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LETTER

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COVID-19 re-infection

1 | INTRODUCTION

The COVID-19 pandemic 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 measles, chickenpox or mumps provides 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 humourous 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 6771 cases during a first phase, *that is*, from January to early May 2020 and 28 360 cases during a second phase, *that is*, 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 (Figure S1).[9,10]

We recently reported a first case of re-infection with different SARS-CoV-2 genotypes.[11] Since then, we decided to actively monitor new re-infections through our computerized database.

2 | METHODS

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 epidemic wave' from 27 January 2020 to 5 May 2020 when it vanished and then stopped, and the second multiple waves epidemic from 15 June 2020 to 12 January 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 positive.

2.1 | 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 were 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, and others were lost to follow up. As a result, some re-infected 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.

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

2.2 | 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] (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 Table S1. When qRT-PCR for Marseille-4 was negative, partial, or full-length, genome sequencing was performed.

3 | RESULTS

Among 90,602 patients tested in the first wave, 6,771 were qRT-PCR positive. Of them, 31 patients were diagnosed with re-infection according to definition (0.47%) (Flow chart). 15 other patients were re-infected in the ongoing second wave.

Mean age was 50 ± 22 years old, 25 patients were men, 20/39 (51.2%) had no comorbidity, the mean time that elapsed between the first and the second infection was 172 days (range 90-308) (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 hospitalized, 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.

TABLE 1 Clinical characteristics of 46 patients with SARS-Cov2 re-infection between the first and the second epidemic wave in Marseille France

		First infection	Second infection	
	Patients (n = 46)	**Nb/total (%)**	**Nb/total (%)**	***P* value**[**]
Age	50 ± 22			
Sex ratio (M/F)	25/21			
Mean delay/SD (days)	172 (90-308)			
Comorbidity				
NA	7/46			
Any	15/39 (38.4%)			
None	20			
Diabetes	5			
Smoker	1			
Hypertension	8			
Heart underlying disease	3			
Obesity BMI > 30 kg/m^2	4			
Asthma	4			
Cancer	0			
Clinical presentation	Symptomatic	39/46 (84.7)	33/46 (71.7)	.102
	Mild/moderate	37/39 (94.8)	26/33 (78.7)	.044
	Severe/critical	2/39(5.1)	7/33 (21.2)	.044
	Asymptomatic	7/46 (15.2)	13/46 (28.2)	.102
	Hospitalized	11/46 (32.1)	12/46 (26)	.500
	ICU	0/46	2/46 (4.3)	.247
	Death	0/46	2/46 (4.3)	.247
	ICU or death in hospitalized patient	0/46	4/46 (8.6)	.058

Abbreviation: NA, not available.

**Fisher exact test.

For the second infection, 33/46 (71.7%) of the patients presented with clinical symptoms compatibles with a SARS-CoV-2 re-infection and twelves were hospitalized ($P = .5$). Twenty-six were classified Mild/Moderate and seven Severe/Critical, significantly more than for the first infection ($P = .044$). Four had a poor outcome ($P = .058$), two were admitted in intensive care unit for severe acute respiratory distress and two died during hospitalization.

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

4 | DISCUSSION

Re-infections 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 re-infection (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 ($P = .0287$). 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 viraemia, such as in measles 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 re-infection 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 suggests 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 titre 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.[4,19] 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.

5 | CONCLUSION

Thus, whether re-infection results from the insufficient efficacy of natural immunity or from its too high specificity

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)

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)[a]

Note: 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.

[a]ICU and or death at the second episode $P = .0287$ (Fisher exact test).

regarding SARS-CoV-2 genomic mutations is a difficult question. More studies are needed on re-infected patients in association with co-morbidities, viral strains and clinical outcome. 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.

KEYWORDS

COVID-19, death, ICU, immunity, mutant, vaccine, prognosis, re-infection, SARS Cov2, variant

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CONFLICT OF INTEREST

All authors are not in conflict of interest with the content of this manuscript.

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REFERENCES

1. Coronavirus Update (Live): 96,750,697 Cases and 2,068,974 Deaths from COVID-19 Virus Pandemic - Worldometer [Internet]. https://www.worldometers.info/coronavirus/#countries [Accessed 12 April 2021].
2. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med*. 2007;357(19):1903-1915.
3. Couch RB, Kasel JA. Immunity to influenza in man. *Annu Rev Microbiol*. 1983;37(1):529-549.
4. Tsang TK, Ghebremariam SL, Gresh L, et al. Effects of infection history on dengue virus infection and pathogenicity. *Nat Commun*. 2019;10(1):1246.
5. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. *N Engl J Med*. 2020;383(20):1920-1931.
6. Latest - Oxford Covid-19 vaccine trial results - Wits University [Internet]. https://www.wits.ac.za/covid19/covid19-news/latest/oxford-covid-19-vaccine-trial-results.html [Accessed 12 April 2021].
7. Amrane S, Tissot-Dupont H, Doudier B, 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;36:101632.
8. Lagier J-C, Million M, Gautret P, 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;36:101791.
9. Colson P, Levasseur A, Delerce J, et al. Dramatic increase in the SARS-CoV-2 mutation rate and low mortality rate during the second epidemic in summer in Marseille. Unpublished [Internet]. https://www.mediterranee-infection.com/wp-content/uploads/2020/04/FD_Raoult_SARS-CoV-2_EID_Sep2020_vL2.pdf [Accessed 12 April 2021].
10. Colson P, Levasseur A, Gautret P, 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;40:101980.
11. Colson P, Finaud M, Levy N, Lagier J-C, Raoult D. Evidence of SARS-CoV-2 re-infection with a different genotype. *J Infect*. 2020. https://doi.org/10.1016/j.jinf.2020.11.011. [Epub ahead pf print]
12. Bedotto M, Fournier P-E, Houhamdi 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*. Cold Spring Harbor Laboratory Press. 2021.
13. Clinical management of COVID-19 [Internet]. https://www.who.int/publications-detail-redirect/clinical-management-of-covid-19 [Accessed 12 April 2021].
14. Cohen JI, Burbelo PD. Reinfection with SARS-CoV-2: implications for vaccines. *Clin Infect Dis*. 2020. https://doi.org/10.1093/cid/ciaa1866. [Epub ahead pf print]
15. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. *Nature Medicine*. 2020;26(11):1691-1693.
16. Choi B, Choudhary MC, Regan J, et al. Persistence and evolution of SARS-CoV-2 in an Immunocompromised Host. *N Engl J Med*. 2020;383(23):2291-2293.
17. Walker BD, Burton DR. Toward an AIDS vaccine. *Science*. 2008;320(5877):760-764.
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;158(7):1445-1459.

19. Vatti A, Monsalve DM, Pacheco Y, Chang C, Anaya J-M, Gershwin ME. Original antigenic sin: a comprehensive review. *J Autoimmun*. 2017;83:12-21.
20. Wilder-Smith A, Flasche S, Smith PG. Vaccine-attributable severe dengue in the Philippines. *Lancet*. 2019;394(10215):2151-2152.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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