Original Article

Antibody response and risk of reinfection over 2 years among the patients with first wave of COVID-19

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A B S T R A C T

Objectives: To describe the dynamics and factors related to natural and hybrid humoral response against the SARS-CoV-2 and risk of reinfection among first-wave patients.
Methods: A prospective longitudinal study with periodic serological follow-up after acute onset of all recovered patients with SARS-CoV-2 infection cared in Udine Hospital (March–May 2020). Nucleocapsid (N) protein and spike receptor-binding domain (S-RBD) antibody tests were used to distinguish natural and vaccine-induced response.
Results: Overall, 153 patients (66 men, mean age 56 years) were followed for a median of 27.3 (interquartile range 26.9–27.8) months. Seroreversion was 98.5% (95% CI: 96.8–99.4) for SARS-CoV-2-N IgM at 1 year and 57.4% (95% CI: 51.5–63.5) for SARS-CoV-2-N IgG at 2 years. Initial serological response (hazard ratio [HR]: 0.99, 95% CI: 0.99–0.99, p 0.002 for IgM and HR: 0.97, 95% CI: 0.97–0.98, p < 0.001 for IgG) and severity of acute infection (HR: 0.62, 95% CI: 0.39–0.96, p 0.033 for IgM and HR: 0.60, 95% CI: 0.37–0.99, p < 0.001 for IgG) were independently associated with persistent SARS-CoV-2-N IgM/IgG response. Older age and smoker status were associated with long-term SARS-CoV-2-N IgM and SARS-CoV-2-N IgG, respectively (HR: 0.75, 95% CI: 0.57–0.98, p 0.038; HR: 1.77, 95% CI: 1.19–2.61, p 0.004 respectively). All patients maintained SARS-CoV-2-S-RBD IgG response at 24-month follow-up. Reinfections occurred in 25 of 153 (16.3%) patients, mostly during the omicron circulation. Reinfection rates did not differ significantly between SARS-CoV-2-N IgG seronegative and seropositive patients (14/89, 15.7% *vs.* 10/62, 16.1%, p 0.947). Unvaccinated patients had higher risk of reinfection (4/7, 57.1% *vs.* vaccinated 21/146, 14.4%, p 0.014).
Discussion: First-wave patients had durable natural humoral immunity in 40% and anti-S-RBD response in 100% up to 2 years after infection. Natural humoral response alone was not protective against reinfections with omicron SARS-CoV-2 variants, whereas vaccination was effective to reduce the risk of a new infection. **Maddalena Peghin, Clin Microbiol Infect 2023;■:1**

Introduction

The evolving pandemic and uptake of the SARS-CoV-2 vaccine require additional understanding of the duration of antibodies acquired through natural infection and the protection conferred by hybrid immunity against reinfection to predict the future COVID-19

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trends and to inform the most appropriate public health policies [1,2].

However, there are significant gaps in the literature on the duration of humoral protection conferred by a prior infection, regarding the effectiveness of both natural and hybrid immunity [3]. Moreover, estimating the degree of this protection in the population may be difficult because of the surge of different variants of concern that can escape pre-existing immunity and variable rates of vaccination [4].

The aim of this study was to describe longitudinally the duration of natural and hybrid antibody response among against SARS-CoV-2 over a 2-year follow-up period after natural infection developed during the first wave in a comprehensive cohort from asymptomatic to severely ill patients and to identify key predictors of duration. We also aimed to examine the protection of natural and hybrid immunity relative to the risk of reinfection.

Methods

Detailed methodology of this prospective cohort study has been described before [5−7] and reported here in accordance to the Strengthening the Reporting of Observational Studies in Epidemiology statement (Table S1) [8].

Summing up, the cohort included adult (≥18 years) ambulatory and hospitalized patients with a diagnosis of COVID-19 attending the Academic Hospital of Udine (Italy) during the first wave (March−May 2020). Patients willing to participate in the study were registered.

Patients were followed with an antibody test against SARS-CoV-2 nucleocapsid (N) IgG protein to follow natural immunity (SARS-CoV-2 IgG and IgM antibodies with iFlash-SARS-CoV-2 test; IgM/IgG thresholds for positivity >10.0 kAU/L). In addition, anti-SARS-CoV-2 spike receptor-binding domain (S-RBD) IgG (performed with Elecsys Roche assay; S-RBD IgG positivity cut-off >0.9 U/mL and maximum value > 2500 U/mL) were performed to follow both natural and vaccine-induced humoral responses, after vaccine introduction. Serological controls were performed every month (±5 days) after symptom onset during the first 4 months, and every other month up to 12 months (±15 days), and every 3 months (±60 days) up to 24 months. S-RBD IgG was introduced from July 2022. The patients' flow diagram is reported in Fig. 1.

Data were collected in a database at the study enrolment and during the follow-up, including vaccination status (date, number and type of vaccine). In addition, reinfections were recorded. Reinfection was defined as a positive SARS-CoV-2 molecular or antigenic test more than 3 months after the onset of the primary infection, independently of presence of symptoms compatible with COVID-19. Viral sequencing for SARS-CoV-2 variants was not usually performed. On the basis of Italian and Friuli Venezia Giulia region sequencing data, the most common variants during the study period were the Alfa, Beta, Delta and from November 2021 Omicron variant was predominant [9].

Patients were categorized as vaccinated/hybrid immunity if they had received the vaccine at least 2 weeks before the reinfection (national vaccination campaign started on 27 December 2020). Patients who received ≥3 shots of SARS-CoV-2 vaccination were considered as fully vaccinated. In Tables S2−S5, the clinical and microbiological definitions, as well as the vaccination status evaluations are detailed.

The 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). Informed consent was obtained from all subjects before the data collection.

Statistical analysis

Patient demographic and clinical characteristics were presented with absolute values and percentages for categorical variables and means or medians (standard deviation 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 χ^2 test or Fisher's exact test, whereas quantitative variables were compared using the t test or Mann-Whitney U test, as appropriate. On 496 patients, univariable and multivariable Cox regression were performed to estimate the association between the antibody persistence of SARS-CoV-2- N IgG (and IgM) and clinical/demographic variables. The outcome was defined as the loss of SARS-CoV-2- N IgG/IgM
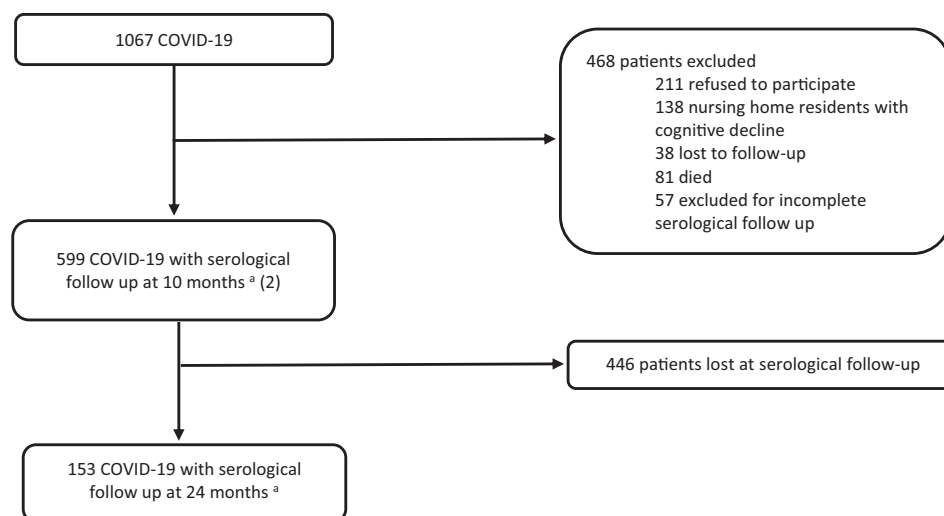

Fig. 1. Flow diagram of in- and out-patients with COVID-19 included in the serological follow-up up to 24 mo after acute infection. [a]Nucleocapsid protein IgM and IgG antibodies (iFlash®) concentrations were measured at the serological follow-up visits each month (±15 d) after symptom onset during the first 4 mo, and every 2 mo up to 12 mo (±15 d) and every 3 mo up to 24 mo (1−2−3−4−6−8−10−12−15−18−21−24 mo after the disease onset). Anti-SARS-CoV-2 spike receptor-binding domain (S-RBD) IgG (performed with Elecsys Roche assay) were performed to follow both natural and vaccine-induced humoral response, after vaccine introduction. In Italy, the SARS-CoV-2 vaccination campaign started on 27 December 2020. S-RBD IgG was introduced from July 2022. Abbreviations: COVID-19, Coronavirus Disease 2019.

Table 1
Serological evolution according to clinical presentation (symptomatic/asymptomatic status) at acute COVID-19 onset

	Total $N = 153$	Symptomatic $N = 143$		Asymptomatic $N = 10$	p
		Moderate, critical and severe[a] $N = 39$	Mild[a] $N = 104$		
IgM seroconversion[b,c] n/N (%)	69 (45.1)	30 (76.9)	38 (36.5)	1 (10)	0.023
IgG seroconversion[b,c] n/N (%)	139 (90.8)	39 (100)	94 (90.4)	6 (60)	0.007
IgM max[b,c] Median (IQR)	8.5 (2−28.5)	31 (12−101)	6.5 (2-20)	1 (0−2)	<0.001
IgG max[b,c] Median (IQR)	82 (57.4−101)	97 (74−115)	75.5 (54−97)	22 (9−36)	<0.001
Persistence of IgG[b], d Median (IQR)	397 (260−915)	637 (366−912)	378.5 (251.5−920)	107 (0−907)	0.082
Persistence of IgM[b], d Median (IQR)	0 (0−92)	77 (0−118)	0 (0−81.5)	0 (0−0)	0.024

Data are n (%), n/N (%), median (IQR).
IQR, interquartile range.
　[a] Disease Severity Scale.
　[b] SARS-CoV-2- N IgM and IgG, measured in kAU/L.
　[c] Within 2 mo from symptoms onset.

antibodies; hence, a hazard ratio <1 indicated factors favouring a higher persistence of the duration of SARS-CoV-2- N IgG/IgM antibodies. Time to SARS-CoV-2- N IgG/IgM antibodies loss was calculated according to Kaplan−Meier method, and log-rank tests were used to compare between groups. The clinical variables considered were the severity of acute COVID-19 (16), the presence of symptoms, the number of acute symptoms of COVID-19, the ICU admission, the days of viral shedding and the maximum SARS-CoV-2- N IgG and IgM values within 2 months. The demographic variables were gender, age, body max index, comorbidities, smoking and alcohol habits, job status and chronic medication. The multivariable analyses included all variables significant at p < 0.05 in the univariable analysis, taking into account potential collinearities. Patients who had a SARS-CoV-2 reinfection diagnosis were followed in a separate cohort. Statistical analyses were performed using STATA 17.0.

Results

Evolution of SARS-CoV-2 antibodies over time

Overall, 1067 patients with COVID-19 were diagnosed at our hospital during the first wave (Fig. 1). The details of this prospective cohort at acute onset have been provided previously [5−7] (Table S6). Overall, almost 3300 blood samples were collected and tested for antibodies against SARS-CoV-2. A total of 153 patients completed the serological follow-up and were tested at a median 27.3 (IQR 26.9−27.8) months (Table S7). A complete description of the natural serological evolution of the first 10 months is presented in our previous work [5]. As previously described, the seroconversion rates for SARS-CoV-2-N IgM within 2 months were 32% and for SARS-CoV-2-N IgG was 90%. Almost all patients developed SARS-CoV-2-N IgM seroreversion at 1 year.

Table 2
Median IgM and IgG titers according to symptomatic and asymptomatic status at acute COVID-19 onset

	Number of observations	Symptomatic		Asymptomatic	p value[a]
		Moderate, critical and severe	Mild		
IgG[b]					
Month from onset					
1	338	74.5 (61−103)	70 (52−95)	14.5 (0−74)	<0.001
2	488	80 (63−95)	66 (34−85)	16 (2−65)	<0.001
3	465	83 (69−93)	57 (22−84)	10.5 (2−47)	<0.001
4	422	78 (58−95)	46 (18−79.2)	8.5 (3−29.8)	<0.001
6	365	61 (35−86)	26 (12−57)	13.5 (4.5−37)	<0.001
8	333	42 (21−67)	17.5 (7−40.3)	8.7 (3−11)	<0.001
10	288	32.9 (19.3−54.4)	14.8 (6.5−33.7)	7.2 (3.1−17.6)	<0.001
12	321	27.5 (17.8−47.4)	12.1 (5.1−27.4)	4.9 (0.6−23.8)	0.005
15	112	23.1 (9.8−31.7)	9.2 (3.6)	4.9 (0.2−6.4)	0.011
24	153	11.2 (6.1−26.3)	7.7 (2.7−42.4)	3.2 (1.1−29.5)	0.198
IgM[b]					
Month from onset					
1	338	37.9 (11−97)	3.5 (1−12)	1 (1−3)	<0.001
2	487	18 (4−43.1)	3 (1−11)	1 (0.8−3)	<0.001
3	464	12 (5−22)	3 (1−8)	1 (0.4−2)	<0.001
4	419	6 (2−12)	2 (1−5)	1 (0−1)	<0.001
6	365	3.6 (1−6.5)	1 (1−3)	1 (0.6−2)	<0.001
8	334	2 (1−4)	1 (1−2.2)	1 (0.4−1.5)	0.028
10	288	1 (0.5−2.2)	0.7 (0.4−1.4)	0.5 (0.3−1.6)	0.371
12	321	0.8 (0.5−1.5)	0.6 (0.4−1.2)	0.7 (0.3−1.1)	0.217
15	112	0.7 (0.3−1.2)	0.6 (0.4−1.1)	0.6 (0.3−0.8)	0.421
24	153	0.4 (0.2−1.8)	0.5 (0.3−1.2)	0.2 (0.2−1.9)	0.239

Data are median (IQR).
IQR, interquartile range.
　[a] Bonferroni correction was applied.
　[b] SARS-CoV-2- N IgM and IgG, measured in kAU/L.

Estimated rate of seropersistence of SARS-CoV-2-N IgG was 42.6% (95% CI: 36.5−48.5) at 2 years of follow-up after COVID-19. Estimated rates of antibody loss were 79.3% (95% CI: 61.3−92.7) for asymptomatic patients, 61.8% (95% CI: 54.8−68.9) for mild patients but only 40.3% (95% CI: 28.8−54.4) for moderate-to-severe COVID-19. The frequency and timing of SARS-CoV-2-N IgM/IgG seroreversion, robustness of SARS-CoV-2-N IgM/IgG max and median SARS-CoV-2-N IgM/IgG titers from symptom onset to follow-up, classified according to different grades of COVID-19 severity at acute onset, were significantly different and are presented in Tables 1 and 2 and Figs. 2 and 3. All patients showed SARS-CoV-2-S-RBD IgG seropersistence at 2 years of follow-up and most of them (152/153, 99.3%) with maximum serological response (S-RBD IgG >2500 U/mL).

Factors associated with persistence of natural humoral response after acute infection

Risk factors associated with the duration of SARS-CoV-2-N 2 IgM/IgG serological response at univariate analysis are listed in Tables 3 and 4. In the multivariable Cox regression analysis higher robustness of the initial SARS-CoV-2-N IgM max titer (maximum titer within 2 months after acute onset), older age and severity of acute COVID-19 were all independent predictors of long-term immunity for SARS-CoV-2-N IgM. The higher robustness of the initial SARS-CoV-2-N IgM/IgG max titer, severity of acute COVID-19 and non-smoker status were also independently associated with IgG response longevity.

Protection of natural and hybrid immunity relative to the risk of reinfection

During the follow-up period, reinfection occurred in 25 of 153 (16.3%) patients, at a median of 22.3 (IQR 21.8−24.2) months after the first acute COVID-19. All but one reinfection occurred during the omicron circulation period. Cases of reinfection were all mild or asymptomatic. The median age was 56 years (IQR 47−59) and 31.8% (7/22) were health care workers. Approximately 40% (10/25) patients had at least one comorbidity.
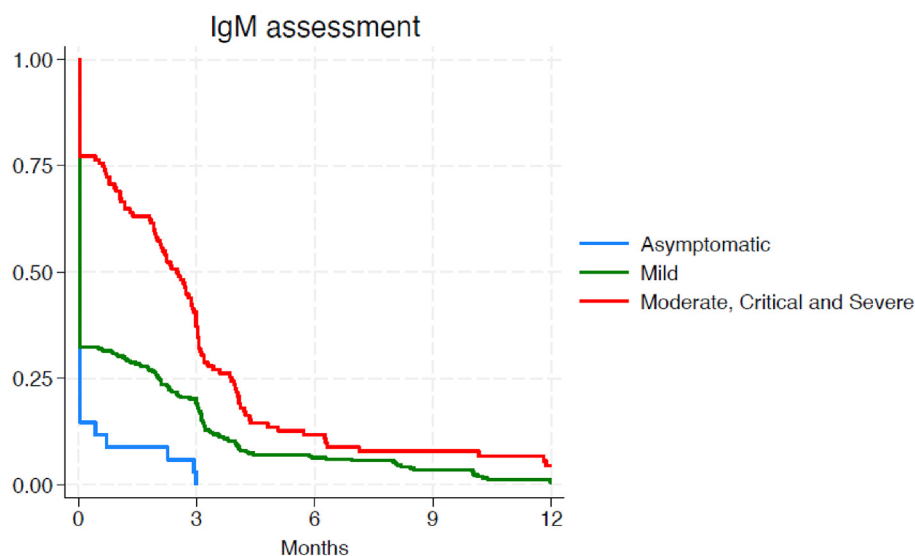
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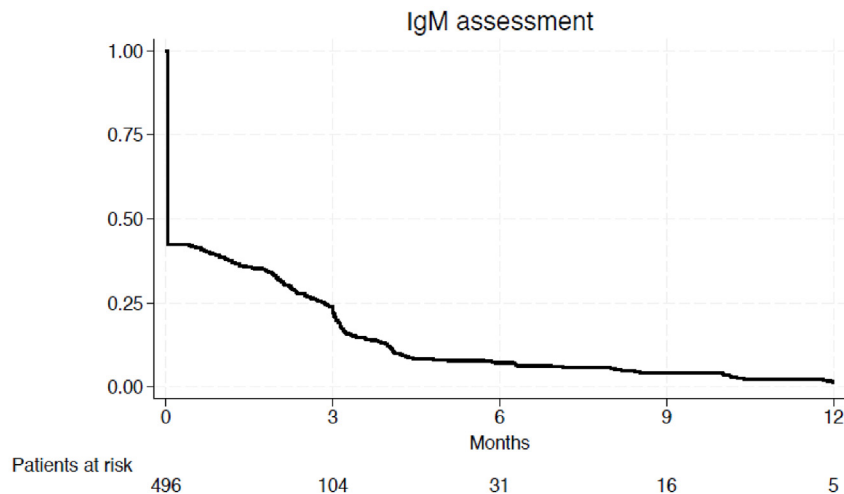
B

Fig. 2. Longitudinal assessment of anti-SARS-CoV-2 IgM in patients who recovered from COVID-19 overall (A) and according to the grade of severity of acute disease (B).

Fig. 3. Longitudinal assessment of anti-SARS-CoV-2 IgG in patients who recovered from COVID-19 overall (A) and according to the grade of severity of acute disease (B).
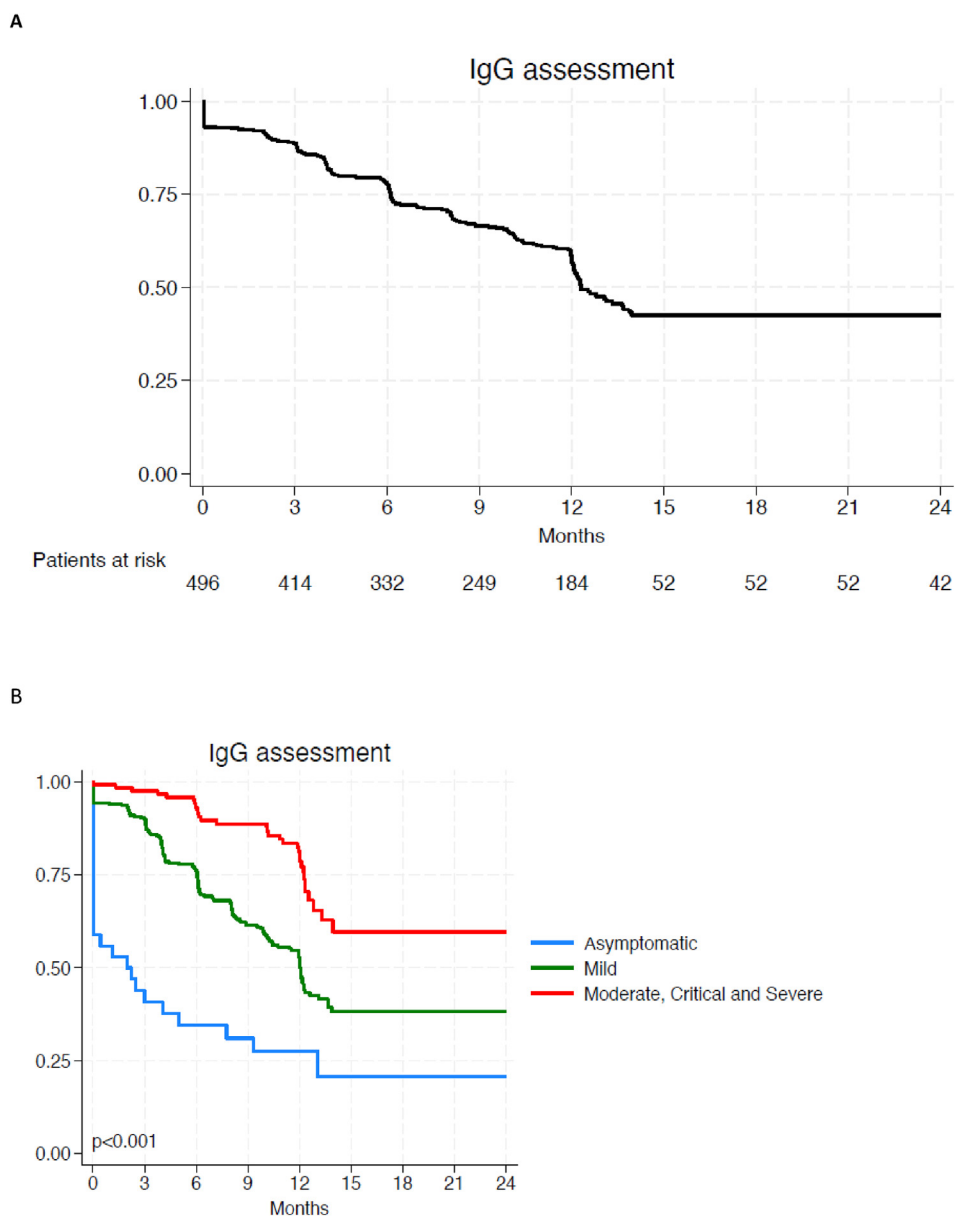
Median SARS-CoV-2-N IgG values before reinfection were not significantly different in reinfected patients compared with non-reinfected patients (7.8 [IQR 3.7–22.1] vs. 7.4 [IQR 2.7–23.8], p 0.861). Reinfection rates did not differ significantly in SARS-CoV-2-N IgG seronegative and/or seroreverted patients compared with SARS-CoV-2-N IgG seropositive patients before reinfection (14/89, 15.7% vs. 10/62, 16.1%, p 0.947). Significant difference in natural humoral SARS-CoV-2-N IgG response at 24 months emerged in reinfected patients compared with non-reinfected patients (median 60 [IQR 5.7–80.9] vs. 7.3 [IQR 2.8–22.3], p < 0.001) (Fig. 4).

Most patients (146/153, 95.4%) had received at least one vaccine dose at 2 years follow-up and 64.7% (99/153) were fully vaccinated. Unvaccinated patients had higher risk of reinfection with respect to vaccinated patients (4/7, 57.1% vs. 21/146, 14.4%, p 0.014). Risk of reinfection was significantly reduced in relation with number of SARS-CoV-2 vaccination shots, being 57.1% (4/7) for unvaccinated patients, 25% (6/24) for partially vaccinated patients (1–2 shots) and 13.1% (13/99) for fully vaccinated patients (p 0.012). All patients showed SARS-CoV-2- S-RBD -IgG seropositivity at time of reinfection.

Discussion

In this prospective longitudinal study on a complete spectrum of unselected patients who recovered from SARS-CoV-2 infection after the first wave, we evaluated the kinetics and durability of SARS-CoV-2 antibodies over a period of 24 months, providing important insights into post-infection natural and hybrid immunity. We found that (a) people who recovered from COVID-19 original strain had durable anti-natural humoral immunity of SARS-CoV-2 in 42% of cases and that all patients maintained SARS-CoV-2- S-RBD -IgG response up to 2 years after the infections with the ancestral virus; (b) the duration of the natural antibody response against SARS-CoV-2 is diverse and varies broadly between individuals; (c) the natural humoral response after the first wave was not protective for omicron reinfection, but omicron reinfection worked as a natural

Table 3
Univariable and multivariable Cox regression for risk factors associated with persistence of SARS-CoV-2- N IgM antibody

	Univariable analysis			Multivariable analysis		
	HR	95% CI	p value	HR	95% CI	p value
Gender	1.02	0.85–1.22	0.800			
Age						
18–40	1			1		
40–60	0.64	0.50–0.83	0.001	0.75	0.57–0.98	0.038
>60	0.52	0.41–0.69	<0.001	0.63	0.46–0.86	0.004
BMI	1.00	0.98–1.02	0.862			
Smoking habit						
Non-smoker	1					
Smoker	1.22	0.93–1.60	0.149			
Ex-smoker	0.80	0.63–1.01	0.066			
Alcohol habit						
Nondrinker	1					
Drinker	1.05	0.87–1.26	0.610			
Number of comorbidities	0.91	0.84–0.99	0.030	1.00	0.89–1.12	0.960
Under chronic medication	0.81	0.67–0.97	0.022	0.99	0.76–1.28	0.933
Severity of acute COVID-19						
Asymptomatic	1			1		
Mild	0.67	0.47–0.96	0.031	0.74	0.51–1.09	0.125
Moderate, critical and severe	0.43	0.29–0.65	<0.001	0.62	0.39–0.96	0.033
Symptomatic	0.61	0.42–0.86	0.006			
Number of symptoms at onset	0.95	0.91–0.99	0.048			
Management						
Out-patients	1					
Ward	0.61	0.49–0.76	<0.001			
ICU	0.66	0.42–1.01	0.058			
Viral shedding, d	0.99	0.98–1.00	0.205			
IgM max within 2 mo[a]	0.99	0.99–0.99	<0.001	0.99	0.99–0.99	0.002
IgG max within 2 mo[a]	0.99	0.99–0.99	0.001	1.00	0.9–1.00	0.109

BMI, body mass index; HCW, health care worker; HR, hazard ratio; ICU, intensive care unit.
  [a] Measured in kAU/L.

immune memory booster; and (d) vaccination was increasingly effective to reduce the risk of a new SARS-CoV-2 acquisition in relation with the unvaccinated or fully vaccinated status.

Global SARS-CoV-2 seroprevalence has been observed to have substantial variation in the proportion of immunity induced by infection or vaccination in different settings worldwide [10]. Recent studies suggest that individuals may have durable and detectable SARS-CoV-2- N IgG levels up to 18 months after natural infection, with inconstant results depending on the selected study population, type of serological tests, the study design and the phase of disease [5,11–14]. The strengths of our study lies in the prospective follow-up of humoral immunity after the first wave, the wide spectrum of the diseases at the onset (including asymptomatic patients, which is an unexplored subgroup in most studies) and the length of the follow-up of 2 years, significantly longer compared with literature in this line [5,11,12]. Detection of IgM has been usually used as a diagnostic test for recognizing active viral infections, but their relevance in later stages has not been clearly defined. As expected in our cohort, seroreversion for IgM was earlier than for IgG and was observed in almost all patients at 1-year follow-up. In keeping with previous literature, we found that anti-N antibodies tend to disappear more quickly than anti-S-RBD antibodies both for vaccinated patients and unvaccinated patients [13]. Indeed, we observed that COVID-19 survivors maintained SARS-CoV-2- N IgG antibodies in around 42% of cases and SARS-CoV-2- S-RBD -IgG hybrid and natural humoral response in all cases up to 2 years after the first wave. These results have important implications on epidemiological models and public health decisions as the estimate of the seroprevalence of SARS-CoV-2 is based on different serological diagnostic tests [13].

The duration of the antibody response against SARS-CoV-2 differs broadly across individuals. In keeping with previous literature, the maximum peak of antibody titers at baseline was a significant factor associated with an increased maintenance of detectable antibody levels over time both for SARS-CoV-2- N IgM and for IgG [5]. Our study confirms that the intensity of the initial antibody response allows the estimation of long-term antibody duration up to 24 months after acute COVID-19. Disease severity at acute onset has been found to be associated with the duration of immune response [11,15]. Ageing was associated with long-term immunity for SARS-CoV-2- N IgM, because immunosenescence contributes to the development of a chronic state of inflammation that leads to an increased humoral response after acute COVID-19, but poorer clinical outcomes and reduced response to vaccination [16]. Active smoking negatively impacts humoral response to COVID-19 vaccines [17] but positively on SARS-CoV-2- N IgG natural humoral response, although the pathophysiologic mechanisms for these associations have not been completely understood [11].

The overall incidence rate of COVID-19 reinfections documented to date ranges from 2.7% and 20.6% [18] and increased risk of SARS-CoV-2 has been associated with reinfection related to omicron variant, compared with previous variants, but with variable protection from severe disease [1,19,20]. In our study after a prolonged longitudinal follow-up of 2 years, we found that the overall rate of reinfections was 16.5%, all reinfections were mild or asymptomatic and mostly (96%) occurred during the omicron circulation.

The role of natural humoral immune response derived from primary infection as a surrogate of protection against reinfection is still debated. We found that SARS-CoV-2- N IgG natural antibody responses were not protective against reinfection, highlighting the importance of immune evasion as a selective pressure driving the emergence of new sub-variants and new clinical pictures [1,18–20]. Interestingly, after reinfection, patients presented a boosted IgG immunization response that persisted at 2 years of follow-up, probably because of the result of a booster effect and cross-protection existing between primary infection and omicron

Table 4
Univariable and multivariable Cox regression for risk factors associated with persistence of SARS-CoV-2-N IgG antibody

	Univariable analysis			Multivariable analysis		
	HR	95% CI	p value	HR	95% CI	p value
Gender	1.21	0.92–1.58	0.169			
Age						
18–40	1			1		
40–60	0.64	0.45–0.90	0.010	1.36	0.95–2.00	0.117
>60	0.46	0.32–0.66	<0.001	0.99	0.65–1.52	0.973
BMI	0.98	0.95–1.01	0.266			
Smoking habit						
Non-smoker	1			1		
Smoker	2.36	1.65–3.36	<0.001	1.77	1.19–2.61	0.004
Ex-smoker	0.97	0.69–1.40	0.881	1.01	0.69–1.47	0.951
Alcohol habit						
Nondrinker	1					
Drinker	1.12	0.86–1.47	0.404			
Number of comorbidities	0.92	0.81–1.04	0.181			
Under chronic medication	0.76	0.58–1.00	0.054			
Severity of acute COVID-19						
Asymptomatic	1			1		
Mild	0.38	0.25–0.58	<0.001	0.60	0.37–0.99	0.045
Moderate, critical and severe	0.15	0.09–0.26	<0.001	0.48	0.29–1.06	0.073
Symptomatic	0.31	0.20–0.47	<0.001			
Number of symptoms at onset	0.85	0.79–0.91	<0.001			
Management						
Out-patients	1					
Ward	0.39	0.27–0.56	<0.001			
ICU	0.37	0.16–0.84	0.0018			
Viral shedding, d	0.98	0.96–0.99	0.016	1.00	0.98–1.02	0.862
IgG max within 2 mo[a]	0.97	0.97–0.98	<0.001	0.97	0.97–0.98	<0.001
IgM max within 2 mo[a]	0.98	0.98–0.99	<0.001	0.99	0.98–0.99	0.002

BMI, body mass index; HCW, health care worker; HR, hazard ratio; ICU, intensive care unit.
[a] Measured in kAU/L.
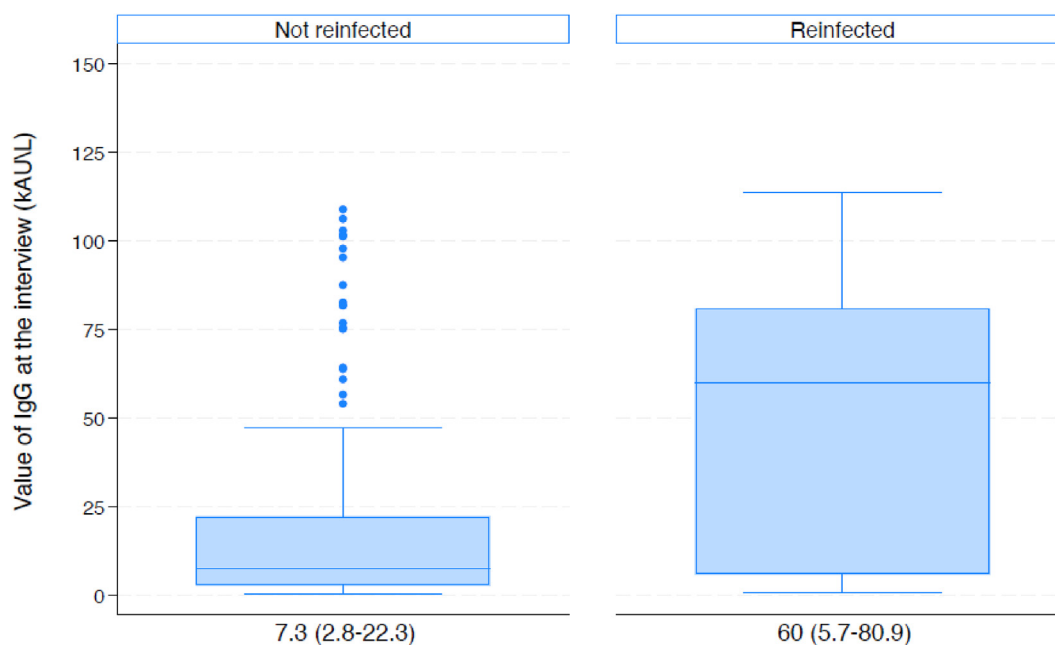
Fig. 4. Serological evolution against SARS-CoV-2 measured with SARS-CoV-2-N IgG in patients with or without reinfection at 2-y follow-up.

reinfection [20,21]. In contrast, we found that vaccination was increasingly effective to reduce the risk of SARS-CoV-2 reinfection in relation with the unvaccinated or fully vaccinated status of previously infected patients. Therefore, we believe that as SARS-CoV-2 epidemiology shifts to endemicity in the context of high levels of immunity, the immune response conferred by past infection should be balanced together with protection from vaccination to provide rational and nuanced vaccination policies [1,20].

This study has several limitations. First, it is a monocentric study performed in a high-income country and a high number of patients

were lost at the follow-up, introducing a selection bias. However, the Kaplan—Meier approach has allowed us to consider in the analysis also the patients lost at the follow-up. Second, humoral response results are assay dependent and our S-RBD IgG positivity cut-off was not quantitative above the maximum threshold (>2500 U/mL). Third, SARS-CoV-2 variants of concern condition different serological response and risk of reinfection but genomic sequencing in reinfected patients was not performed because of logistic challenges and test unviability. Fourth, rates of reinfection may be underestimated, because reinfections were not routinely checked. Lastly, further immunological tests to assess cell-mediated adaptive immunity and neutralizing antibody were not accomplished.

In conclusion, in our comprehensive longitudinal study on natural immune response of an unselected population after the first wave, we found that receptor-binding domain antibodies are longer lasting compared with anti-N antibodies, independently of vaccination status. The duration of natural serological response against SARS-CoV-2 is depended on burden of disease at acute onset, and intensity of the initial antibody response, age and smoker status. Reinfections may occur independently of natural serological response at time of reinfection but may work as a booster of humoral immune memory. Vaccination shots in previously infected patients were increasingly effective to reduce the risk of a SARS-CoV-2 reinfection. Further long-term standardized prospective studies are needed to determine the role and longevity of natural and hybrid humoral response during the evolving pandemic and to understand the best pathways for public health policies to design future vaccination plans.

Author contributions

Conceptualization: MP, AP, MI, CT. Methodology: MP, AP, MI, CT. Software: MDM, MI. Validation: MDM, MI. Formal analysis: MDM MI. Investigation: MP, AP, MI, MDM, CT. Resources: AS, FC. Data curation: FF, SC, VG. Writing—original draft: MP. Writing—review and editing: MP, AP, MI, PAG, CT. Visualization: MP, MI, MDM. Supervision: AP, MI, PAG, CT. Project administration: MP, AP, MI, FC, CT. Funding acquisition: MI.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.12.017.

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