

A cross-sectional study of SARS-CoV-2 seropositivity among healthcare workers and residents of long-term facilities in Italy, January 2021

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Abstract

Long-term care facilities (LTCFs) are high-risk settings for SARS-CoV-2 infection. This study aimed to describe SARS-CoV-2 seropositivity among residents of LTCFs and health-care workers (HCWs). Subjects were recruited in January 2021 among unvaccinated HCWs of LTCFs and hospitals and residents of LTCFs in Northern Italy. Information concerning previous SARS-CoV-2 infections and a sample of peripheral blood were collected. Anti-S SARS-CoV-2 IgG antibodies were measured using the EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG). For subjects with previous COVID-19 infection, gender, age, type of subject (HCW or resident), and time between last positive swab and blood draw were considered as possible determinants of two outcomes: the probability to obtain a positive serological result and antibody titer. Six hundred and fifty-eight subjects were enrolled. 56.1% of all subjects and 65% of residents presented positive results (overall median antibody titer: 31.0 RU/ml). Multivariable models identified a statistically significant 4% decrease in the estimated antibody level for each 30-day increase from the last positive swab. HCWs were associated with significant odds for seroreversion over time (OR: 0.926 for every 30 days, 95% CI: 0.860–0.998), contrary to residents (OR: 1.059, 95% CI: 0.919–1.22). Age and gender were not factors predicting seropositivity over time. Residents could have a higher probability of maintaining a seropositive status over time compared to HCWs.

KEYWORDS

antibodies, COVID-19, enzyme-linked immunosorbent assay, Italy, nursing homes, serology

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1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) was declared a Public Health Emergency of International Concern (PHEIC) on January 30, 2020.¹ As of June 18, 2021, there have been 177 million confirmed cases worldwide, including 3.8 million deaths.^{2,3} Italy was one of the first EU countries hit by the pandemic. The Italian Council of Ministers declared a state of emergency throughout the country on January 31, 2020.⁴ Since then, Italy has faced three epidemic waves and in response, the government has implemented increasingly strict containment measures.^{5–8} Currently, Italy has reported 7 611 614 cases of COVID-19 and 138 651 deaths.⁹

The gold standard for COVID-19 diagnosis is the detection of SARS-CoV-2 by real-time reverse-transcription polymerase chain reaction (real time-RT-PCR).^{10,11} However, it is possible that many asymptomatic or mildly symptomatic patients remain undetected, contributing to the spread of the virus.¹² In this context, serological investigations can be used to evaluate previous exposure to the virus, as well as the presence of an immune response.¹¹ Seroprevalence studies could represent a fundamental tool to generate a more realistic estimate of the cumulative incidence of disease,¹³ especially in countries where PCR testing was insufficient in the initial stages of the epidemic due to a contingent allocation of resources.¹⁴

It is not yet clear whether the antibody titer is a marker of protective immunity, nor whether there is a protective immunity threshold against the virus.¹⁵ Further, the duration of adaptive immunity to SARS-CoV-2 after natural infection remains to be determined.^{16–18}

Long-term care facilities (LTCFs) are high-risk settings for SARS-CoV-2 infection, both for residents and personnel. The purpose of this multicentric study was to describe the antibody response to SARS-CoV-2 among individuals at high risk of exposure due to the environment in which they live or work: residents of LTCFs and health-care workers (HCWs) of acute-care hospitals and LTCFs, following the first two pandemic waves in Italy (January 2021). It is important to state that the vaccination campaign against SARS-CoV-2 in Italy began on December 27, 2020. All subjects analyzed in this study were therefore unvaccinated at the time of blood sample collection.

2 | MATERIALS AND METHODS

2.1 | Enrollment of subjects

Subjects were recruited on a voluntary basis in January 2021, among a convenience sample of HCWs (medical doctors, nurses, and ancillary staff) of LTCFs and one hospital (total $n = 495$; LTCFs = 372; hospital = 123) and residents of LTCFs ($n = 163$) in the region of Piedmont, in Northern Italy. Subjects were enrolled at six LTCFs and the main hospital of the city of Alessandria, two LTCFs of Cuneo, and five LTCFs of Turin. All the subjects were unvaccinated, as the samples were collected the day before vaccination was scheduled.

2.2 | Data collection

Demographic characteristics of enrolled subjects, as well as information concerning previous SARS-CoV-2 infections confirmed by RT-PCR testing, were collected from the Health Directorates of the involved facilities and checked on the regional database in which all official swabs are registered. Further, participants were asked whether they had previously been infected by SARS-CoV-2 and if so, when. After acquiring the written consent from all subjects, a sample of peripheral blood was collected.

2.3 | Laboratory analysis

The analysis was carried out at the Laboratory of Serology and Microbiology applied to Hygiene of the Department of Public Health and Paediatrics of the University of Turin. Blood samples were delivered to the laboratory and, after centrifugation, sera were extracted and stored at -20°C until analysis. SARS-CoV-2 IgG antibodies were measured using the EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG). The kit allows the specific detection of IgG antibodies using the S1 domain of the spike protein including the immunologically relevant receptor-binding domain (RBD). Sera were analyzed in a 100-fold and 1000-fold dilution, and IgG results were expressed in relative units per milliliter (RU/ml) using a 6 point calibration curve. A peroxidase-based revelation system was used, and, after color development, optical density at 450 nm was determined. The IgG antibodies titers were determined using the calibration curve obtained from standards.

The result should be interpreted as negative if lower than 8 RU/ml, borderline if between 8 and 11 RU/ml, and positive if ≥ 11 RU/ml, according to the instructions of the kit manufacturer. A conversion factor of 3.2 has been identified by the manufacturer to convert relative units to binding antibody units/ml (BAU/ml); this measurement unit has been indicated by the WHO as a standard unit and conversion factors have been identified by the manufacturers.¹⁹

2.4 | Statistical analysis

Descriptive characteristics were presented as medians and interquartile ranges or means and standard deviations following the results of corresponding statistics yielded by the Shapiro-Wilk normality test.

For subjects with a previous positive swab confirming COVID-19 infection, gender, age, type of subject (HCW or resident), and time between last positive swab and blood draw were considered as possible determinants of two outcomes: the probability to obtain a positive serological result and, for subjects who showed detectable antibodies, the actual antibody titer. Consequently, multivariable regression models (logistic and log-linear, respectively) were built to evaluate the impact of explanatory variables on each outcome, by

TABLE 1 Descriptive characteristics of participants

(A) According to subject types: Health-care workers (HCWs) versus residents of long-term care facilities (LTCFs)				
	All participants (n = 658)	HCWs (n = 495)	LTCFs residents (n = 163)	p-value
Gender, female	531 (80.7%)	416 (84.0%)	115 (70.6%)	0.0003
Age (years)	-	-	-	< 0.0001
Median (Q1–Q3)	51 (43–65)	47 (38–54)	86 (80–90)	-
Range	19–106	19–76	51–106	-
≥1 previous positive swab	402 (61.1%)	298 (60.2%)	104 (63.8%)	0.4589
Days between last positive swab and blood test ^a	-	-	-	< 0.0001
Median (Q1–Q3)	72 (45–262)	83 (57–267)	61 (30–262)	-
Range	13–327	13–327	22–289	-
Serological test result	-	-	-	0.0028
Negative (<8 RU/ml)	261 (39.6%)	214 (43.2%)	47 (28.9%)	-
Borderline (8–11 RU/ml)	28 (4.3%)	18 (3.7%)	10 (6.1%)	-
Positive (>11 RU/ml)	369 (56.1%)	263 (53.1%)	106 (65.0%)	-
IgG titer ^b (RU/ml)				0.0078
Median (Q1–Q3)	31.0 (10.7–77.3)	27.7 (10.3– 65.1)	42.6 (13.6–114.3)	-
(B) According to presence/absence of previously diagnosed SARS-CoV-2 infection				
	≥1 previous positive swab (n = 402)	No previous positive swabs (n = 256)		p-value ^c
Gender, female	326 (81.1%)	205 (80.0%)		0.7618
Age (years)	-	-		0.4863
Median (Q1–Q3)	51 (43–67)	51 (42–65)		-
Range	19–106	22–95		-
Subject type	-	-		0.4589
HCWs	298 (74.1%)	197 (77.0%)		-
LTCFs residents	104 (25.9%)	59 (23.0%)		-
Serological test result	-	-		< 0.0001
Negative (<8 RU/ml)	73 (18.2%)	188 (73.4%)		-
Borderline (8–11 RU/ml)	16 (4.0%)	12 (4.7%)		-
Positive (>11 RU/ml)	313 (77.8%)	56 (21.9%)		-
IgG titer ^b (RU/ml)	-	-		< 0.0001
Median (Q1–Q3)	35.6 (14.0–82.5)	11.6 (3.3–46.9)		-

^aConsidering only subjects with at least one positive swab.

^bConsidering only subjects with detectable antibodies.

^cFisher's exact test and Mann–Whitney–Wilcoxon *U* test were used for categorical and quantitative variables respectively.

allowing also for the presence of possible interactions between determinants. Nonlinear effects of continuous variables (age and time between swab and blood draw) were also investigated through restricted cubic splines regression.

The significance of interactions in the model was evaluated through likelihood ratio tests, to keep the simplest model with explanatory power.²⁰ Relevant diagnostics for final models were conducted, including the analysis of variance inflation factors (VIFs) to check for multicollinearity and the verification of residual normality assumption. Optimism due to overfitting was quantified through validation via bootstrap by resampling 1000 times.²¹

2.5 | Statistical parameters and computing software

The significance level was set at $\alpha = 0.05$ for all analyses, except for likelihood ratio tests, where a level of 0.1 was chosen for a more conservative approach towards interactions

The statistical software R (version 4.0.5)²² was used for all computation and plotting: models and diagnostics were performed using the “rms”²³ and “lmtest” packages.²⁴

3 | RESULTS

3.1 | Descriptive statistics

A total of 658 subjects were enrolled in the study, including 495 (75.2%) HCWs (LTCFs: 372; hospital: 123) and 163 (24.8%) residents. The vast majority of participants were female (80.6%). Age distribution was bimodal (median 51 years), consistently with the two categories of participants. Among all enrolled subjects, 402 (61.1%) had previously been diagnosed with COVID-19, while 256 (38.9%) had no previous positive RT-PCR results.

For the 402 previously positive participants, owing to the dynamics of the pandemic waves (with a limited number of cases during the warm season) and to the study design (all serum samples acquired approximately at the same time), the lag between swab date and blood draw varied among subjects, with 239 samples (59.5%) drawn 55 ± 23 days after the last positive swab (range: 13–139) and 162 (40.3%) collected 276 ± 24 days after the last positive swab (range: 216–327). For one participant, this information was not available.

Serological tests proved negative for 261 subjects (39.7%): 162 (24.6%) were completely negative (no detectable circulating antibodies) and 99 (15.1%) had antibody titers below 8 RU/ml. Only a few borderline results were observed (28, 4.2%), whereas 369 (56.1%) obtained a frankly positive result (above 11 RU/ml). In two of these positive samples, antibody titers exceeded the maximum quantifiable level (1200 RU/ml). As expected, negative results (<8 RU/ml) were reported mostly (72%) in subjects never diagnosed with COVID-19: while the proportion of negative results attained 73.4% in subjects with no positive swabs (188/256), it appeared to be

as low as 18.2% in previously infected patients (73/402). Among all residents, 65.0% (106/163) reported a frankly positive antibody titer (>11 RU/ml). Positive antibody titers were identified in 53.58% of HCWs (81.30% among hospital personnel and 43.31% among LTCFs workers).

On the other hand, considering all samples with detectable antibodies, the median antibody titer was 31.0 RU/ml (Q1–Q3 range: 10.7–77.3, geometric mean: 31.5 RU/ml), and antibody levels detected were higher ($p < 0.0001$) among previously infected participants (median: 35.6 RU/ml, IQR: 14.0–82.5) than among participants with no previous positive swab (median: 11.6 RU/ml, IQR: 3.3–46.9). Descriptive characteristics are reported in further detail in Table 1, and the distribution of antibody titers according to previous PCR positivity is presented in Figure 1.

3.2 | Regression analyses

As age and type of subject were evidently collinear, each of the two variables was used separately in any multivariable model. The multivariable logistic regression analysis failed to identify any significant influence of age in modifying the persistence of a positive antibody titer over time ($p = 0.2679$). A scatterplot representing antibody titers according to age and number of days since the last positive swab is presented in Figure 2. Conversely, persistence of SARS-CoV-2 antibodies appeared to differ between subject types in the logistic regression analysis, the probability of a positive titer decreasing significantly for HCWs (OR: 0.926 for every 30 days, 95% CI: 0.860–0.998) but not for residents (OR: 1.059, 95% CI: 0.919–1.22). No significant differences emerged in relation to gender ($p = 0.1764$, Table 2A).

On the other hand, considering the subset of subjects with detectable SARS-CoV-2 antibodies, the log-linear regression showed that age was not significant, neither when considered linearly ($p = 0.0809$) nor in the restricted cubic splines model ($p = 0.1752$), in determining the estimate of the antibody titer at a given time. Gender and type of subject did not show any particular confounding effect. Interestingly, all adjusted models suggest a similar value for the probability of antibody loss over time, with a decrease in antibody titer by 4.01%–4.61% every 30 days (Table 2B).

It must be noted that, in log-linear models, the interaction between age (or subject type) and time since the last positive swab could not be evaluated because of collinearity, which conversely was not an issue in any of the logistic regression models (VIFs ≤ 2.5). Optimism evaluated by bootstrapping was lower than 0.25, thus confirming the validity of the results obtained. No violation of residual normality assumption was detected in the log-linear regression models.

4 | DISCUSSION

Understanding the profile of serum antibody responses to SARS-CoV-2 is critically important to guide epidemiological surveillance, infection control measures, antiviral treatment, and vaccination.²⁵

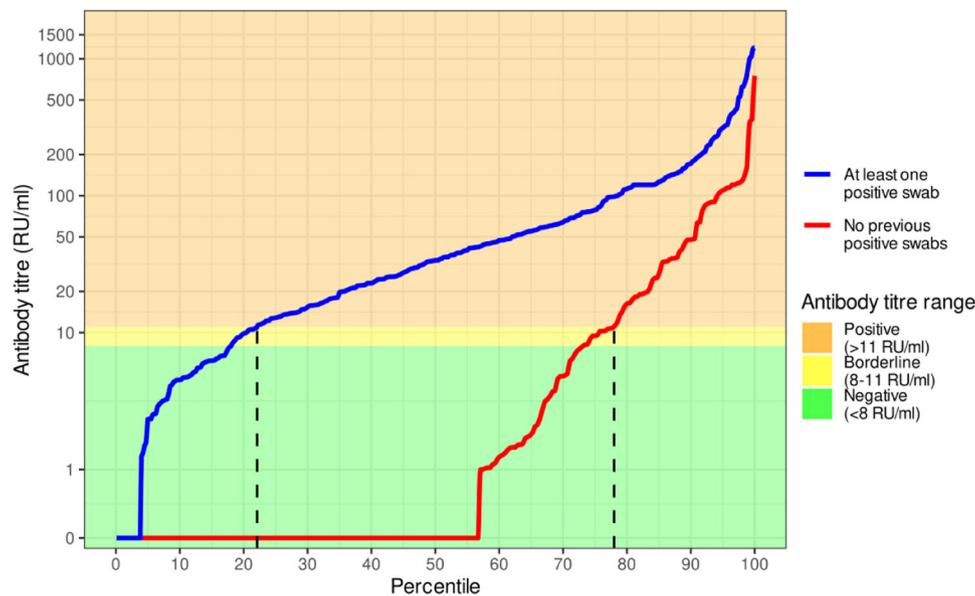


FIGURE 1 Distribution of SARS-CoV-2 anti-S IgG titers, stratified according to the presence/absence of previous positive swabs. All subjects are included ($n = 658$). The x-axis represents the percentile of each distribution, while the y-axis denotes the antibody titer (in RU/ml, positive values are expressed on a logarithmic scale)

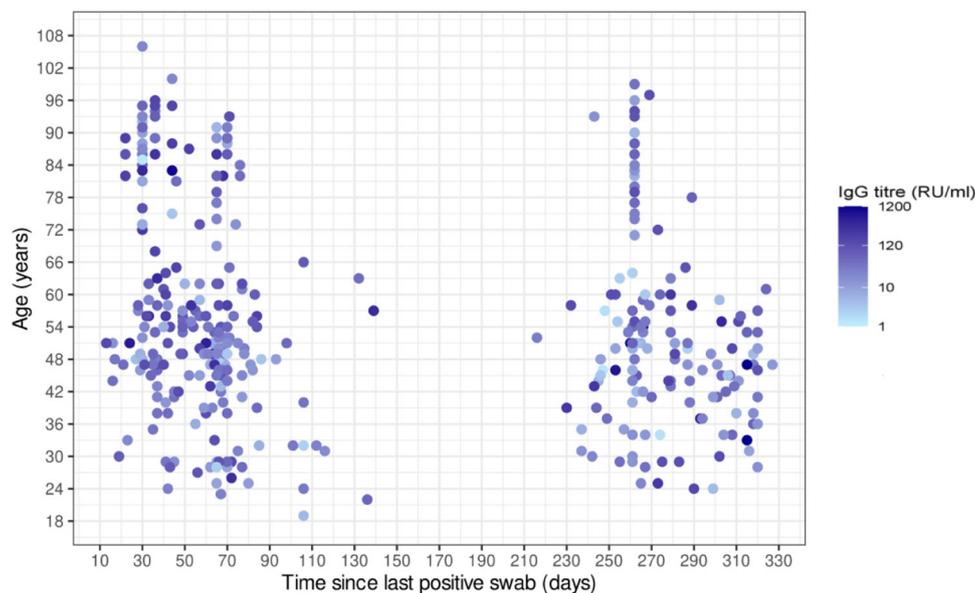


FIGURE 2 Scatterplot representing SARS-CoV-2 anti-S IgG titers (outcome) according to age and number of days since the last positive swab. The outcome is represented in scaled colors from light (low titers) to dark blue (high titers). Only a gradient on the horizontal axis (lower IgG levels at longer times) can be appreciated, as forecast by adjusted models

Conflicting results have been published regarding the durability of IgG levels over time, an issue with crucial relevance for vaccination strategy.¹⁶ This study adds to a growing body of literature on the duration of IgG persistence following natural immunity, by presenting seroprevalence data from 658 unvaccinated subjects at high risk of exposure to SARS-CoV-2.

In this analysis of serum samples provided by residents of LTCFs and HCWs working in LTCFs and hospitals in Northern Italy during the third epidemic wave, positive IgG titers were identified in 65% of

residents and over 50% of HCWs (81.30% for hospital personnel and 43.31% for LTCFs workers). A previous study of adult volunteers from two bordering Northern Italian regions performed in March–April 2020 estimated a seroprevalence in the general population of 11% and in LTCF residents of 41.5%; in a 2021 evaluation on healthy blood donors, a $\approx 15\%$ prevalence was identified.^{26,27} The seroprevalence for HCWs in our survey was over five times higher than the overall seroprevalence among HCWs in screening settings estimated by a meta-analysis of studies published through August

TABLE 2 Output of the multivariable regression models, related to participants with at least one previous positive swab for SARS-CoV-2

(A) Logistic regression: The outcome is represented by the probability of a frankly positive (>11 RU/ml) antibody test. Subject category is considered as an effect modifier, according to the likelihood ratio test for the interaction term ($p = 0.0935$)

Subject category	Variable	β coefficient (95% CI)	Odds ratio (95% CI)	p -value
Health-care workers	Time: swab to blood test	-	-	0.042
	For a 1-day increase	-0.0025 (-0.005, -0.0001)	0.997 (0.995, 1.000)	-
	For a 30-day increase	-0.0764 (-0.1507, -0.002)	0.926 (0.860, 0.998)	-
Long-term care residents	Time: swab to blood test	-	-	0.4164
	For a 1-day increase	0.0019 (-0.0028, 0.0066)	1.002 (0.997, 1.007)	-
	For a 30-day increase	0.0572 (-0.0846, 0.1989)	1.059 (0.919, 1.220)	-
	Gender (Ref. = female)	0.4679 (-0.2105, 1.1462)	1.597 (0.810, 3.146)	0.1764

(B) Log-linear regression: These models include participants with detectable antibody titer only (>0 and <1200 RU/ml). In each of the two models, the outcome is the anti-S IgG titer and the main explanatory variable is represented by the number of days between swab and blood test. The first column shows variables for which each model is adjusted

Variables used for adjustment	β coefficient (log) per day (95% CI)	Antibody loss every 30 days (95% CI)	p -value
Sex + age	-0.0014 (-0.0026, -0.0002)	-4.01% (-7.41%, -0.49%)	0.0261
Sex + subject category	-0.0016 (-0.0028, -0.0004)	-4.61% (-7.98%, -1.13%)	0.0101

2020, which included several studies performed in Northern Italy.¹² The higher seroprevalence found among HCWs in our study could be due to the inclusion of personnel working in both acute-care settings and LTCFs. A recent study comparing seropositivity among HCWs in hospitals and nursing homes in Rhode Island found a significantly higher seroprevalence in nursing home staff compared to hospital staff (13.1% vs. 3.1%).²⁸

In this study, nearly 30% of subjects without a previous positive swab presented frank antibody positivity, compared with an assay specificity of 99.8%. This finding suggests a considerable number of infections in the healthcare settings we investigated were undetected, which might have contributed to the circulation of SARS-CoV-2 within facilities and complicated infection control efforts.²⁹ These infections were presumably asymptomatic or mildly symptomatic as they did not lead to PCR testing and were associated with significantly lower titers, consistent with previous findings of a positive correlation between clinical severity and antibody levels.^{15,30,31}

On the other hand, 4% of subjects in our study with a documented previous positive swab did not present any detectable antibodies, and a further 14% did not reach the threshold of 8 RU/ml after a median time of 70 days. These may represent an artifact of assay performance, as the tests we employed have a sensibility of 93.2% after 20 or more days from symptom onset or positive swab. Despite this test being one of the first releases in the market, it has been duly validated and comparison studies with subsequently released assays have confirmed the equivalence and accuracy.^{32–34} Although other possible explanations are that these subjects seroconverted in the considered timeframe or never seroconverted at all. Declining antibody titers have been documented after several months of observation,^{13,15,16,35} as seen with seasonal

coronaviruses.³⁶ Consistent with these findings, our model identified a statistically significant 4% decrease in the estimated antibody level for each 30-day increase in time from the last positive swab. However, the potential for waning immunity remains a debated issue and further investigation is required to assess the timing and extent of this phenomenon.¹³

According to the results of the multivariable analysis we performed, being an HCW was associated with significant odds for seroreversion over time whereas being a resident of an LTCF was not, after adjustment for age and gender. This finding suggests residents could have a higher probability of maintaining a seropositive status over time. LTCFs represent high-risk congregate settings with long-standing infection control challenges,^{28,37} and LTCF residents are exposed to a significantly higher risk of SARS-CoV-2 infection compared to LTCF personnel.^{29,38} Higher peak viral loads and prolonged viral shedding among residents represent additional risk factors for continued inter-facility transmission of SARS-CoV-2.^{25,28} The natural boosting of antibodies due to repeated exposure could explain the more durable antibody response over time found in residents in this study. Moreover, LTCF residents represent a frail and older population at increased risk for severe disease, which has been associated with more robust and durable antibody responses.³⁹

This study has several limitations. First, participation was voluntary among a convenience sample, which may have affected representativeness therefore a selection bias cannot completely be excluded, also due to the fact that in Italy, especially in the first waves, Piedmont and Lombardy were the epicenters of the epidemic. The high proportion of previously positive HCWs and LTCF residents found in this study supports this concern. The cross-sectional design of this study has inherent limitations, particularly in light of the

potential for waning immunity. Furthermore, it was not possible to collect information regarding the timing or clinical characteristics of previous infections. Our results could also have been affected by the sensitivity and specificity of the assays we employed, as previously discussed. It must also be noted that seropositivity may not reflect immunity to reinfection, as other components of the immune response may contribute to protective immunity (e.g., T cell immunity),^{15,40} although results of previous studies suggest a strong correlation exists between anti-S antibody levels and neutralization activity.³¹ In our study, being an LTCF resident was more important than the effect of age, as no association with seropositivity was found with age, inconsistent with previous reports.³¹

5 | CONCLUSIONS

Antibody tests are an essential tool in the long-term management of infectious diseases. Seroprevalence studies have the potential of identifying previously infected subjects and could allow generating more accurate estimates of the cumulative incidence of infection compared to the number of infections reported through public health surveillance systems, although an adjustment for waning antibody kinetics is required.^{13,41} A reliable estimate of the cumulative incidence of SARS-CoV-2 infection is essential to assess disease severity, monitor immunity levels in a population, inform public health policies and predict the impact of vaccination strategies.¹³ This study allowed to obtain a more comprehensive evaluation of previous exposure to SARS-CoV-2 and to assess the level of natural immunity in specific high-risk populations, providing context for assessing the success of past infection control policies and interventions. Results of this study reinforce the concern that antibody levels following infection wane over time and highlight the importance of improving infection control practices in LTCFs in our region.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

The research protocol was in accordance with the Declaration of Helsinki and fulfilled the requirements of Italian (Law 2003/196) and European regulations (GDPR EC/2016/679) concerning data protection and privacy. All study procedures were reviewed and approved by the Ethical Boards of the relevant institutions (“SS. Antonio e Biagio e Cesare Arrigo” Hospital of Alessandria, Local Health Authorities of Alessandria, Cuneo, and Turin) and the approval was subsequently confirmed by the Ethical Board of the University of Turin (protocol numbers COV 28/2020, 10077, and 0016945).

AUTHOR CONTRIBUTIONS

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DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request.

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REFERENCES

1. Coronavirus (COVID-19) events as they happen. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen>
2. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis.* 2020;20(5):533-534. [https://www.thelancet.com/journals/laninf/article/PIIS1473-3099\(20\)30120-1/fulltext](https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30120-1/fulltext)
3. WHO. Coronavirus (COVID-19) dashboard. <https://covid19.who.int>
4. Ministero della Salute. Nuovo coronavirus, Consiglio dei ministri dichiara stato d'emergenza. https://www.salute.gov.it/portale/news/p3_2_1_1_1.jsp?lingua=italiano%26menu=notizie%26p=dalministero26id=4035
5. Vinceti M, Filippini T, Rothman KJ, Di Federico S, Orsini N. SARS-CoV-2 infection incidence during the first and second COVID-19 waves in Italy. *Environ Res.* 2021;197:111097. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8012166/>
6. Vicentini C, Bordino V, Gardois P, Zotti CM. Early assessment of the impact of mitigation measures on the COVID-19 outbreak in Italy. *Public Health.* 2020;185:99-101.

7. Riccardo F, Andrianou X, Bella A, et al. *Epidemia COVID-19 Aggiornamento nazionale 7 aprile 2021 - Prodotto dall'Istituto Superiore di Sanità (ISS)*. Primo piano—ISS. https://www.iss.it/primo-piano/-/asset_publisher/o4oGR9qmvUz9/content/id/5477037
8. Scoppetta C, Casciato S, Di Gennaro G. Lethality rate of the two waves of the COVID-19 pandemic in Italy. *Eur Rev Med Pharmacol Sci*. 2021;25(1):9-10.
9. Task force COVID-19 del Dipartimento Malattie Infettive e Servizio di Informatica, Istituto Superiore di Sanità. *Epidemia COVID-19*. Aggiornamento nazionale: 12 gennaio; 2022. https://www.epicentro.iss.it/coronavirus/bollettino/Bollettino-sorveglianza-integrata-COVID-19_12-gennaio-2022.pdf
10. Böger B, Fachi MM, Vilhena RO, Cobre AF, Tonin FS, Pontarolo R. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *Am J Infect Control*. 2021;49(1):21-29.
11. Moura DTH, McCarty TR, Ribeiro IB, et al. Diagnostic characteristics of serological-based COVID-19 testing: a systematic review and meta-analysis. *Clinics (Sao Paulo)*. 2020;75:e2212. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7410353/>
12. Galanis P, Vraka I, Fragkou D, Bilali A, Kaitelidou D. Seroprevalence of SARS-CoV-2 antibodies and associated factors in healthcare workers: a systematic review and meta-analysis. *J Hosp Infect*. 2021; 108:120-134.
13. Shioda K, Lau MSY, Kraay ANM, et al. Estimating the cumulative incidence of SARS-CoV-2 infection and the infection fatality ratio in light of waning antibodies. *Epidemiology*. 2021;32(4): 518-524.
14. Vicentini C, Bazzolo S, Gamba D, Zotti CM. Analysis of the fatality rate in relation to testing capacity during the first 50 days of the COVID-19 epidemic in Italy. *Am J Trop Med Hyg*. 2020;103(6): 2382-2390. <https://www.ajtmh.org/view/journals/tpmd/103/6/article-p2382.xml>
15. Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. *Nature*. 2021;591(7851):639-644.
16. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol*. 2020;5(52):eabe5511. <https://www.science.org/doi/10.1126/sciimmunol.abe5511>
17. Kim JY, Kwon J-S, Bae S, et al. SARS-CoV-2-specific antibody and T cell response kinetics according to symptom severity. *Am J Trop Med Hyg*. 2021;105(2):395-400.
18. Chvatal-Medina M, Mendez-Cortina Y, Patiño PJ, Velilla PA, Rugeles MT. Antibody responses in COVID-19: a review. *Front Immunol*. 2021;12:633184. <https://www.frontiersin.org/article/10.3389/fimmu.2021.633184>
19. Infantino M, Pieri M, Nuccetelli M, et al. The WHO International Standard for COVID-19 serological tests: towards harmonization of anti-spike assays. *Int Immunopharmacol*. 2021;100:108095. <https://www.sciencedirect.com/science/article/pii/S1567576921007311>
20. Greenberg RS, Kleinbawm DG. Mathematical modeling strategies for the analysis of epidemiologic research. *Annu Rev Public Health*. 1985;6(1):223-245. <https://www.annualreviews.org/doi/10.1146/annurev.pu.06.050185.001255>
21. Efron B, Gong G. A leisurely look at the bootstrap, the jackknife, and cross-validation. *Am Stat*. 1983;37(1):36-48. <https://www.jstor.org/stable/2685844>
22. R: The R project for statistical computing. <https://www.r-project.org/>
23. Harrell FE Jr.rms: regression modeling strategies R package version 6.2.0;2021. <https://cran.r-project.org/web/packages/rms/rms.pdf>
24. Zeileis A, Hothorn T. Diagnostic checking in regression relationships. *R News*. 2002;2(3):7-10. <https://cran.r-project.org/web/packages/lmtest/vignettes/lmtest-intro.pdf>
25. To KK, Tsang OT, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. 2020;20(5):565-574. [https://www.thelancet.com/article/S1473-3099\(20\)30196-1/fulltext](https://www.thelancet.com/article/S1473-3099(20)30196-1/fulltext)
26. Vena A, Berruti M, Adessi A, et al. Prevalence of antibodies to SARS-CoV-2 in Italian adults and associated risk factors. *J Clin Med*. 2020; 9(9):2780 <https://www.mdpi.com/2077-0383/9/9/2780>.
27. Valenti L, Pelusi S, Cherubini A, et al. Trends and risk factors of SARS-CoV-2 infection in asymptomatic blood donors. *Transfusion*. 2021;61(12):3381-3389.
28. Akinbami LJ, Chan PA, Vuong N, et al. Severe acute respiratory syndrome coronavirus 2 seropositivity among healthcare personnel in hospitals and nursing homes, Rhode Island, USA, July–August 2020. *Emerg Infect Dis*. 2021;27(3):823-834. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7920685/>
29. Malenfant JH, Eslami M, Dao BL, et al. Seroprevalence of SARS-CoV-2 among skilled nursing facility residents and staff members—Los Angeles County, August–September 2020. *J Infect Dis*. 2021. 225:367-373.
30. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis*. 2020;71(16):2027-2034.
31. Post N, Eddy D, Huntley C, et al. Antibody response to SARS-CoV-2 infection in humans: a systematic review. *PLoS One*. 2020;15(12): e0244126.
32. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, et al. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. *J Clin Virol*. 2020;129:104512. <https://www.sciencedirect.com/science/article/pii/S1386653220302547>
33. Tré-Hardy M, Wilmet A, Beukinga I, Dogné J-M, Douxfils J, Blairon L. Validation of a chemiluminescent assay for specific SARS-CoV-2 antibody. *Clin Chem Lab Med*. 2020;58(8):1357-1364. <https://www.degruyter.com/document/doi/10.1515/cclm-2020-0594/html>
34. Nicol T, Lefeuvre C, Serri O, et al. Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). *J Clin Virol*. 2020;129:104511. <https://www.sciencedirect.com/science/article/pii/S1386653220302535>
35. Choe PG, Kang CK, Suh HJ, et al. Waning antibody responses in asymptomatic and symptomatic SARS-CoV-2 infection. *Emerg Infect Dis*. 2021;27(1):327-329. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7774548/>
36. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol Infect*. 1990;105(2):435-446.
37. Taylor J. Serial testing for SARS-CoV-2 and virus whole genome sequencing inform infection risk at two skilled nursing facilities with COVID-19 outbreaks—Minnesota, April–June 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69:69-1295. <https://www.cdc.gov/mmwr/volumes/69/wr/mm6937a3.htm>
38. Telford CT. Preventing COVID-19 outbreaks in long-term care facilities through preemptive testing of residents and staff members—Fulton County, Georgia, March–May 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69:1296-1299. <https://www.cdc.gov/mmwr/volumes/69/wr/mm6937a4.htm>
39. Wang X, Guo X, Xin Q, et al. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients. *Clin Infect Dis*. 2020;71(10):2688-2694.

40. Chia WN, Zhu F, Ong SWX, et al. Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *The Lancet Microbe*. 2021;2(6):e240-e249. [https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247\(21\)00025-2/fulltext](https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(21)00025-2/fulltext)
41. Lumley SF, Wei J, O'donnell D, et al. The duration, dynamics, and determinants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody responses in individual healthcare workers. *Clin Infect Dis*. 2021;73(3):e699-e709.

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