COVID-19 re-infection

Philippe Brouqui[1,3], Philippe Colson[1,3], Cléa Melenotte[1] 3, Linda Houhamdi[1,3], Marielle Bedotto[3] Christian Devaux [1,3], Philipe Gautret[1,2], Matthieu Million[1,3], Philippe Parola[1,2], Didier Stoupan[1,2], Bernard La Scola[1,3], Jean-Christophe Lagier[1,3], Didier Raoult[1,3]

[1]Aix-Marseille Univ, IRD, APHM, MEPHI, Marseille, France

[2]Aix-Marseille Univ, IRD, APHM, VITROME, Marseille, France

[3]IHU-Méditerranée Infection, Marseille, France

Text Words: 1515

Abstract: 51

References: 20

Tables: 2

Supplementary material 3: Figure S1, Table S1, Table S2 : total (150 Ko)

Acknowledgment: We thank technicians from our laboratory for their investment in genomic analysis and clinicians for their help in retrieving clinical data.

Funding: This study was funded by ANR-15-CE36-0004-01 and by ANR “Investissements d’avenir”, Méditerranée infection 10-IAHU-03.

Conflict of interest: all authors are not in conflict of interest with the content of this manuscript.

English edition: This manuscript has been edited in English thank to Maria Fillanino an English native

ABSTRACT: We report 46 cases of reinfection with SARS-CoV-2 virus. Among them 12 were reinfected with SARS-CoV-2 Marseille 4_20A/18877T-1a. The second episode is more severe suggesting that primary infection might exacerbate the clinical expression of the disease at second episode. The extreme genomic variability of SARS Cov 2 will challenge vaccine campaigns.

The Covid-19 epidemic ongoing since January 2020 , caused so far 95 million cases and 2 million deaths worldwide (1). COVID-19 was initially considered a disease caused by a stable virus that could provide immunity, as it is the case with most respiratory viruses (with the notable exception of rhinoviruses), for which immunity is lasting a year or more. In childhood naturally acquired infection with measle, chickenpox, or mumps provide protection for the entirety of life (2). In other viruses, such as influenza, acquired immunity is dependent on variants, and warrants annual vaccination as well as mixing strains adapted to the epidemiology of the previous year (3) . Finally, in other situations, such as dengue fever, the protection provided by the first episode may, on the contrary, generate facilitating antibodies resulting in the second infection being more severe than the first, and this phenomenon has been found in cases of infections following vaccination against dengue fever (4).

In COVID-19, it quickly became apparent that naturally acquired immunity would not, in all cases, provide protection for the months following the first infection. This may be due to a lack of efficient natural immunity after infection or to the existence of variants on major epitopes theoretically leading to resistance to infection. This point was particularly important for the Spike protein, as it is the target of bioengineered viruses (5) , and already observed variations, particularly in the South African variant, show that mutations in this protein lead to humorous and apparently clinical resistance to the AstraZeneca vaccine developed on Spike (6).

Since February 2020, at IHU Méditerranée Infection in Marseille, France, we offer non-restrictive access to SARS-CoV-2 screening tests for all patients, whether symptomatic or not (7). This led us to diagnose more than 5,000 cases during a first phase *i.e.* from January to early May 2020 and more than 17,000 cases during a second phase, *i.e.* from June to January 2021 (8) . Viral genotypes closely related to the Wuhan strain were identified during the first phase in Marseille, but since then this genotype has disappeared and left room to several new variants identified during the second phase (***supplementary Figure S1***) (9) (10).

We recently reported a first case of reinfection with different SARS-CoV-2 genotypes (11). Since then, we decided to actively monitor new reinfections through our computerized database.

THE STUDY

At IHU Méditerranée Infection, since the end of January 2020, 445 611 SARS-CoV-2 qRT-PCR on nasopharyngeal samples have been performed to 232 195 patients. Based upon the epidemic curve, we defined two periods for this study, one called "the first bell curve epidemics" from January 27th 2020 to May 5th 2020 when it vanished and then stopped, and the second multiple waves epidemic from June 15th 2020 to January 12th 2021. A computerized alert system was set up to detect patients who had two positive SARS-CoV-2 qRT-PCRs on nasopharyngeal samples collected more than 90 days apart and clinical recovery and at least one negative qPCR after the first episode.

Clinical data collection

Patients with confirmed COVID-19 were invited for a clinical evaluation at day 1. Clinical data including, age, sex, medical history, clinical and laboratory assessment including oxygen saturation, blood pressure, respiratory frequency, QT interval measurement and blood potassium was performed. Patient's outcomes were recorded in the hospital information system, and extracted retrospectively from medical record (8). Approximately 2/3 of positive patients presented for care, others were lost to follow up. As a result, some reinfected patients have not been recorded in our files and missing clinical data were obtained by telephone. The study was approved by our institutional review board committee (Méditerranée Infection N°: 2020-021). The analysis of collected data followed the reference methodology MR-004 registered on N° MR 5010010520 in the AP-HM register.

Virus genotype identification strategy

We performed 3282 SARS-CoV-2 genomes until now at IHU Méditerranée infection. Our first 691 complete genome sequence analysis demonstrated that since July 2020, 10 new clades emerged that we named Marseille-1 to 10, replacing the original viral strains that disappeared in early May (9). The Marseille-4 variant (Marseille 4_20A/18877T-1a) which exhibit a mutation in the receptor

binding domain of the Spike protein became the dominant genotype in Marseille (9) (***supplementary Figure S1***) .

We first aim to determine whether the viruses responsible for re-infection in our patients were Marseille-4 variants. For this we assessed viral genotype in the second sample for each patient by a Marseille-4-specific qRT-PCR based on previous SARS-CoV-2 genome descriptions (12). Their sequence and the qPCR conditions are shown in ***supplementary Table S1***. When qRT-PCR for Marseille-4 was negative, partial, or full-length genome sequencing was performed.

According to definition, 46 patients with SARS-CoV-2 re-infection were reported here, 31 which were infected during the first wave among 5000 (0.6%) and 15 during the second epidemic.

Mean age was 50±22 years-old, 25 patients were men, the mean delay between the first and the second infection was 172 days (range 90 to 308) (**Table 1**).

As for the first episode of infection 39/46 (84.7%) patients presented with clinical symptoms compatible with a SARS-CoV-2 infection (8). All but 2 were classified as Mild / Moderate (13) . Seven, were asymptomatic and resulted from systematic testing. Thirty-five were followed as ambulatory patients and eleven were hospitalised, among them four asymptomatic patients hospitalized for another reason than covid and seven for COVID-19. None of the 46 patients were admitted to ICU.

For the second infection, 33/46 (71.7%) of the patients presented with clinical symptoms compatibles with a SARS-CoV-2 reinfection and twelves were hospitalized (p= 0.5). Twenty-six were classified Mild/Moderate and seven Severe/Critical, significantly more than for the first infection (p= 0.044). Four had a poor outcome (p=0.058), two were admitted in intensive care unit for severe acute respiratory distress and two died during hospitalisation.

Among the 46 patients with reinfection according to definition, 31 who were infected during the first phase were reinfected with another genomic variant different with the original Wuhan (9) (**Table S2 in supplementary data file**) . Of them 12 were reinfected with SARS-CoV-2 Marseille 4_20A/18877T-1a genotype. Of the 15 other patients infected during the second phase, 7 were reinfected with SARS-CoV-2 Marseille 4_20A/18877T-1a genotype.

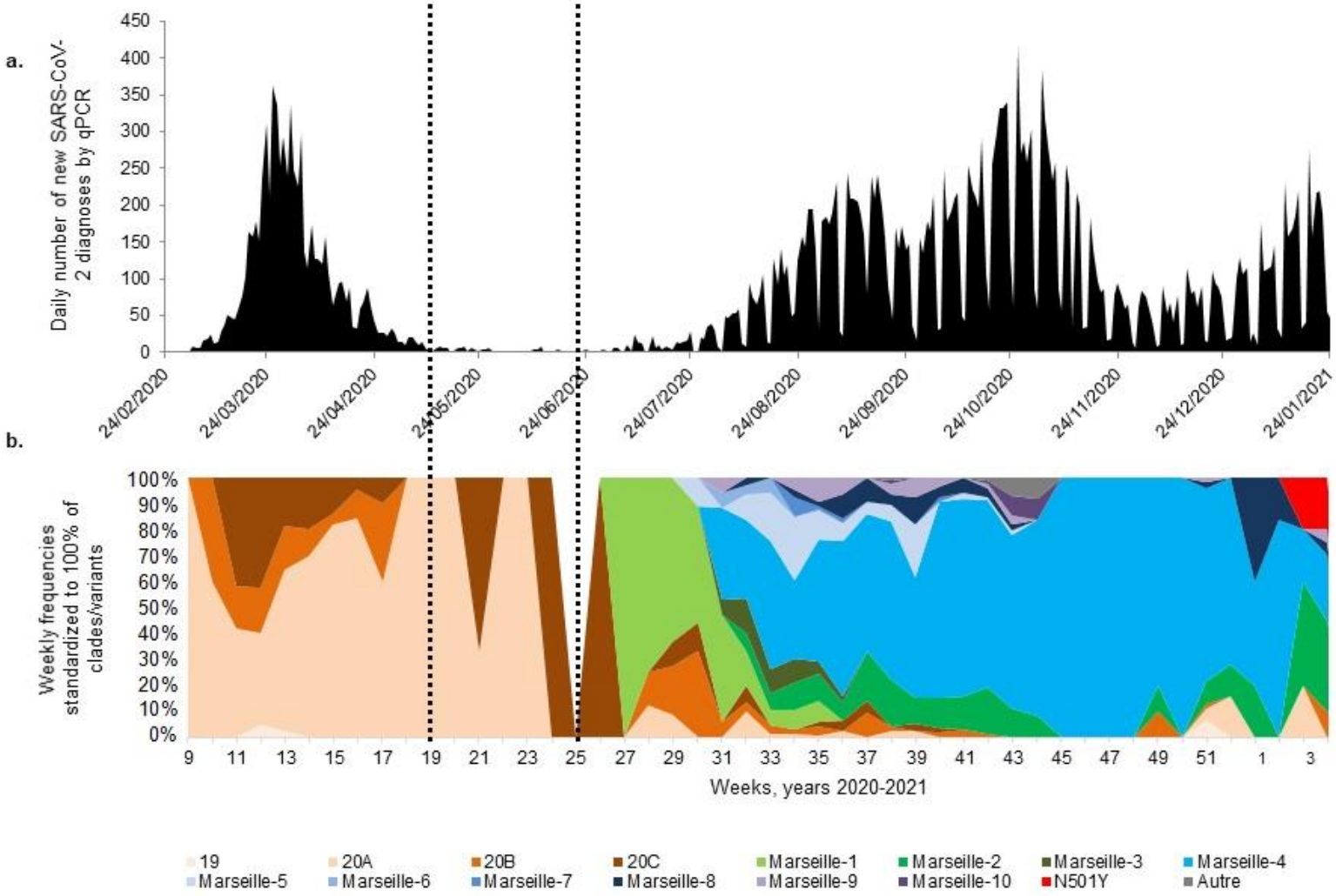
Reinfections with SARS-CoV-2 have recently been reported by several authors worldwide and reviewed recently (14). Evolution of clinical status between the two episodes is reported in **Table 2** for 61 patients with reinfection (46 reported here and the 15 patients reviewed in Cohen et al) (14). Among them, 26/61 (42.6 %) presented similar clinical status in both episode, 18/61 (29.5%) had a milder form of the disease in the second episode, 17/61 (27.8 %) worsen from asymptomatic to Mild/Moderate - Severe/Critical. It is important to notice that 5 patients experienced ICU and/or died in the second episode of infection (p=0.0287). Although this needs to be confirmed in larger studies, it suggests that the second episode is more clinically severe eventually leading to ICU and death.

Protection from naturally acquired respiratory virus , influenza, RSV, and seasonal coronaviruses, is generally of short-lived and in most cases not more than a year (15) . These infections are mucosal infections and rarely associated with a viremia, such as in measle which is known to provide more prolonged protection (2). The mean delay between two infections observed in our patients is 5.7 months. On another side, by analogy with HIV or influenza for example, the specific characteristics of SARS-CoV-2, including the extreme genetic variability in circulating viral isolates worldwide along with a high mutation rate in immunocompromised host (16), likely allows for rapid escape from adaptive immune responses (17) . 12 of the 31 patients with documented reinfection were due to the Marseille 4_20A/18877T-1a variant developing in our area since August 2020 and which became the dominant variant in Marseille. The genetic variability of SARS-CoV-2 questions the putative efficacy of commercialized vaccine based on the Spike protein (6), knowing that in most countries it is likely that circulating viruses are not the original virus used for vaccine production but genetic variant (9). The apparently more severe form reported in the second episode suggest that the primary infection might exacerbate the clinical expression of the disease such has been reported for dengue. It was reported that people who had a low neutralizing antibody titer after a first dengue episode are at a higher risk to experience a severe dengue in case of secondary infections (18) . To explain such adverse effects of the immune response it was hypothesized that the patient's

antibodies produced during the primary dengue episode cross-react with the other serotypes and enhance the secondary infection, thereby increasing the proinflammatory process associated to disease (19) (4). Based on this hypothesis it can also be suggested that vaccination of previously infected individuals might also, as reported in dengue (20), be deleterious suggesting careful monitoring of the vaccination campaign.

Thus, if reinfection results from the insufficient efficacy of natural immunity or from its too high specificity regarding SARS-CoV-2 genomic mutations is a difficult question. However, it seems that the second episode is likely more severe as reported in dengue. These features should be kept in mind in monitoring the vaccination efficacy and its adverse events.

Figure S1 . Number of new SARS-CoV-2 diagnoses by qPCR (a) and proportion per week of clades/variants based on genome sequencing (b). Bars delimit the first phase from the second phase



Supplementary Table S1. Primers and probe of the Marseille-4 variant-specific qPCR

Name	Sequence (5'-3')	Positions *
Primers:		
Pri_IHU_ C4_5_MBF	GAGGTTTAGAAGAGCTTTTGGTGA	9,460-9,483
Pri_IHU_ C4_5_MBR	CCAGGTAAGAATGAGTAAACTGGTG	9,549-9,573
Probe (6FAM-labelled):		
Pro_IHU_ C4_5_MBP	CCTTATTTCATTCACTGTACTCTG	9,520-9,543

* in reference to genome NC_045512.2 (Wuhan-Hu-1 isolate). The nucleotide specific of the Marseille-4 variant is covered by the probe and underlined.

PCR conditions are as follows: The qPCR was performed by adding 5 µL of extracted viral RNA to 15 µL of reaction mixture containing 5 µL of 4X TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Grand Island, NY, USA), 0.5 µL of forward primer (10 pmol/µL), 0.5 µL of reverse primer (10 pmol/µL), 0.4 µL of probe (10 pmol/µL), and 8.6 µL of water. PCR conditions were as follows: reverse transcription at 50°C for 10 min, then a hold at 95°C for 20 sec followed by 40 cycles comprising a denaturation step at 95°C for 15 sec and a hybridization-elongation step at 60°C for 60 sec. This qPCR was run on a LC480 thermocycler (Roche Diagnostics, Mannheim, Germany).

Table 1: Clinical and virological characteristics of 46 patients with SARS-Cov2 reinfection between the first and the second epidemic wave in Marseille France

	Patients (n= 46)	First infection Nb/total (%)	Second infection Nb/total (%)	P value*
Age	50+/-22			
Sex ratio (M/F)	25/21			
Mean delay / SD (days)	172 (90-308)			
Clinical presentation	Symptomatic	39/46 (84.7)	33/46 (71.7)	0.102
	Mild/Moderate	37/39 (94.8)	26/33 (78.7)	0.044
	Severe/Critical	2/39(5.1)	7/33 (21.2)	0.044
	Asymptomatic	7/46 (15.2)	13/46 (28.2)	0.102
	Hospitalized	11/46 (32.1)	12/46 (26)	0.500
	ICU	0/46	2/46 (4.3)	0.247
	Death	0/46	2/46 (4.3)	0.247
	ICU or Death in hospitalized patient	0/46	4/46 (8.6)	0.058

*Fischer exact test

Supplementary Table S2. Characteristics of 46 patients with SARS-CoV-2 reinfection

N°	Age/Sex	1st infection		2sd infection		Delay between infections (days)	1st infection 1st SARS-CoV-2 (Ct value)	2sd infection 1st SARS-CoV-2 (Ct value)	Genotype of the first infection	Genotype of the second infection
		Symptoms	Hospit°	Symptoms	Hospit°					
1	46/F	Mild	No	Mild	No	235	20	27	(Wuhan-Hu-1) NC_045512.2	In progress
2	50/F	Mild	No	Mild	No	143	27	28	(Wuhan-Hu-1) NC_045512.2	In progress
3	35/F	No	Yes[1]	No	Yes [1]	262	29	15.0	(Wuhan-Hu-1) NC_045512.2	NA
4	77/M	Moderate	No	Mild	Yes	109	21	POS	(Wuhan-Hu-1) NC_045512.2	In progress
5	88/F	Mild	Yes	No	No	236	33	19	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
6	55/M	Mild	No	Mild	No	203	21	27	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
7	40/F	Mild	No	Mild	No	208	31	28	(Wuhan-Hu-1) NC_045512.2	In progress
8	33/M	Mild	No	Mild	No	167	25	20	(Wuhan-Hu-1) NC_045512.2	In progress
9	41/F	Mild	No	Mild	No	207	18	25	(Wuhan-Hu-1) NC_045512.2	NA
10	58/M	Mild	No	Mild	No	142	31	26	(Wuhan-Hu-1) NC_045512.2	In progress
11	46/F	Mild	No	Critical	ICU	217	29	24	(Wuhan-Hu-1) NC_045512.2	In progress
12	92/F	Moderate	Yes	Severe	Yes	239	26	27	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a

13	27/F	Mild	No	Mild	No	124	25	18	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
14	70/M*	Moderate	No	No	No	118	27	17	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
15	90/F	Moderate	Yes	No	No	147	24	19	(Wuhan-Hu-1) NC_045512.2	In progress
16	41/M	Mild	No	No	No	162	27	16	(Wuhan-Hu-1) NC_045512.2	In progress
17	59/F	Mild	No	Mild	No	217	30	28	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
18	15/F	No	Yes[1]	No	Yes[1]	230	30	28	(Wuhan-Hu-1) NC_045512.2	In progress
19	92/F	No	Yes [1]	Critical	Yes	90	NA	NA	(Wuhan-Hu-1) NC_045512.2	NA
20	24/M	Mild	No	Moderate	No	152	20	18	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
21	33/M	Mild	No	Mild	No	222	21	29	(Wuhan-Hu-1) NC_045512.2	NA
22	53/F	Mild	No	Mild	No	213	32	26	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
23	57/F	Mild	No	Mild	No	234	33	Pos	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
24	77/M	Mild	Yes	No	No	231	20	19	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
25	60/M	No	Yes [1]	Critical	ICU	141	POS	26	(Wuhan-Hu-1) NC_045512.2	NA
26	34/M	Mild	No	Severe	Yes ‡	150	24	30	(Wuhan-Hu-1) NC_045512.2	In progress
27	65/M	Moderate	Yes	Moderate	Yes	99	15	26	(Wuhan-Hu-1) NC_045512.2	In progress

28	60/M	Moderate	No	Mild	No	210	22	23	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
29	24/M	Mild	No	Mild	No	308	23	28	(Wuhan-Hu-1) NC_045512.2	Marseille 4_20A/18877T-1a
30	64/F	Mild	No	Mild	No	242	34	33	(Wuhan-Hu-1) NC_045512.2	In progress
31	54/M	Moderate	No	Moderate	No	300	14	30	(Wuhan-Hu-1) NC_045512.2	In progress
32	75/M	Severe	yes	No	No	100	21	33	In progress	Marseille 4_20A/18877T-1a
33	45/F	Mild	No	Mild	No	132	26	Pos	In progress	In progress
34	62/M	Moderate	No	Mild	No	145	23	33	In progress	In progress
35	2/F	Mild	No	Severe	Yes[1]	107	28	30	In progress	In progress
36	45/M	Mild	No	No	No	129	26	31	In progress	Marseille 4_20A/18877T-1a
37	19/M	Mild	No	No	No	91	26	30	In progress	Other*
38	57/M	Mild	No	Mild	No	170	29	20	In progress	Marseille 4_20A/18877T-1a
39	69/M	Severe	Yes	Moderate	Yes	162	33	27	In progress	Marseille 4_20A/18877T-1a
40	52/M	No	No	Mild	No	121	30	19	In progress	Marseille 4_20A/18877T-1a
41	26/F	Mild	No	No	No	158	29	27	In progress	Other
42	46/F	No	No	No	No	107	34	22	In progress	Marseille 4_20A/18877T-1a

43	26/M	Mild	No	Mild	No	126	31	32	In progress	Other
44	20/M	No	no	Mild	no	94	30	28	In progress	Marseille 4_20A/18877T-1a
45	22/F	Mild	No	No	No	163	19	27	In progress	Other
46	79/M	Mild	No	Critical	Yes‡	145	Pos	13	In progress	Other

*Previously reported by Colson et al. [11]
‡: Death; F: Female; M: Men.
NA: Not enough material to conclude; *: Another genotype than Marseille 4_20A/18877T-1a.
Hospit°: hospitalization; ICU: Intensive care unit.

[1]Hospitalization for other reason than COVID-19 infection
Patient 3: Hospitalized for thrombopenia and Idiopathic thrombocytopenic purpura. The patient received repeated systemic steroid therapy and then splenectomized.
Patient 18: Hospitalization for psychiatric disorder, no clinical symptom for COVID-19 infection, qRT PCR SARS-CoV-2 test was performed systematically
Patient 19: Patient screened for COVID-19 in rehabilitation for a femoral fracture, hospitalized without COVID-19 symptoms
Patient 25: Hospitalization for post-traumatic uvula oedema
Patient 35: Hospitalization for dehydration

Table 2: Evolution of clinical status between the first and the second episode of infection in our 46 patients and the 15 reported in the literature Cohen et al. (7). Of them, 27.8 % get worse in the second episode, and 8.1% were admitted in ICU or died, 29.5% get better and 42.6% presented the same clinical status for both episodes.

Clinical presentation		This study (46)	Cohen et al. (15)	Patient/Total (%)
Worse	Asymptomatic to Mild-Moderate	3	2	5/61 (8.1)
	Asymptomatic to Severe -Critical	7	5	12/61 (19.6)
Better	From Moderate to Mild or asymptomatic	14	4	18/61 (29.5)
Unchanged status		22	4	26/61 (42.6)
ICU and or Death		4	1	5/61 (8.1) *

* ICU and or death at the second episode p=0.0287 (Fisher exact test)

1. Coronavirus Update (Live): 96,750,697 Cases and 2,068,974 Deaths from COVID-19 Virus Pandemic - Worldometer [Internet]. [cited 2021 Jan 20]. Available from: https://www.worldometers.info/coronavirus/#countries

2. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007 Nov 8;357(19):1903–15.

3. Couch RB, Kasel JA. Immunity to Influenza in Man. Annu Rev Microbiol. 1983 Oct;37(1):529–49.

4. Tsang TK, Ghebremariam SL, Gresh L, Gordon A, Halloran ME, Katzelnick LC, et al. Effects of infection history on dengue virus infection and pathogenicity. Nat Commun. 2019 Mar 18;10(1):1246.

5. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020 Nov 12;383(20):1920–31.

6. Latest - Oxford Covid-19 vaccine trial results - Wits University [Internet]. [cited 2021 Feb 9]. Available from: https://www.wits.ac.za/covid19/covid19-news/latest/oxford-covid-19-vaccine-trial-results.html

7. Amrane S, Tissot-Dupont H, Doudier B, Eldin C, Hocquart M, Mailhe M, et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious diseases referral hospital in Marseille, France, - January 31st to March 1st, 2020: A respiratory virus snapshot. Travel Med Infect Dis. 2020 Aug;36:101632.

8. Lagier J-C, Million M, Gautret P, Colson P, Cortaredona S, Giraud-Gatineau A, et al. Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis. Travel Med Infect Dis. 2020 Aug;36:101791.

9. Colson P, Levasseur A, Delerce J, Chaudet H, Bossi V, Ben Kheder M, Fournier PE, Lagier JC, Raoult D. Dramatic increase in the SARS-CoV-2 mutation rate and low mortality rate during the second epidemic in summer in Marseille. Unpublished [Internet]. Available from: https://www.mediterranee-infection.com/wp-content/uploads/2020/04/FD_Raoult_SARS-CoV-2_EID_Sep2020_vL2.pdf

10. Colson P, Levasseur A, Gautret P, Fenollar F, Thuan Hoang V, Delerce J, et al. Full-length title: Introduction into the Marseille geographical area of a mild SARS-CoV-2 variant originating from sub-Saharan Africa: an investigational study. Travel Med Infect Dis. 2021 Jan 31;101980.

11. Colson P, Finaud M, Levy N, Lagier J-C, Raoult D. Evidence of SARS-CoV-2 re-infection with a different genotype. Journal of Infection. 2020 Nov;S0163445320307064.

12. Bedotto M, Fournier P-E, Houhamdi L, Levasseur A, Delerce J, Pinault L, et al. Implementation of an in-house real-time reverse transcription-PCR assay for the rapid detection of the SARS-CoV-2 Marseille-4 variant. medRxiv. 2021 Feb 8;2021.02.03.21250823.

13. Clinical management of COVID-19 [Internet]. [cited 2021 Jan 26]. Available from: https://www.who.int/publications-detail-redirect/clinical-management-of-covid-19

14. Cohen JI, Burbelo PD. Reinfection with SARS-CoV-2: Implications for Vaccines. Clin Infect Dis. 2020 Dec 18;

15. Edridge AWD, Kaczorowska J, Hoste ACR, Bakker M, Klein M, Loens K, et al. Seasonal coronavirus protective immunity is short-lasting. Nature Medicine. 2020 Nov;26(11):1691–3.

16. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. N Engl J Med. 2020 Dec 3;383(23):2291–3.

17. Walker BD, Burton DR. Toward an AIDS Vaccine. Science. 2008 May 9;320(5877):760–4.

18. Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch Virol. 2013 Jul;158(7):1445–59.

19. Vatti A, Monsalve DM, Pacheco Y, Chang C, Anaya J-M, Gershwin ME. Original antigenic sin: A comprehensive review. J Autoimmun. 2017 Sep;83:12–21.

20. Wilder-Smith A, Flasche S, Smith PG. Vaccine-attributable severe dengue in the Philippines. Lancet. 2019 Dec 14;394(10215):2151–2.