 PMID: 34960181; PubMed Central PMCID: PMC8704374.

25. Lustig Y, Sapir E, Regev-Yochay G, Cohen C, Fluss R, Olmer L, et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. Lancet Respir Med. 2021; 9(9):999–1009. Epub 2021/07/06. https://doi.org/10.1016/S2213-2600(21)00220-4 PMID: 34224675; PubMed Central PMCID: PMC8253545.

26. Tartof SY, Slezak JM, Fischer H, Hong V, Ackerson BK, Ranasinghe ON, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *Lancet*. 2021; 398(10309):1407–16. Epub 2021/10/08. https://doi.org/10.1016/S0140-6736(21)02183-8 PMID: 34619098; PubMed Central PMCID: PMC8489881.

27. Dakovic Rode O, Bodulic K, Zember S, Cetinic Balent N, Novokmet A, Culo M, et al. Decline of Anti-SARS-CoV-2 IgG Antibody Levels 6 Months after Complete BNT162b2 Vaccination in Healthcare Workers to Levels Observed Following the First Vaccine Dose. Vaccines (Basel). 2022; 10(2). Epub 2022/02/27. https://doi.org/10.3390/vaccines10020153 PMID: 35214612; PubMed Central PMCID: PMC8876023.

28. Muller L, Andree M, Moskorz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent Immune Response to the Biontech/Pfizer BNT162b2 Coronavirus Disease 2019 Vaccination. *Clin Infect Dis*. 2021; 73(11):2065–72. Epub 2021/04/28. https://doi.org/10.1093/cid/ciab381 PMID: 33906236; PubMed Central PMCID: PMC8135422.

29. Wolszczak-Biedrzycka B, Bienkowska A, Dorf J. Assessment of Post-Vaccination Antibody Response Eight Months after the Administration of BNT1622b2 Vaccine to Healthcare Workers with Particular Emphasis on the Impact of Previous COVID-19 Infection. Vaccines (Basel). 2021; 9(12). Epub 2021/12/29. https://doi.org/10.3390/vaccines9121508 PMID: 34960254; PubMed Central PMCID: PMC8704861.

30. Subbarao S, Warrener LA, Hoschler K, Perry KR, Shute J, Whitaker H, et al. Robust antibody responses in 70-80-year-olds 3 weeks after the first or second doses of Pfizer/BioNTech COVID-19 vaccine, United Kingdom, January to February 2021. Euro Surveill. 2021; 26(12). Epub 2021/03/27. https://doi.org/10.2807/1560-7917.ES.2021.26.12.2100329 PMID: 33769252; PubMed Central PMCID: PMC7995559.

31. Tretyn A, Szczepanek J, Skorupa M, Jarkiewicz-Tretyn J, Sandomierz D, Dejewska J, et al. Differences in the Concentration of Anti-SARS-CoV-2 IgG Antibodies Post-COVID-19 Recovery or Post-Vaccination. Cells. 2021; 10(8). Epub 2021/08/28. https://doi.org/10.3390/cells10081952 PMID: 34440721; PubMed Central PMCID: PMC8391384.

32. Nour D, Rafei R, Lamarca AP, de Almeida LGP, Osman M, Ismail MB, et al. The Role of Lebanon in the COVID-19 Butterfly Effect: The B.1.398 Example. *Viruses*. 2022; 14(8). Epub 2022/08/27. https://doi.org/10.3390/v14081640 PMID: 36016262; PubMed Central PMCID: PMC9412248.

33. Maaske J, Sproule S, Falsey AR, Sobieszczyk ME, Luetkemeyer AF, Paulsen GC, et al. Robust humoral and cellular recall responses to AZD1222 attenuate breakthrough SARS-CoV-2 infection compared to unvaccinated. *Front Immunol*. 2022; 13:1062067. Epub 2023/01/31. https://doi.org/10.3389/fimmu.2022.1062067 PMID: 36713413; PubMed Central PMCID: PMC9881590.

34. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med*. 2021; 385(7):585–94. Epub 2021/07/22. https://doi.org/10.1056/NEJMoa2108891 PMID: 34289274; PubMed Central PMCID: PMC8314739.

35. Stouten V, Hubin P, Haarhuis F, van Loenhout JAF, Billuart M, Brondeel R, et al. Incidence and Risk Factors of COVID-19 Vaccine Breakthrough Infections: A Prospective Cohort Study in Belgium. *Viruses*. 2022; 14(4). Epub 2022/04/24. https://doi.org/10.3390/v14040802 PMID: 35458532; PubMed Central PMCID: PMC9029338.

36. Almufty HB, Mamani MMA, Ali AH, Merza MA. COVID-19 vaccine breakthrough infection among fully vaccinated healthcare workers in Duhok governorate, Iraqi Kurdistan: A retrospective cohort study. *J Med Virol*. 2022; 94(11):5244–50. Epub 2022/07/12. https://doi.org/10.1002/jmv.27985 PMID: 35811398; PubMed Central PMCID: PMC9350230.

37. Lipsitch M, Krammer F, Regev-Yochay G, Lustig Y, Balicer RD. SARS-CoV-2 breakthrough infections in vaccinated individuals: measurement, causes and impact. *Nat Rev Immunol*. 2022; 22(1):57–65. Epub 2021/12/09. https://doi.org/10.1038/s41577-021-00662-4 PMID: 34876702; PubMed Central PMCID: PMC8649989.

38. Eyre DWea. The impact of SARS-CoV-2 vaccination on Alpha and Delta variant transmission. *medRxiv*. 2021. https://doi.org/10.1101/2021.09.28.21264260.

39. Wolszczak Biedrzycka B, Bienkowska A, Smolinska-Fijolek E, Biedrzycki G, Dorf J. The Influence of Two Priming Doses of Different Anti-COVID-19 Vaccines on the Production of Anti-SARS-CoV-2 Antibodies After the Administration of the Pfizer/BioNTech Booster. *Infect Drug Resist*. 2022; 15:7811–21. Epub 2023/01/06. https://doi.org/10.2147/IDR.S390351 PMID: 36600955; PubMed Central PMCID: PMC9807069.

40. Keyel AC, Russell A, Plitnick J, Rowlands JV, Lamson DM, Rosenberg E, et al. SARS-CoV-2 Vaccine Breakthrough by Omicron and Delta Variants, New York, USA. *Emerg Infect Dis*. 2022; 28(10):1990–8. Epub 2022/09/02. https://doi.org/10.3201/eid2810.221058 PMID: 36048774; PubMed Central PMCID: PMC9514330.

41. Kustin T, Harel N, Finkel U, Perchik S, Harari S, Tahor M, et al. Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2-mRNA-vaccinated individuals. *Nat Med*. 2021; 27(8):1379–84. Epub 2021/06/16. https://doi.org/10.1038/s41591-021-01413-7 PMID: 34127854; PubMed Central PMCID: PMC8363499.

42. Rosenberg ES, Dorabawila V, Easton D, Bauer UE, Kumar J, Hoen R, et al. Covid-19 Vaccine Effectiveness in New York State. *N Engl J Med*. 2022; 386(2):116–27. Epub 2021/12/24. https://doi.org/10.1056/NEJMoa2116063 PMID: 34942067; PubMed Central PMCID: PMC8693697.

43. Regenhardt E, Kirsten H, Weiss M, Lubbert C, Stehr SN, Remane Y, et al. SARS-CoV-2 Vaccine Breakthrough Infections of Omicron and Delta Variants in Healthcare Workers. *Vaccines (Basel)*. 2023; 11(5). Epub 2023/05/27. https://doi.org/10.3390/vaccines11050958 PMID: 37243062; PubMed Central PMCID: PMC10220865.

44. Goga A, Bekker LG, Garrett N, Reddy T, Yende-Zuma N, Fairall L, et al. Breakthrough SARS-CoV-2 infections during periods of delta and omicron predominance, South Africa. *Lancet*. 2022; 400

(10348):269–71. Epub 2022/07/10. https://doi.org/10.1016/S0140-6736(22)01190-4 PMID: 35809579; PubMed Central PMCID: PMC9259009.

45. Trunfio M, Verga F, Ghisetti V, Burdino E, Emanuele T, Bonora S, et al. Clinical Phenotype and Contagiousness of Early Breakthrough SARS-CoV-2 Infections after BNT162b2 COVID-19 mRNA Vaccine: A Parallel Cohort Study in Healthcare Workers. *Vaccines (Basel)*. 2021; 9(12). Epub 2021/12/29. https://doi.org/10.3390/vaccines9121377 PMID: 34960123; PubMed Central PMCID: PMC8705309.

Conclusion and perspectives

Coronavirus disease 2019 (COVID-19) has touched more than 700 million people around the world, and the number of related deaths has exceeded 7 million, as of June 23, 2024 [1]. This new emerging disease constituted an attractive field for scientific research, in different disciplines. Genomic characterization of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mutations and emerging lineages is paramount in building efficient and reliable health measures, designing therapies, and developing vaccines to eradicate the widespread disease. Assessing the immune response generated after SARS-CoV-2 infection or after vaccine intake is crucial for understanding the immune status of the population and the validity of vaccination.

In this thesis, we reemphasized the importance of implementing a national screening investigation of circulating SARS-CoV-2 lineages and tracking the antibody titers generated after vaccination in Lebanon. Even though previous studies went in a similar direction [2-8], our work was original owing to its new findings and perspective.

In our first study, our sequencing results have more than doubled the number of Lebanese sequences available for the first year of the pandemic in GISAID. In addition, we improved the sampling of the B.1.398 lineage, which was associated with the first surge in the number of COVID-19 cases in Lebanon and has played a crucial role in drawing SARS-CoV-2's pathways within the country, with the district of Tripoli constituting the center of B.1.398 dispersal. We also unveiled the capacity of Lebanon to spread B.1.398 to different countries, with a higher frequency of intercontinental than intracontinental dispersion.

Our second study constituted a live example of the effectiveness of using RT-qPCR targeting specific variant mutations to track SARS-CoV-2's variants. The results have shown a concordant image to that obtained by analyzing the SARS-CoV-2 Lebanese sequences available in GISAID. This was the first Lebanese study investigating VOCs and Omicron subvariants using RT-qPCR, presenting it as an accurate, reliable, and cost-effective screening tool.

Last but not least, in our third study, we have targeted a widely diverse adult population, including citizens from different regions in North Lebanon, health care workers, and Lebanese University staff, with stratified age groups. We could determine that age was significantly associated with the decline in the immune response, and BTN162b2 was more efficient than the AZD1222 vaccine.

In conclusion, SARS-CoV-2 descendant lineages are still emerging. In light of the

previously addressed discussions, we recommend conducting surveys with larger sample sizes and broader geographical scales, in order to get a more realistic and accurate representation of the disease status in Lebanon. Moreover, analyzing our quantitative reverse transcription polymerization chain reaction (RT-qPCR) samples by whole genome sequencing (WGS) will be an important advancing step to confirm our result and detect the exact variants, sub-variants and/or recombinant lineages. Finally, considering the three FDA-approved vaccines for 2023-2024, namely BNT162b2, mRNA-1273, and NVX-CoV2373 [9, 10], further serological surveys should target these vaccines, since they include a monovalent component related to the Omicron variant XBB.1.5, the current prevalent lineage.

References

1. The World Health Organization (WHO). WHO COVID-19 dashboard [July 11, 2024]. Available from: https://data.who.int/dashboards/covid19/.

2. Feghali R, Merhi G, Kwasiborski A, Hourdel V, Ghosn N, Tokajian S. Genomic characterization and phylogenetic analysis of the first SARS-CoV-2 variants introduced in Lebanon. PeerJ. 2021;9:e11015. Epub 2021/10/07. doi: 10.7717/peerj.11015. PubMed PMID: 34611501; PubMed Central PMCID: PMCPMC8447710.

3. Fayad N, Abi Habib W, Kandeil A, El-Shesheny R, Kamel MN, Mourad Y, et al. SARS-CoV-2 Variants in Lebanon: Evolution and Current Situation. Biology (Basel). 2021;10(6). Epub 2021/07/03. doi: 10.3390/biology10060531. PubMed PMID: 34198622; PubMed Central PMCID: PMCPMC8232177.

4. Merhi G, Trotter AJ, de Oliveira Martins L, Koweyes J, Le-Viet T, Abou Naja H, et al. Replacement of the Alpha variant of SARS-CoV-2 by the Delta variant in Lebanon between April and June 2021. Microb Genom. 2022;8(7). Epub 2022/07/26. doi: 10.1099/mgen.0.000838. PubMed PMID: 35876490; PubMed Central PMCID: PMCPMC9455693.

5. AlKalamouni H, Abou Hassan FF, Bou Hamdan M, Page AJ, Lott M, Matthews M, et al. Genomic surveillance of SARS-CoV-2 in COVID-19 vaccinated healthcare workers in Lebanon. BMC Med Genomics. 2023;16(1):14. Epub 2023/01/29. doi: 10.1186/s12920-023-01443-9. PubMed PMID: 36707851; PubMed Central PMCID: PMCPMC9880935.

6. Kodsi IA, Rayes DE, Koweyes J, Khoury CA, Rahy K, Thoumi S, et al. Tracking SARS-CoV-2 variants during the 2023 flu season and beyond in Lebanon. Virus Res. 2024;339:199289. Epub 2023/12/01. doi: 10.1016/j.virusres.2023.199289. PubMed PMID: 38036064; PubMed Central PMCID: PMCPMC10704499.

7. Abdel Sater F, Younes M, Nassar H, Nguewa P, Hamze K. A rapid and low-cost protocol for the detection of B.1.1.7 lineage of SARS-CoV-2 by using SYBR Green-based RT-qPCR. Mol Biol Rep. 2021;48(11):7243-9. Epub 2021/10/07. doi: 10.1007/s11033-021-06717-y. PubMed PMID: 34613565; PubMed Central PMCID: PMCPMC8493944.

8. Moghnieh R, El Hajj C, Abdallah D, Jbeily N, Bizri AR, Sayegh MH. Immunogenicity and Effectiveness of Primary and Booster Vaccine Combination Strategies during Periods of SARS-CoV-2 Delta and Omicron Variants. Vaccines (Basel). 2022;10(10). Epub 2022/10/28. doi: 10.3390/vaccines10101596. PubMed PMID: 36298460; PubMed Central PMCID: PMCPMC9607825.

9. Chakraborty C, Bhattacharya M, Chopra H, Islam MA, Saikumar G, Dhama K. The SARS-CoV-2 Omicron recombinant subvariants XBB, XBB.1, and XBB.1.5 are expanding rapidly with unique mutations, antibody evasion, and immune escape properties - an alarming global threat of a surge in COVID-19 cases again? Int J Surg. 2023;109(4):1041-3. Epub 2023/03/15. doi: 10.1097/JS9.0000000000000246. PubMed PMID: 36917125; PubMed Central PMCID: PMCPMC10132296 Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

10. U.S. Food and Drug Admission (FDA). COVID-19 Vaccines for 2023-2024 2024 [July 9, 2024]. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines-2023-2024.