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SARS-CoV-2 with positive re-test: a case study and bibliographic review

Diana Rebeca Acosta-Fócil[1], Andrea Alejandra Toledo-Rojas[1,2] and Agnès Fleury[1,3]*

[1]Instituto Nacional de Neurología y Neurocirugía, Unidad Mixta de Estudios de la Neuroinflamación, Instituto de Investigaciones Biomédicas, [2]Facultad de Medicina, División de Investigación, [3]Instituto de Investigaciones Biomédicas, Depto. De Medicina Genómica y Toxicología Ambiental, Universidad Nacional Autónoma de México, Av. Universidad # 3000, Alcaldía de Coyoacán 04510, Ciudad de México, México.
E-mail: *afleury@iibiomedicas.unam.mx, ORCID ID 0000-0002-8930-2202

Abstract

One of the questions that remains regarding infection with SARS-CoV-2 is whether re-infection is possible and if so, what factors might be promoting it. To answer this question, we first presented the case of a patient with two probable successive infections by SARS-CoV-2. Then, we performed a review of the literature (using Pubmed, Scielo, Google Scholar and Elsevier) to assess the importance of having 2 RT-PCR positive tests separated by 1 negative. Three different circumstances have been identified: subjects with reinfection confirmed by genetic analysis of the virus; positive re-test in asymptomatic subjects; and subjects with possible reinfection. Different circumstances could be involved in the fact of a reinfection. One of them, the infection with a second virus genetically different from the first and that has not been affected by the immune response developed after the first infection, and the second, the fact that not all patients will develop a persistent protective immune response after a first infection. Many unknowns remain on this subject and more research is needed to better understand the characteristics of the immune response, as well as its efficacy on the different variants of the virus.

Keywords: COVID-19 reinfection case report; SARS-CoV-2 reinfection; SARS-CoV-2 antibodies; SARS-CoV-2 immunity; re-test positive to SARS-CoV-2.

SARS-CoV-2 con nueva prueba positiva: estudio de caso y revisión bibliográfica

Resumen

Una de las preguntas que surge con respecto a la infección por SARS-CoV-2 es si la reinfección es posible y, de ser así, qué factores podrían estar promoviéndola. Para responder a esta pregunta, primero presentamos el caso de un paciente con dos probables infecciones sucesivas por SARS-CoV-2. Posteriormente, realizamos una revisión de la literatura (utilizando Pubmed, Scielo, Google Scholar y Elsevier) para evaluar la importancia de tener 2 pruebas de RT-PCR positivas separadas por 1 negativa. Se identificaron tres circunstancias diferentes: sujetos con reinfección confirmada por análisis genético del virus; nueva prueba positiva en sujetos asintomáticos; y sujetos con posible reinfección. Diferentes circunstancias podrían verse involucradas en el hecho de una reinfección. Una de ellas, la infección con un segundo virus genéticamente diferente del primero y que no se haya visto afectado por la respuesta inmune desarrollada después de la primera infección, y la segunda, el hecho de que no todos los pacientes desarrollarán una respuesta inmune protectora permanente después de una primera infección. Aún quedan muchas incógnitas sobre este tema y son necesarias más investigaciones destinadas a comprender mejor las características de la respuesta inmune, así como su eficacia sobre las diferentes variantes del virus.

Palabras clave: reporte de caso de reinfección por COVID-19; reinfección por SARS-CoV-2; anticuerpos contra SARS CoV-2; inmunidad contra SARS-CoV-2; re-test positivo a SARS-CoV-2.

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Introduction

On March 2020, a global pandemic caused by the new SARS-CoV-2 virus, was declared (Organización Panamericana de la Salud, 2020). Although the scientific knowledge about it has increased rapidly, many aspects of this infection are still unknown (Gao *et al*., 2020). Quantification of reinfection risk and evaluation of associated factors to this risk is still an unsolved question (Organización Panamericana de la Salud, 2020).

Here, we present a case of a young Mexican medical doctor who experienced two symptomatic clinical pictures of COVID-19 a month apart, both confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Before the second one, she had tested negative by RT-PCR and was asymptomatic.
In this context, a review of the literature was carried out to decipher the meaning of having two positive RT-PCR tests separated by a negative result.

Case report

A 25-year-old female, with history of endometriosis and controlled asthma, who worked taking samples for detection of SARS-CoV-2 by RT-PCR. Her symptoms began on 8th June, 2020 (day 1**)** **(**Figure 1**)**, when she presented odynophagia and mild cough without expectoration at night. On day 4 she tested positive for RT-PCR SARS-CoV-2.

During the next week she continues with mild symptoms, plus anosmia, moderate intensity headache and asthenia. She did not present dyspnea nor fever and maintained O_2 Sat levels >98%. After 25 days of quarantine, she returned to work on day 29 completely asymptomatic. On day 32, an IgG antibody test for SARS-CoV-2 was negative and on day 33 a RT-PCR was also negative.

On day 35 she reported diarrhea at night, odynophagia, dry cough and in the morning, she had a fever at 38.4 °C. A RT-PCR for SARS-CoV-2 performed at day 36 resulted positive. Chest X-ray did not show alterations on day 38, and laboratory studies reported: leucocytes 4,200; lymphocytes 53%; D-Dimer 0.54; ferritin 42.5 and C-Reactive Protein 0.6. In the following days new symptoms appeared: anosmia, dysgeusia, intense headaches and persistent fever at >38.3 °C. O_2 Sat decreased to 91%, so she attended emergency medical services, where new laboratory samples were taken, reporting: leucocytes 4,100; lymphocytes 63%; D-Dimer 368.99; ferritin 59.8 and C-Reactive protein: negative. She was discharged with indication to monitor O_2 Sat levels.

Day 42 (day 8 of the second infection) was the last day with fever, and the rest of the symptoms gradually subsided over the following weeks. On day 52, still presenting anosmia, dysgeusia and asthenia, she tested positive to IgG antibody for SARS-CoV-2. On November 23rd, four months after the onset of symptoms, she was asymptomatic, and SARS-CoV-2 IgG was still positive.

Literature review

We made a web search to include the cases of patients presenting two RT-PCR positive tests separated by a RT-PCR negative test or by an asymptomatic period. The search was performed on Pubmed, Scielo, Google Scholar and Elsevier using *"COVID-19 reinfection case report", "SARS-CoV-2 antibodies", SARS-CoV-2 immunity", "re-test positive"* as keywords, up to December 2020. Appropriate references of the reviewed articles were also included, as pre-print and per reviewed articles. World Health Organization and Center for Disease Control and Prevention webpages were consulted.

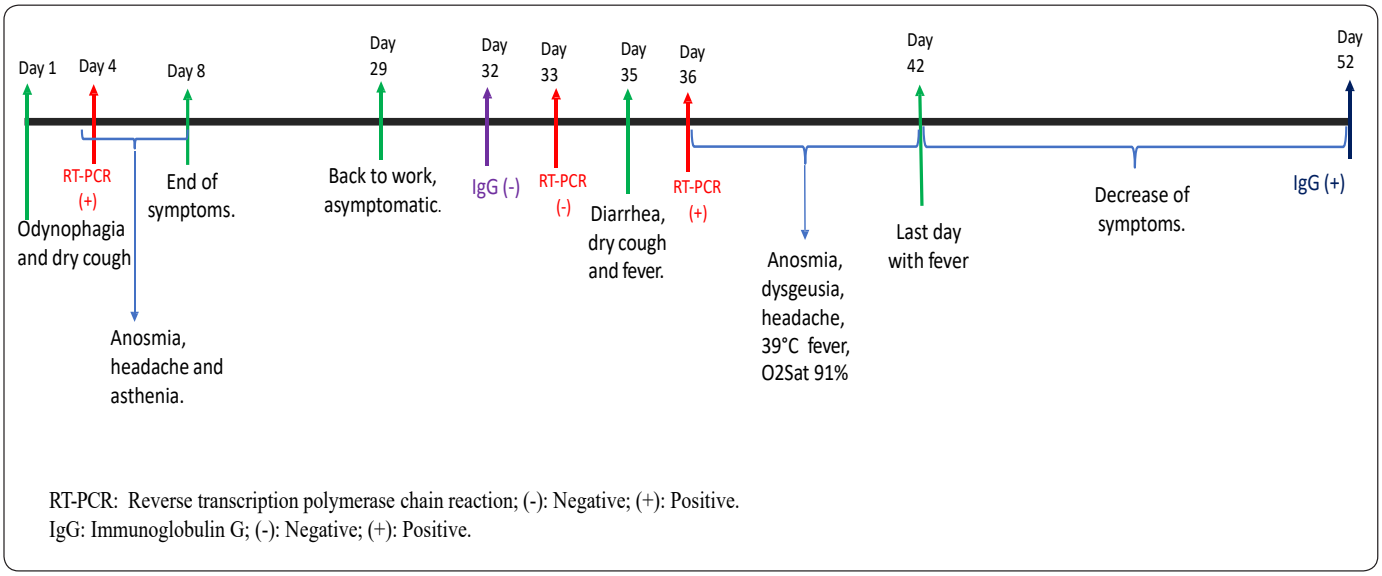

Figure 1. Case report timeline.

Three situations were clearly defined

Subjects with reinfection confirmed by genetic analysis of the virus (Table I).

We found 10 such cases published in the literature (Tillett *et al.*, 2021; Larson *et al.*, 2020; Gupta *et al.*, 2020; To *et al.*, 2020; Prado-Vivar *et al.*, 2021; Van Elslande *et al.*, 2020; Goldman *et al.*, 2020; Mulder *et al.*, 2020; Selhorst *et al.*, 2020). The reinfection was confirmed because, in all cases, the virus of the second infection presented genetic differences compared to the first infection's virus.

The main characteristics of these cases were: mostly male (6/10), and an average age of 44 years old (25-89 range), consisting on eight adults and two seniors. 60% presented mild symptoms during first infection, one a moderate clinical picture, two were asymptomatic and one, with a previous pulmonary disease, had a severe presentation. Of the four patients tested for the presence of SARS-CoV-2 IgG before the second infection, three were negative, and one was positive. By comparing the severity of the two clinical pictures, in 4 patients (40%) it was similar on both, in 3 the first was the most severe, while in 3 the second infection was the most severe. The only patient with positive antibodies after the first disease presented a milder second disease. The time between both infections was 98.5 days on average (range 48-185 days).

Positive re-test in asymptomatic subjects (Table II).

Different case series described this situation (Lu *et al.*, 2020; Lan *et al.*, 2020; An *et al.*, 2020; Huang *et al.*, 2020). All these early series come from China. In this country, at the start of the pandemic, all the hospital discharged patients were to be isolated for 14 days, and at the end of this period new RT-PCR tests were to be carried out. Discharge criteria included particularly to have 3 negative RT-PCR tests with a 24-hour difference between each of them.

The number of asymptomatic subjects included in these studies was 198, and their main characteristics are presented in Table II. As shows, there was a short time between negative and positive RT-PCR (less than 3 weeks), with most patients (94.4%) being asymptomatic and not contagious at the moment of the second positive RT-PCR. Indeed, in 3 of the 4 series, a contact's follow

Table I. Cases of reinfection confirmed by viral sequence analysis.

Reference	Country	Age, Sex	Comobirdities	Initial symptoms	Severity	First positive RT-PCR	Negative RT-PCR	IgG test, +/-	Second presentation	Severity	2nd. Positive RT-PCR	IgG(+)
Tillet *et al.*, 2021	USA	25, M	None	March 25	Mild	April 18	May 09,26	NR	May 31	Moderate	June 05	June 06
Larson *et al.*, 2020	USA	42, M	None	March 19	Mild	March 20	NM	NR	May 19	Moderate	May 24	June 01
Gupta *et al.*, 2020	India	25, M	None	NR	Asympt.	May 05	May 13	NR	Asympt.	Asympt.	Aug 21	NR
Gupta *et al.*, 2020	India	28, F	None	NR	Asympt.	May 07	May 27	NR	Asympt.	Asympt.	Sep 05	NR
To *et al.*, 2020	China	33, M	None	March 26	Mild	March 26	April 13	May 05, (-)	Asympt.	Asympt.	Aug 15	Aug 20
Prado *et al.*, 2021	Ecuador	46, M	None	May 12	Mild	May 23	June 03	May 16, (-)	July 20	Mild	July 22	Aug 18
Van Elslande *et al.**, 2020	Belgium	51, F	Asthma	March	Mild	March 09	NR	NR	June	Mild	June 10	June
Goldman *et al.**, 2020	USA	60-69, M	Pulmonary emphysema, SAH	March	Severe	March	Day 39 & 41	July, (-)	June	Moderate	Day 140	NR
Mulder *et al.**, 2020	Netherlands	89, F	Waldenström's Macro-globulinemia	NR	Mild	NR	NR	NR	NR	Mortal	NR	(-)
Selhorst *et al.*, 2020	Belgium	39, F	None	March	Moderate	March 16	NR	June 18, (+)	September	Mild	Sep 17	Sep 23

(+): positive; (-): negative; M: male. F: female. SAH: Systematic Arterial Hypertension. NR: Not reported. Asympt.: asymptomatic. NM: Not mentioned. Neg: negative. Pos: positive. Aug: August. Sep: September. *Peer-reviewed.

Table II. Subjects with positive re-test.

References	Dates	Number of cases/%	Days between negative and positive RT-PCR	Contacts' follow up	New symptoms	N IgG(+) / N tested
Lu *et al*., 2020	january-february	87/619 (14%)	2-19 days	No positive contacts	10 unproductive cough at night	58/59
Lan *et al.*, 2020	january-february	4/19 (21.05%)	5-13 days	No positive contacts	No	NR
An *et al.*, 2020	january-march	38/262 (14.5%)	<14 days	No positive contacts	No	NR
Huang *et al.*, 2020	january-april	69/414 (16.7%)	<14 days	NR	No	40/40

N: number of patients. NR: not reported.

up was done and no cases were detected. In this scenario, the second positive test was a random finding. The detection of IgG antibodies was done in two studies (Mulder *et al*., 2020; Lan *et al*., 2020) on 156 patients. From these, 155 resulted positive (99.3%).

Subjects with possible reinfection (Table III).

Ten published cases, and the one presented in this paper, might be cases of reinfection (Bonifácio *et al*., 2020; Duggan, Ludy, Shannon, Reisner & Wilcox, 2021; Fernandes Valente Takeda *et al*., 2020; Ozaras, Ozdogru & Yilmaz, 2020). Genetic analysis of the virus was not performed in any patient during both clinical pictures. Most of the patients were male (7, 63.6%), mean age being 42.5 years (range of 23 to 82 years), consisting on eight young adults and three seniors. Seven (63.6%) had a RT-PCR test at the end of the first COVID clinical picture, all resulting negative. 3 patients were also tested for IgG antibodies at this moment and all of them were negative. 4 patients were tested for antibodies at the end of the second infection, with 2 of them being positive and 2 being negative. Ten of the patients (90.9%), presented mild symptoms both times. The time between clinical pictures was on average 56.7 days (range of 25 to116 days). All of these patients presented symptoms in both clinical pictures.

Discussion

One of the main observations of this review is that the possibility of symptomatic reinfections with SARS-CoV-2 virus seems to be extremely low. Although it is known that the publications present only a partial view of reality, given that to date more than 70 million COVID-19 cases have been reported worldwide, the number of reinfections published is really low. In this context, a study from Qatar estimates the risk of reinfection at 0.02% (Abu-Raddad *et al*., 2020).

This low frequency of reinfections is probably related with the fact that the infection is, in most patients, followed by the development of a specific immune response that protects the host (Deeks *et al*., 2020).

Frequency and duration of the immune response

Different studies have evaluated the frequency of the antibody response after infection. In particular, a wide study from China found that from the fifth week after presentation of symptoms, more than 95% of patients developed specific IgG and by week 12, 100% of subjects had IgG (Li *et al*., 2020). Regarding this, a Cochrane systematic review of the literature found compatible results. Here, 91.4% of patients presented IgG antibodies 2-3 weeks after symptoms onset, and 96.0% 4-5 weeks after (Deeks *et al*., 2020). In addition, in most of the studies a positive correlation between disease severity and post-disease antibodies levels was observed. Seronegativity was significantly more frequent in asymptomatic individuals than in symptomatic patients (Li *et al*., 2020; Röltgen *et al*., 2020; Weis *et al*., 2021; Shirin *et al*., 2020).

Another question that remains is the duration of the antibodies' response. This also seems to depend, although not exclusively, on the severity of the COVID-19. It was shown that the decrease of the antibodies was faster and more evident in asymptomatic subjects and patients with mild symptoms (Röltgen *et al*., 2020). In a study from India, in which 201 asymptomatic people who had positive IgG were retested 45 to 65 days after the first test, 141 (70.15%) had negative results (Nag, Chaudhry, Mishra, Rai & Gupta, 2020). Another study showed a decrease of antibodies' titer in a sample taken approximately 60 days after the first test in 94% (146/156) of participants, of which 28% (44/156) had results below positive range (Self *et al*., 2020). Here the negativization of the response was also significantly more frequent in asymptomatic people vs. symptomatic (Self *et al*., 2020).

It seems to this day, that the decrease of antibody titer with time is an evidence (Dan *et al*., 2021). However, this does not

Table III. Cases of not confirmed reinfections.

Reference	Country	Age, sex	Comorbidities	Initial symptoms	Severity	First positive RT-PCR	Negative RT-PCR	IgG test (date, +/-)	Second presentation	Severity	2nd. Positive RT-PCR	IgG(+)
Zhou *et al.*, 2021	China	40, M	None	Jan 18	Severe	Jan 23	Feb 04,06	NR	Feb 13	Moderate	Feb 14	Feb 19 Neg
Bonifacio *et al.*, 2020	Brazil	24, F	None	May 06	Mild	May 13	NR	July 02, (-)	Jun 27	Mild	July 02	July 16
Duggan *et al.*, 2021	USA	82, M	SAH, CKD, DM, Parkinson	April	Severe	April	May	NR	May	Severe	May	NR
Fernandes Valente Takeda *et al.*, 2020	Brazil	26, M	None	March 16	Mild	March 27	NR	NR	May 08	Mild	May 13	NR
Fernandes Valente Takeda *et al.*, 2020	Brazil	63, M	SAH	March 16	Mild	March 27	NR	NR	May 13	Mild	May 18	NR
Fernandes Valente Takeda *et al.*, 2020	Brazil	40, F	Asthma, spondylitis	March 18	Mild	March 18	March 30	NR	May 27	Mild	Jun 01	NR
Fernandes Valente Takeda *et al.*, 2020	Brazil	67, M	SAH, apnea, obesity	March 20	Mild	March 24	April 08	NR	May 13	Mild	May 16	NR
Fernandes Valente Takeda *et al.*, 2020	Brazil	47, M	None	March 23	Mild	March 23	April 07	NR	May 18	Mild	May 22	NR
Fernandes Valente Takeda *et al.*, 2020	Brazil	31, M	None	April 09	Mild	April 15	NR	NR	Jun 05	Mild	Jun 08	NR
Ozaras *et al.*, 2020	Turkey	23, F	None	April 09	Mild	April 09	April 22,27	NR	Aug 04	Mild	Aug 04	Aug 17 (-)
Present case	Mexico	25, F	Asthma, endometriosis	June 08	Mild	June 11	July 10	July 09, (-)	July 12	Mild	July 13	July 29

(-): Negative; (+): positive; M: male; F: female; SAH: Systematic Arterial Hypertension; CKD: Chronic Kidney Disease; DM: Diabetes mellitus; Jan: January; Feb: February; Aug: August; NR: Not reported; Neg: negative.

mean that immunity does not persist with time. Memory cells are still present and might allow a fast response if necessary. It has been reported that T CD4 and CD8 cells of patients recovered from moderate to severe COVID-19, can recognize multiple regions of SARS-CoV-2 virus' N-protein (Le *et al.*, 2020; Grifoni *et al.*, 2020).

Ability of the immune response to provide protection

As we know, all the viral infections are followed by the development of an immune response, considered as protective (Mueller & Rouse, 2008). Regarding protective immunity following natural infection by SARS-CoV-2, information is currently scarce (Dan *et al.*, 2021). Therefore, we cannot specify an approximate efficacy rate when only a few cases of reinfection have been reported, without their natural immune response having been systematically analyzed. However, there are viral diseases whose healing depends mainly, if not exclusively, on the antibody response, and others where the destructive action of the killer lymphocytes is fundamental (Dan *et al.*, 2021; Mueller & Rouse, 2008**).** What the situation is in the case of COVID-19 is not yet clearly defined, although several data suggest that the major protective effect is to be attributed to antibodies against the Spike protein, and in

particular against its receptor-binding domain (Dan *et al*., 2021; Forni & Mantovani, 2021; Shah, Firmal, Alam, Ganguly & Chattopadhyay, 2020).

Although the immunity developed after vaccination may be different from the immunity acquired after direct contact with a virus (Galipeau, Greig, Liu, Driedger & Langlois, 2020), the results of phase III evaluation of different vaccines have shown that a strong protective immunity is obtained (Table IV). The duration of this protective immunity remains unknown, but these results confirm the ability of the immune response (after vaccine or infection) to provide protection.

It remains to be determined whether the vaccines currently being developed will be effective against the new variants of the virus. SARS-CoV-2 is an RNA virus, and these viruses generally have a high mutation rate (Lauring & Andino 2010; Duffy, 2018). Genetic instability has long been considered to represent a challenge for the development of effective vaccines against RNA viruses (Forni & Mantovani, 2021). Thousands of mutations have already appeared, but only a very small minority are likely to be able to change the virus appreciably (Wise, 2020). In December 2020, the presence of a new variant of the SARS-Cov-2 virus called B1.1.7 was reported in the U.K.; in South Africa another variant called B.1.351 emerged independently, and in Brazil a variant called P.1 was identified in early January (CDC March 2021, https://www.cogconsortium.uk/_(Wise, 2020; Zhou *et al*., 2021). These variant strains, compared to that of Wuhan, show multiple changes (deletions and substitutions) in the spike protein, 9 for B.1.1.7, 10 for B.1.351, and 12 for P.1. Most of the concern comes from mutations in the receptor-binding domain (RBD) of the spike protein that the virus uses to bind to the human ACE2 receptor, as it is the main target of the three leading vaccines (Wise, 2020; Zhou *et al*., 2021; Villoutreix, Calvez, Marcelin & Khatib, 2021).

Recently, two letters were published regarding the effectiveness of the Pfizer and Moderna vaccines on the new variants (Wu *et al*., 2021; Liu *et al*., 2021). It seems that their efficacity is good on the B.1.1.7 variant since the antibodies obtained from the plasma of vaccinated subjects neutralize equally the original strain of the virus and this mutant. However, in both cases the ability to neutralize the mutant B.1.351 is reduced by 50% (Wu *et al*., 2021; Liu *et al*., 2021). The response of previously infected or vaccinated individuals to these new variants will be the subject of further studies in the coming months. It is possible that although the antibody response against new variants may not prevent infection, its severity may be less. Indeed, T cell responses to the spike protein in particular, might not be disturbed by the mutational changes and might help limit the spread of infection to the lower respiratory tract, thus preventing severe disease (Zhou *et al*., 2021).

Over time, as more mutations occur, the vaccine may need to be modified. This happens with seasonal flu, which mutates every year, the vaccine being adjusted accordingly (Wise, 2020).

Table IV. Sars-Cov2 Vaccines.

Vaccine name	Country	Laboratory	Vaccine type	Efficacy (phase III Results)	Activity on new variants
BNT162b2	USA-Germany	Pfizer BioNTech	mRNA	95%	Effective for B.1.1.7. Less effective against B.1.351.
ARNm-1273	USA	Moderna Tx, Inc	mRNA	94.1%	Effective for B.1.1.7. Less effective against B.1.351.
Sputnik V Gam-Covid-VAC	Rusia	Gamaleya National Center	Viral vector	91.6%	N.R.
NVX-CoV2373	USA	Novavax	Protein subunit	89.3%	N.R.
AZD1222	United Kingdom-Sweden	Oxford-Astra Zeneca	Viral vector	70.4%	N.R.
CVnCov	Germany	Curevac/GlaxoSmithKline	mRNA	Unknown	N.R.
BBIBP-CorV	China	Sinopharm	Inactivated virus	79.3%	Less effective against B.1.351.*
Ad26.COV2.S	USA-Belgium	Johnson & Johnson	Viral vector	66%	N.R.
CoronaVac	China	Sinovac-Biotech	Inactivated virus	50.4%	N.R.

N.R. Not reported.

SARS-CoV-2 virus does not appear to mutate as quickly as the influenza virus, and mRNA vaccines that have been shown to be effective so far can be modified more easily than traditional vaccines if necessary (Wise, 2020).

With these data, what can we say on the reported cases with two positive RT-PCR?

First, it is very probable that the cases presented in Table II does not correspond to reinfections. Several characteristics distinguish them from patients with confirmed reinfections presented in Table I. Particularly time between positive RT-PCR tests was in average of 98.5 days for confirmed reinfections, and less than 21 days for the cases presented in Table II. Also, regarding clinical presentations, 70% of confirmed reinfections were symptomatic, while only 5.6% of the Table II patients were. Thus, it seems that 2 groups of distinct subjects are considered in each table. The negativity of a RT-PCR test between two positive tests can be favorized by two factors. It is possible that after a decrease in the viral load associated with the administration of antiviral treatment, it becomes detectable again when treatment is stopped (Gao *et al*., 2020). Also, false negative RT-PCR tests occurs, particularly due to testing, transportation or laboratory procedure's errors (Wang, Kang, Liu & Tong, 2020; Woloshin, Patel & Kesselheim, 2020). On the other hand, the persistence of positivity in RT-PCR tests can be linked to the persistence of pieces of viral particles or fragments without active replication (Kang, Wang, Tong & Liu, 2020). Indeed, many viruses demonstrate prolonged presence of genetic material in its host even after clearance of the live virus and resolution of symptoms (Duggan *et al*., 2021)**.** Therefore, detection of genetic material by RT-PCR alone does not imply active infection or infectivity (Dao, Hoang & Gautret 2021). In this sense, it is interesting to note that patients in Table II seems to be not contagious at the moment of the second positive RT-PCR as none of their contacts become infected.

About patients with confirmed reinfections (Table I), it draws attention that from the 4 patients with specific antibodies test performed after the first infection, all but one, were negative. In the case of the patient with positive antibodies having neutralizing capacity after the first infection, the sample was taken 3 months after the first presentation, but three months before the second presentation; it is therefore impossible to know whether the antibodies persisted in sufficient amount right before the second infection (Selhorst *et al*., 2020). It is also important to note that in this case the re-infecting virus did not harbor any known spike mutation that could have enabled the escape from neutralizing antibodies induced during primary infection. Her second clinical picture was milder than the first one and antibodies' response was faster the second time (Selhorst *et al*., 2020).

In the cases of patients with possible reinfections (Table III), the specific antibodies tests performed after the first disease were negative. The vast majority of these patients presented a mild first disease and thus it is very likely that their antibody response after the first infection was absent or weak enough to allow a second infection. In the case of the patient that had two severe presentations, reinfection is particularly doubtful, because the new symptoms occurred only 10 days after discharge and new positive RT-PCR was observed during a confirmed bacterial superinfection (Duggan *et al.,* 2021). The other patient with a severe first disease developed a milder presentation during the second infection. Although the presence of antibodies was not assessed, he likely developed a strong protective immune response after the first infection, which could be involved in the lesser severity of the second episode.

In conclusion, different conditions are most likely involved in the possibility of reinfections. In particular, infection with a second virus genetically different from the first and unaffected by the immune response developed after the first infection, and the fact that not all patients will develop a persistent protective immune response after a first infection (Figure 2). The currently published cases do not allow us to know the respective weight of each of these factors in the risk of developing reinfection. One fact seems however certain: the risk of reinfection is higher when the first infection is mild because the antibody response that results from it is weaker and lasts for a shorter time. The increase in knowledge generated every day will make it possible to have more precise information in the future; meanwhile, our best weapon remains prevention with the help of vaccine, face masks, social distancing and correct handwashing, both in the cases of having and not having been previously infected.

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