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ORIGINAL ARTICLE

Low risk of reinfections and relation with serological response after recovery from the first wave of COVID-19

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Abstract

The aim of the study was to assess reinfection rates in relation to long-term antibody dynamics against SARS-CoV-2 after the first wave. A prospective longitudinal study with monthly serological follow-up during the first 4 months, and then at 6, 8, and 10 months after the disease onset of all recovered adult in- and outpatients with COVID-19 attending Udine Hospital (Italy) from March to May 2020. During the follow-up, reinfections were collected. A total of 546 unselected individuals with COVID-19 acquired from March to May 2020 were included (292 female, mean age 53 years). After a median follow-up of 10 months (IQR 6.2–10.4), reinfection occurred in 6 (1.1%) patients, median age of 44.5 years (IQR 33−49). All had a previous history of mild COVID-19 (all were healthcare workers) and reinfection occurred a median of 9 months (IQR 8.2−10.2) after the onset of the first episode. Patients with reinfection were either seronegative (2/56, $n=3.6\%$), seroreverted (2/137, 1.5%), or seropositive (2/353, 0.6%) ($p=0.085$). All reinfections were mild ($n=5$) or asymptomatic ($n=1$). After reinfection, none of patients developed IgM response and only two had a transitory boosted IgG immunization response. In an unselected population after the first wave of COVID-19, after a prolonged observation period (mean 10 months), reinfection was very uncommon; occurred in patients with a previous history of mild infection, mostly with weak or absent serological response; and manifested with mild or asymptomatic clinical presentation.

Keywords SARS-CoV-2 reinfection · COVID-19 reinfection · SARS-CoV-2 antibodies · SARS-CoV-2 serology · Longitudinal study · SARS-CoV-2 IgM · SARS-CoV-2 IgG

Abbreviations

BMI	Body mass index
COVID-19	Coronavirus Disease 2019
ICU	Intensive care unit
IQR	Interquartile range
HCW	Healthcare worker
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard deviation

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Introduction

Cases of Coronavirus Disease 2019 (COVID-19) reinfection have been sporadically reported and questions remain on the incidence, time of occurrence, duration, and protective role of immunity after natural infection. [1–5] Knowledge available on reinfections is still scarce due to the frequently retrospective nature of published reports and the limited follow-ups post-symptom onset. In addition, all studies provide estimates in selected biased populations derived from laboratory testing data sets, specific outbreak settings, or subgroups such as healthcare workers (HCWs), and it is unclear how generalizable and applicable the findings are to an unselected adult population [6–13].

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) humoral immunity suggests that more than 90% of seroconversion rates occur after acute primary infection with variable degrees of decline in antibody levels over time [14, 15]. However, the literature available provides limited evidence on the presence of SARS-CoV-2 antibodies as a surrogate of individual protection against reinfection [1–7]. A better understanding of the long-term dynamics of immune response to SARS-CoV-2 infection and the risk of reinfection would help in defining and monitoring the extent of virus spread and the herd immunity, as well as in identifying the most appropriate public health strategies including vaccination planning to control the of COronaVIrus Disease 19 (COVID-19) pandemic [16].

The aim of this prospective longitudinal study was to comprehensively characterize the relative incidence of COVID-19 reinfections in relation to serological response among individuals who had recovered from COVID-19 after the first wave. The study included a wide spectrum of unselected patients ranging from asymptomatic to severely infected, assessed over a 10-month follow-up period.

Methods

Study setting and patient population

We performed the study at Udine Hospital (Italy), a 1000-bed tertiary-care teaching hospital identified as a regional referral center for COVID-19 patients and serving approximately 350,000 citizens. Methods and findings are reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [17].

The target population was a cohort of all consecutive adult in- and outpatients (≥ 18 years) attending the Infectious Disease Department with a diagnosis of COVID-19 from March 1 (the day of the first COVID-19 diagnosis at our hospital) until May 30, 2020. Further definitions of acute COVID-19 and baseline conditions are summarized in Supplementary Table 1.

Serological test collection, reinfection follow-up, and definitions

SARS-CoV-2 antibody concentrations were measured at the serological follow-up visits each month (± 15 days) after symptom onset for the first 4 months, and every other month up to 10 months (± 15 days), from March 2020 to February 2021 (CORMOR 3–4® protocol). Patients attending at least two serological follow-ups were included in the study (Fig. 1).

Clinical reinfection was defined as clinical recurrence of symptoms compatible with COVID-19, accompanied by a positive PCR test (Ct < 35), more than 90 days after the onset of the primary infection, supported by close-contact exposure or outbreak settings, and no evidence of another cause of infection. Epidemiological reinfection

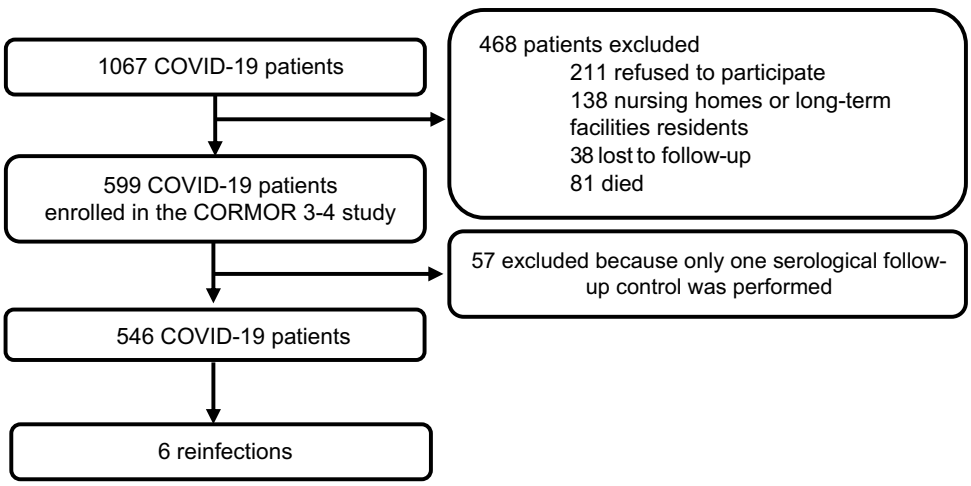

Fig. 1 Reinfection and serological follow-up (up to February 2021): flow diagram of in- and outpatients with COVID-19 included

was defined as any positive PCR test (Ct < 35) more than 90 days from first episode, regardless of symptoms [18].

Specifically, patients with symptoms or signs of recurrent illness (fever, rhinorrhea, sore throat, cough, dyspnea, sputum, myalgia, fatigue, thoracic pain, vomiting, diarrhea, dysgeusia or anosmia, conjunctivitis, rash) and/or a positive PCR test for SARS-CoV-2 were instructed to contact the research team by phone, in order to schedule a prompt visit (within 24 h) at the infectious disease outpatient clinic or go to the emergency department for medical examination and PCR test for SARS-CoV-2. In addition, during this contact, patients were asked about previous not-reported episodes of symptoms/signs. Systematic SARS-CoV-2 PCR test was performed at regular intervals (every 2/4 weeks) only for healthcare workers (HCWs) in accordance to Hospital and Nursing homes/long-term facility protocols.

At the time of reinfection, for definitive analysis, we classified patients according to their most recent (within 2 months) antibody status into three groups: (1) seronegative in the absence of any IgM-/IgG-positive serological samples; (2) seroreverted in the presence of a decline in IgM/IgG antibody levels below the positivity threshold after initial seroconversion; and (3) seropositive in the presence of persistence of IgM-/IgG-positive serological sample.

Antibody measurements

Serum concentrations of the anti-SARS-CoV-2 specific antibodies IgG and IgM were assessed using iFlash-SARS-CoV-2 (Shenzhen YHLO Biotech Co., Ltd., China, distributed in Italy by Pantec SRL), a paramagnetic particle chemiluminescence immunoassay (CLIA) for the determination of IgM and IgG antibodies against SARS-CoV-2 N and S protein. In accordance with the manufacturer's instructions, the IgM and IgG thresholds for positivity were considered to be 10.0 kAU/L.[19].

Statistical analysis

Patients' demographic and clinical characteristics were presented with absolute values and percentages for categorical variables and means or medians (standard deviation (SD) or interquartile ranges (IQRs)) for continuous variables. The Shapiro–Wilk test was used to assess whether data were normally or non-normally distributed. Categorical variables were compared using the chi square (χ^2) test or Fisher's exact test, while quantitative variables were compared using the *t*-test or Mann–Whitney *U* test, as appropriate. Statistical analysis was performed using STATA 16.1.

Results

Study population at onset of acute COVID-19

Overall, during the study period, a total of 1067 patients received the COVID-19 diagnosis. After excluding 211 patients for refusing to participate in the research, 138 nursing home/long-term facility residents who were not capable of giving their consent due to cognitive decline, 38 who were lost to follow-up, 51 for incomplete serological follow-up and 81 deaths, a total of 546 patients were included (Fig. 1). Demographic and clinical characteristics of patients at baseline are summarized in Table 1. The mean age of our study population was 53 years (SD 15.4; range 18−94), 292 (53.5%) were female and the majority (480/521, 92.1%) were native Italians. One hundred and fifteen were HCWs. During the acute phase, most patients (502, 91.9%) were symptomatic and presented mild illness (374, 68.5%). One hundred and forty-seven (27.2%) had been hospitalized (22 in the intensive care unit) (Table 1).

Serological dynamics of SARS-CoV-2 IgM and IgG after primary infection

A complete description of the serological evolution of the study population is presented in another work that is currently in progress. In brief, the overall seroconversion rate within 2 months was 32% for IgM: 25% in mild cases and 61% in moderate to critical cases. IgM was generally not detected after 4 months (90th percentile equal to 135 days). The overall seroconversion rate for IgG within 2 months was higher for IgG (90%): 91% in mild patients, 100% in moderate to critical patients, and only 54% in asymptomatic cases. About half of the patients (47%) had experienced IgG seroreversion at 10 months, and rates of antibody loss were almost complete (88%) for asymptomatic patients, around half for mild cases (53%) but only 13% for moderate to severe COVID-19.

Reinfection

Patients were followed up for a median of 10 months (IQR, 6.2–10.4). The reinfection rate was 1.1% (6/546). Cases of reinfection occurred at a median of 9 months (IQR 8.2−10.2) after the acute onset of the first episode. The median age was 44.5 years (IQR 33−49) and all were HCWs (Table 2). As reported in Table 2, all patients experienced mild infection (6/370, 1.6%) during the first episode and manifested mild ($n = 5$) or asymptomatic ($n = 1$) reinfections. Reinfection rates did not differ significantly in seronegative (2/56, $n = 3.6\%$), seroreverted (2/137, 1.5%), or seropositive

Table 1 Patients' baseline characteristics and clinical presentation at acute COVID-19 onset

	Total $n=546$
Gender, *n* (%)	
Female	292 (53.5)
Male	254 (46.5)
Age, median (IQR)	54 (42–64)
BMI, median (IQR)	25.2 (22.7–28.3)
Ethnicity, *n*/*N* (%)	
Native Italian	480/521 (92.1)
European	38/521 (7.3)
Non-European	3/521 (0.6)
Smoking habit, *n*/*N* (%)	
Smoker	78/544 (14.3)
Non-smoker	356/544 (65.4)
Ex-smoker	110/544 (20.2)
Alcohol habit, *n*/*N* (%)	
Non-drinker	269/538 (50)
Drinker	266/538 (49.4)
Abuser	3/538 (0.6)
Occupation, *n*/*N* (%)	
Exposed to public	141/504 (28.0)
Not exposed to public	92/504 (18.2)
HCWs	119/504 (23.6)
Retired	93/504 (18.4)
Other	59/504 (11.7)
Comorbidities, number, *n* (%)	
0	259 (47.4)
1	163 (29.8)
2	69 (12.6)
3	35 (6.4)
≥4	20 (3.7)
Comorbidities, *n*/*N* (%)	
Hypertension	122/534 (22.8)
Obesity	89 (16.3)
Diabetes	31/541 (5.7)
Chronic respiratory disease[†]	20/541 (3.7)
Cardiovascular disease*	7/541 (1.3)
Liver disease	10/541 (1.8)
Psychiatric disorders[‡]	6/541 (1.1)
Immunosuppression	8/539 (1.5)
Under chronic medication, *n*/*N* (%)	260/539 (48.2)
Acute COVID-19 severity[#], *n* (%)	
Asymptomatic	44 (8.1)
Mild	374 (68.5)
Moderate	89 (16.3)
Severe	25 (4.6)
Critical	14 (2.6)
Symptoms at onset, number, *n*/*N* (%)	
0	44/541 (8.1)
1	110/541 (20.3)
2	102/541 (18.8)
3	94/541 (17.4)
4	84/541 (15.5)
≥5	107/541 (19.8)
Management, *n*/*N* (%)	
Outpatients	394/541 (72.8)
Inpatients	
Ward[§]	125/541 (23.1)
ICU	22/541 (4.1)

BMI, body mass index; *HCWs*, healthcare workers; *ICU*, intensive care unit

*Cardiovascular disease: heart failure, ischemic heart disease, tachyarrhythmia, valvular heart disease, venous thromboembolism

†Pulmonary disease: asthma, chronic obstructive *pulmonary disease*

‡*Depression, anxiety*

#Asymptomatic; mild (without pneumonia); moderate (with pneumonia); severe (with severe pneumonia); critical including acute respiratory distress syndrome (ARDS), sepsis and/or septic shock

§Infectious disease, emergency or pneumology department

(2/353, 0.6%) patients ($p=0.085$) but were significantly higher in HCWs than in non-HCWs (6/119, 5.0% versus 0/385, $p<0.001$). Only one patient had a high-titer serological response against SARS-CoV-2 at the time of reinfection (Table 2) (Fig. 2). After reinfection, none of the patients developed an IgM response and only two had a transitory boosted IgG immunization response (Fig. 2). After repeating isolation and tracing of close contacts, we found no transmission to other individuals. The serological evolution after the first and second infections is described in Fig. 2.

Discussion

Our prospective longitudinal study of an unselected population with COVID-19, acquired during the first wave of the pandemic, with different degrees of severity, shows that there was a very low risk of reinfection after a mean follow-up of 10 months and that the primary serological response was not accurately predictive of reinfection.

Ideally, episodes of reinfection are confirmed when the primary and secondary episode are caused by different variants of SARS-CoV-2, using whole genomic sequencing [20]. However, there are considerable logistic challenges and these tests are often unavailable [1–10]. In the absence of such molecular confirmation, other criteria of clinical and epidemiological reinfection have recently been proposed and were met by our patients.

Table 2 Demographic, clinical, and laboratory characteristics of patients with possible SARS-CoV-2 reinfections

	Gender, age, occupation setting	Comorbidities	First positive and first negative NAAT Ct values	First episode Disease severity	IgM/IgG seroconversion after first episode	Serological response at time of reinfection *	Reinfection Positive NAAT No. of days to reinfection Ct values	Reinfection Disease severity
Patient 1 Blue line	F, 33 y HCW in a disability center	No	31/03/2020 08/04/2020 35	Mild (cough, fever, anosmia/ageusia)	Yes	21/09/2020 Seropositive IgG 104 IgM 6	27/11/2020 241 days 32	Mild (fatigue)
Patient 2 Green line	F, 28 y HCW in a nursing home	No	16/04/2020 24/04/2020 34	Mild (fatigue, cough, fever, myalgia)	No	14/07/2020 Seronegative IgG 0 IgM 1	24/11/2020 222 days 21	Mild (fatigue, cough, fever, myalgia)
Patient 3 Yellow line	M, 55y HCW in a nursing home	No	28/03/2020 13/04/2020 NA	Mild (cough, fever)	Yes	20/11/2020 Seropositive IgG 15.9 IgM 1	11/01/2021 289 days 34	Asymptomatic
Patient 4 Red line	F 49 y HCW in a nursing home	No	10/03/2020 08/04/2020 NA	Mild (cough, nausea/vomit fatigue, myalgia, anosmia/ageusia)	Yes	04/01/2021 Seroreverted Seronegative IgG 8.4 IgM 0.9	03/02/2021 323 days 34	Mild (headache)
Patient 5	F 44y HCW in non COVID-19 hospital ward	Migraine	14/04/2020 24/04/2020 36	Mild (nose cold, odynophagia, chest pain)	No	Seronegative IgG 0 IgM 1	15/12/2020 251 days NA	Mild (nose cold, sneezing, odynophagia)
Patient 6	F 45y HCW in a nursing home	No	17/03/2020 07/04/2020 30	Mild (cough, diarrhea, fatigue, myalgia, anosmia/ageusia)	Yes	14/01/2021 Seroreverted Seronegative IgG 6.8 IgM 3.9	21/01/2021 310 days 34	Mild (odynophagia)

Ct, cycle threshold; *F*, Female; *HCW*, healthcare worker; *M*, Male; *NA*, not available; *NAAT*, nucleic acid amplification test

*Measured in kAU/L. according to their most recent (within 2 months) antibody status

The overall incidence rate of COVID-19 reinfections documented to date ranges from 0.15 to 2.2% and has proved to be low compared with the incidence rate among naïve patients. Data are generally collected in specific populations, without a prospective search and with shorter follow-up periods compared to that in our study [1–10, 13, 21]. After a prolonged longitudinal follow-up of 10 months, we found that the overall rate of reinfections was 1.1%. Notably, during this period, rates of infection in the general population of the Friuli Venezia Giulia (FVG) Region were high (prevalence 8%; cases 99,730; deaths 3402) [22].

In our cohort, reinfections have been manifested with low severity and only in patients with a previous history of mild infection, whereas patients with moderate to critical illness did not experience reinfection. These results are in line with available studies suggesting that reinfections are less severe than primary infections and mostly occur in patients with an initial history of asymptomatic or mild COVID-19 [1, 2, 10–12, 23, 24].

The fact that all our reinfections occurred in HCWs is surprising. This could be due either to a higher grade of exposure to COVID-19 cases or to a more rapid access to SARS-CoV-2 molecular PCR testing compared with the general population [1, 2, 6, 12, 18].

In the present study, the median age of reinfected patients was low, and none of the elderly (> 65 years) subjects included was reinfected. These findings add those available in recent studies showing conflicting results on the degree of natural humoral protection against reinfection among older people and found high rates of reinfection in the young population. [8, 10, 25, 26].

It is worth noting that although there is currently no strong evidence that previous SARS-CoV-2 infection reduces the transmission risk [23], we performed tracing of

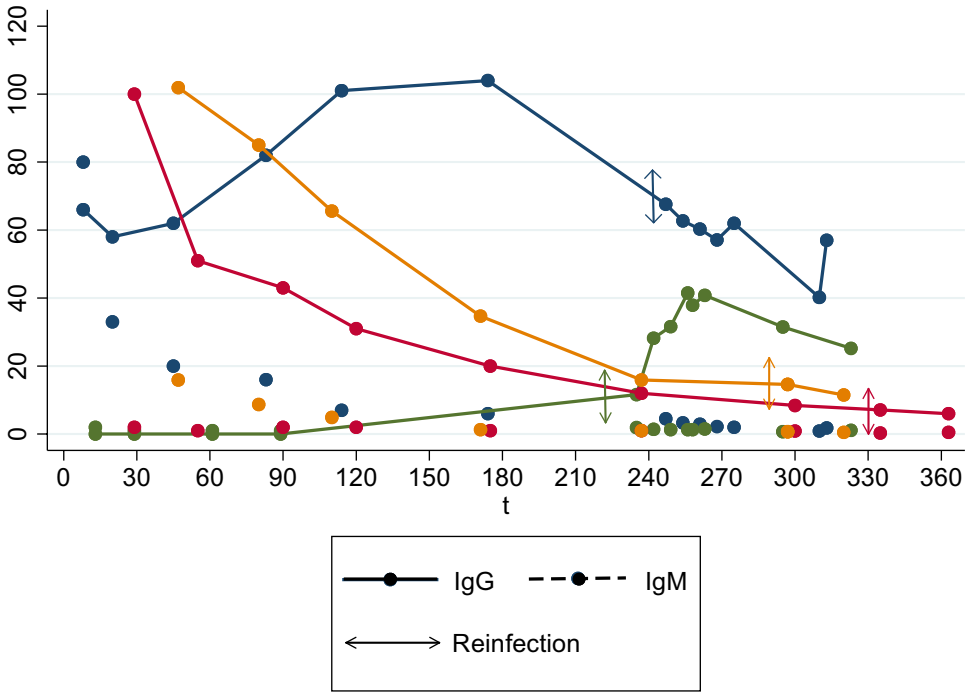

Fig. 2 Humoral IgM and IgG response of reinfected patients. Patient 1—blue line; patient 2—green line; patient 3—yellow line; patient 4—red line

close contacts among our four patients and did not find any secondary cases.

As in previous studies, the serological follow-up of our population showed the development of virus-specific antibodies within 2 months in most patients (90%), followed by a waning of antibody responses in the late convalescent period, with an IgG antibody loss of up to 50% at 10 months [14, 23]. Although there is no conclusive evidence that SARS-CoV-2 antibody responses protect against reinfection, it has been observed that they reduce the risk in the case of breakthrough reinfection [1, 7, 10, 18, 25]. In our study, only one reinfected patient maintained a robust humoral response at the time of reinfection. The role of humoral immune memory derived from primary infection as a surrogate of individual protection against reinfection is still controversial [1–10]. Interestingly, after reinfection, only two patients presented a transitory boosted IgG immunization response, with no associated change in IgM response. This humoral evolution after reinfection may be the result of a cross-protection associated with primary infection, elicitation of T cell–mediated adaptive immunity, and/or immune evasion due to a genetically distinct form of SARS-CoV-2 [14, 24, 27, 28].

This study has several limitations. First, it was performed at a single center where a number of patients refused to participate and were lost at the follow-up. Second, in reinfected patients, viral cultures were not performed and genomic sequencing searching for variants was not possible. Third, our rates of reinfection may be underestimated, since asymptomatic reinfections were not routinely checked. However, our hospital was used as a referral center for COVID-19 where the majority of the COVID-19 cases were confirmed, and none of them was admitted during the study period. Fourth, seropositive patients may have at-risk behavior and be less likely to seek medical evaluation if reinfected, but this was not observed in other studies [7]; furthermore, the high number of HCWs may be associated with a selection bias because HCWs are more likely to self-monitor for any symptoms and to continue to be involved in a research study. Fifth, due to the low number of reinfections, it was not possible to evaluate whether past seroconversion or current antibody persistence determined protection against the risk of reinfection. Lastly, the role of B cell– and T cell–mediated adaptive immunity was not assessed in our study, and, therefore, it was not possible to determine whether protection from reinfection was conferred through the measured antibodies or cellular immunity. However, the complexity of this test prevents routine testing on a large scale.

Conclusions

Most people who have recovered from COVID-19 have a low risk of reinfection; secondary infections may occur mainly in patients with a primary mild COVID-19 infection and with low or absent serological response at the time of reinfection. These findings may suggest that natural humoral protective immunity may be transient and may not confer herd immunity. Given the short supply of vaccine in some countries or settings, public health interventions should be extended as not to prioritize patients with a previous history

of SARS-CoV-2 infection except for those at risk for poor outcomes and/or high-risk settings of exposure. Further large-scale standardized longitudinal studies with a longer follow-up focused both on humoral and on cell-mediated adaptive SARS-CoV-2 immunity are needed to determine the longevity of protection after infection for subsequent episodes in order to understand the evolution of the pandemic and to design a vaccination plan, taking into consideration emerging variants of concern.

Supplementary information

The online version contains the supplementary material.

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Author contribution Maddalena Peghin: conceptualization, methodology, formal analysis, investigation, and writing—review and editing. Emilio Bouza: conceptualization and writing—review; Martina Fabris: laboratory analysis and review; Maria De Martino: conceptualization, methodology, formal analysis, and writing; Alvisa Palese: conceptualization and writing—review and editing; Giulia Bontempo: data collections; Elena Graziano: data collections; Valentina Gerussi: data collections; Valentina Bressan: data collections; Assunta Sartor: laboratory analysis; Miriam Isola: conceptualization, methodology, formal analysis, and writing; Carlo Tascini: methodology, formal analysis, investigation, writing—review and editing; and Francesco Curcio: laboratory analysis and review.

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Data availability Original data will be made available upon request.

Code availability Not applicable.

Declarations

Ethical approval This study was approved by the Ethics Committee of the Friuli Venezia Giulia Region (CORMOR 3–4 protocol; CEUR-2020-OS-219; and CEUR-2020-OS-205). All procedures were carried out in accordance with the ethical standards of the University of Udine and the *ASUFC* and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all subjects before collecting the data and performing serological tests.

Consent to participate Oral and written informed consent was obtained from the participants.

Consent for publication The authors have seen the final version of the manuscript and approved submission for publication.

Conflict of interest MP reports receiving grants and personal fees from Pfizer, MSD, Thermofisher and Dia Sorin outside the submitted work. CT has received grants in the last two years from Correvio, Biotest, Biomerieux, Gilead, Angelini, MSD, Pfizer, Thermofisher, Zambon, Shionogi, Avir Pharma, and Hikma unrelated to the study submitted. The other authors declare no competing interests.

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