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PII: S1201-9712(21)00558-0
DOI: https://doi.org/10.1016/j.ijid.2021.07.003
Reference: IJID 5539

To appear in: International Journal of Infectious Diseases

Received date: 15 April 2021
Revised date: 29 June 2021
Accepted date: 2 July 2021

Please cite this article as: Francesca Rovida, Irene Cassaniti, Elena Percivalle, Antonella Sarasini, Stefania Paolucci, Catherine Klersy, Sara Cutti, Viola Novelli, Carlo Marena, Francesco Luzzaro, Giovanni De Vito, Roberta Schiavo, Giuliana Lo Cascio, Daniele Lilleri, Fausto Baldanti, Incidence of SARS-CoV-2 infection in health care workers from Northern Italy based on antibody status: immune protection from secondary infection- A retrospective observational case-control study., International Journal of Infectious Diseases (2021), doi: https://doi.org/10.1016/j.ijid.2021.07.003

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Incidence of SARS-CoV-2 infection in health care workers from Northern Italy based on antibody status: immune protection from secondary infection- A retrospective observational case-control study.

Francesca Rovida¹*, Irene Cassaniti¹*, Elena Percivalle¹, Antonella Sarasini³, Stefania Paolucci¹, Catherine Klersy², Sara Cutti³, Viola Novelli³, Carlo Marena³, Francesco Luzzaro⁴, Giovanni De Vito⁵, Roberta Schiavo⁶, Giuliana Lo Cascio⁶, Daniele Lilleri¹§, Fausto Baldanti¹,⁷.

¹Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
²Biometry and Clinical Epidemiology Service, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
³Medical Direction, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
⁴Clinical Microbiology and Virology Unit, Lecco A. Manzoni Hospital, Lecco, Italy
⁵Occupational Medicine Unit, A. Manzoni Hospital, Lecco, School of Medicine & Surgery Milano Bicocca University
⁶Microbiology Unit, Clinical Pathology Department, Guglielmo da Saliceto Hospital, Piacenza, Italy
⁷Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia Italy

*Equally contributed

§Corresponding author: Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy; email: d.lilleri@smatteo.pv.it

Abstract word count: 150
Text word count: 1163
Highlights

- Immunity from natural SARS-CoV-2 infection is protective in healthcare workers
- Secondary infection is associated with low or absent serum neutralizing titer
- Anti-Spike IgG were not significantly lower in subjects with secondary infections
- Secondary infection is usually asymptomatic or mildly symptomatic
- Vaccination of SARS-CoV-2-seronegative subjects might be prioritized

Abstract

Objective. The protection from SARS-CoV-2 infection induced by SARS-CoV-2 anti-S1 and anti-S2 IgG antibody positivity resulting from natural infection was evaluated.

Methods. The frequency of SARS-CoV-2 infection (as determined by virus RNA detection) was evaluated in a group of 1,460 seropositive and a control groups of 8,150 seronegative healthcare workers in three Centres of Northern Italy in the period June-November 2020. Neutralizing serum titers were analysed in seropositive subjects with or without secondary SARS-CoV-2 infection.

Results. During the 6-month survey, 1.78% seropositive subjects developed secondary SARS-CoV-2 infection while 6.63% seronegative controls developed primary infection (odds ratio: 0.26; 95% confidence interval: 0.17-0.38). Secondary infection was associated with low or absent serum neutralizing titer (p<0.01) and was mildly symptomatic in 45.8% cases vs 71.4% symptomatic primary infections (odds ratio: 0.34; 95% confidence interval: 0.16-0.78).

Conclusions. Immunity from natural infection appears protective from secondary infection; therefore, vaccination of seronegative subjects might be prioritized.

Keywords: SARS-CoV-2 infection; secondary infection; neutralizing antibody; immune protection.
**Introduction**

Natural SARS-CoV-2 infection elicits humoral (Percivalle et al., 2020; Ni et al., 2020; Muecksch 2020) and cellular responses (Grifoni et al., 2020). However, little is known about protection against secondary infection. Recently, a longitudinal study conducted in the United Kingdom (Lumley et al., 2021) showed that SARS-CoV-2 seropositivity was associated with a lower SARS-CoV-2 RNA detection rate.

Furthermore, some authors reported cases of SARS-CoV-2 secondary infection (To et al., 2020; Van Elslande et al., 2020; Tillett et al., 2021) suggesting that previous exposure might not guarantee a complete protection.

On the other side, the recently introduced vaccines showed high efficacy in preventing COVID-19 disease (Baden et al., 2021; Voysey et al., 2021), and the hypothesis of vaccination for previously SARS-CoV-2 infected subjects has been debated.

We analysed data from a large retrospective observational case-control study, providing results on immune protection from secondary SARS-CoV-2 infection in seropositive subjects.

**Methods**

A cohort of 9,610 healthcare workers (2,567 male and 7,043 female; median age 47 years, range 21-70 years) from three hospitals in Northern Italy (Fondazione IRCCS Policlinico San Matteo, Pavia; Alessandro Manzoni Hospital, Lecco; Guglielmo da Saliceto Hospital, Piacenza), involved in Covid-19 diagnosis and clinical care, were stratified according to SARS-CoV-2 seropositivity in the period April 29th -May 29th. Data on the role of the workers are reported in Table 1.

Data were matched with SARS-CoV-2 RNA positivity in nasopharyngeal swabs in the period June 1st -November 30th, 2020.
Detection of SARS-CoV-2 RNA in seronegative subjects was defined as a primary infection, whereas, in according with ECDC case definition (ECDC, 2021), detection of SARS-CoV-2 RNA ≥60 days following previous positive serology (anti-spike IgG antibodies) was defined as a secondary infection.

Serological analysis was performed using chemiluminescent assay (Liason SARS-CoV-2 S1/S2 IgG, Diasorin, Saluggia, Italy) for the measurement of SARS-CoV-2 anti-S1 and anti-S2 IgG antibody. Neutralizing antibody serum titre was determined as previously reported (Percivalle et al., 2020).

The health condition of all the workers was constantly monitored in all the three hospitals. The PCR assays to detect SARS-CoV-2 RNA were performed in the laboratories of each center. Nasopharyngeal sampling was scheduled every 14 days in health-care workers of fragile wards in Pavia and Lecco hospitals and every 7 days in Piacenza hospital. In addition, in Piacenza hospital monitoring was scheduled every 14 days in Covid-19 wards. In all the centres, nasopharyngeal sampling was collected in all the symptomatic individuals and contacts. Data on Symptoms were collected during an interview by a physician and insert in a specific database.

Results
At the end of the first epidemic wave, in the period April 29th -May 29th 2020, were collected 9,610 venous blood samples of healthcare workers to evaluate the SARS-CoV-2 anti-S1 and anti-S2 IgG antibody status, 1,460 health care workers (15.2%) were SARS-CoV-2 seropositive (cases) while 8,150 did not (controls). During the second epidemic wave, SARS-CoV-2 infection was detected in 26/1,460 SARS-CoV-2-seropositive subjects (1.78%), and in 540/8,150 SARS-CoV-2-seronegative controls (6.63% primary infections), with an odds ratio of 0.26 (95% confidence interval [CI] 0.17-0.38). The populations from each Center considered independently showed a similar trend (Table
Median age was similar in subjects with primary (47 years, range 23-64) or secondary infections (49 years, range 26-59), as well as the rate of subjects with direct contact with patients (75.66% vs 71.43%, respectively; p=0.68).

Data on symptoms were available in 24/26 (92.3%) secondary infections and in 391/540 (72.4%) primary infections. Mild symptoms were reported in 11/24 (45.8%) secondary vs 279/391 (71.4%) primary infections (odds ratio 0.34, 95% CI 0.16-0.78, p=0.012), while the other subjects were asymptomatic.

Data on anti-Spike IgG antibody quantitative levels were available for 313 seropositive subjects without, and 7 seropositive subjects with a SARS-CoV-2 secondary infection (Fig 1A), all from the Pavia hospital, for the other two hospitals were available only qualitative results. Although the difference between the two groups was not significant (p=0.16; Mann-Whitney U-test), 5/7 subjects with SARS-CoV-2 secondary infection had anti-Spike IgG antibody levels within the lower quartile of subjects with no subsequent positive swab. Neutralizing serum titre was determined for the 7 subjects with and 13 seropositive subjects without secondary infection (Fig 1B). Neutralizing serum titre was significantly lower in the subjects with secondary infection (p<0.01; Mann-Whitney U-test) and, in particular, was undetectable in 5 of them.

Discussion

The results of this case-control study confirmed that immunity resulting from natural infection is associated with a significant protection from secondary infection. It could be hypothesized that ineffective SARS-CoV-2 immunity was elicited in the subjects developing a secondary infection. Indeed, SARS CoV-2 neutralizing antibodies were undetectable in 5/7 (71.4%) subjects with secondary infection analysed. While we cannot rule out false-positive serostatus
results in these individuals, it could be also speculated that the presence of high SARS-CoV-2 neutralizing titres might be crucial for protection.

During the survey period, the odds for developing SARS-CoV-2 infection was 4 times lower in seropositive subjects. Therefore, we can assume that immunity elicited by natural infection is around 75% protective, similarly to what observed with adenovirus-vectored vaccine (70%), and slightly lower than what reported for mRNA vaccines (95%) (Baden et al., 2021; Voysey et al., 2021). However, it is difficult to directly compare protective effect of natural with vaccine-induced immunity, as there is a difference in the immune response that they elicit. In our study, about half of individuals with SARS-CoV-2 secondary infection were asymptomatic virus carriers diagnosed occasionally due to screening of contacts of infected subjects. Therefore, it appears that immunity occurring after natural infection is highly protective from symptomatic SARS-CoV-2 secondary infection. In view of vaccination strategies, prioritizing the immunization of seronegative individuals while deferring it in previously infected individuals would reduce the need of vaccine doses and speed up the process to reach immune protection in the population.

Major limitations of this study were its retrospective nature, the availability of quantitative anti-SARS-CoV-2 IgG antibody levels only for one center, and the testing of neutralization titres in a limited subset. On the other hand, this study support and further extend the results of the large SIREN study conducted in England on healthcare workers (Hall et al., 2021) and of other recent studies conducted in healthcare workers or the general population (reviewed by O Murchu et al., 2021), although these studies did not evaluate the levels of neutralizing antibodies in subjects with secondary infections. Only one study evaluated the role of neutralizing antibody for SARS-CoV-2 reinfection in ferrets (Kim Y et al., 2021). Kim et al. reported that SARS-CoV-2 reinjected ferrets showed active virus replication in the upper respiratory and gastro-intestinal tracts. The high neutralizing antibody titre group showed attenuated viral replication and rapid viral clearance.
Furthermore, direct-contact transmission was observed only from reinfected ferrets with low neutralizing antibody titres (<20) and not from other groups. More data are needed to evaluate the protective role of neutralizing antibody in humans.

In conclusion, we confirmed that immunological memory elicited by SARS-CoV-2 infection is protective from secondary infections up to 6 months. Further analysis in the general population are warranted.

**Conflict of Interest**

The authors have no conflict of interest to declare.

**Funding Source**

This work was supported by Fondazione Cariplo [grant CoVIM, no. 2020-1374] and Ministero della Salute, Ricerca Finalizzata [grant BIAS no. 2020-12371760] and Ricerca Corrente, [grant no. 80206] and from European Commission – Horizon 2020 [EU project 101003650 – ATAC]

**Ethical Approval**

The SARS CoV-2 antibody status of health care workers was performed in Lombardy Region (Pavia and Lecco Hospitals) in agreement with a specific Regional screening protocol (Circolare Regione Lombardia “Utilizzo test sierologici”, protocollo G1.2020.0017959, 22 April 2020). In Emilia-Romagna Region (Piacenza Hospital) screening was performed in agreement with a specific Regional protocol (Emilia-Romagna Region, Giunta Regionale, “COVID-19: disciplina dei test sierologici”, Delibera N° 350, 16 April 2020). A specific written informed consent was collected.
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Table 1. Role of the study participants

| Staff group                          | Fondazione IRCCS Policlinico San Matteo Pavia | Alessandro Manzoni Hospital Lecco | Guglielmo da Saliceto Hospital Piacenza | All participants |
|--------------------------------------|-----------------------------------------------|----------------------------------|----------------------------------------|------------------|
| nursing or health care assistant     | 1647                                          | 1374                             | 1661                                   | 4682             |
| doctor                               | 924                                           | 514                              | 571                                    | 2009             |
| pharmacist, biologist, chemist, physicist | 82                                             | 16                               | 259                                    | 357              |
| researcher                           | 31                                            | 0                                | 0                                      | 31               |
| healthcare technicians               | 608                                           | 336                              | 199                                    | 1143             |
| administrative                       | 306                                           | 287                              | 325                                    | 918              |
| other                                | 164                                           | 0                                | 306                                    | 470              |
| Total                                | 3762                                          | 2527                             | 3321                                   | 9610             |
Table 2. Occurrence of SARS-CoV-2 infection in seropositive and seronegative healthcare workers

| Center   | SARS-CoV-2 serostatus | No. subjects (%) | No. subjects with SARS-CoV-2 RNA positive swab (% on seropositive or seronegative subjects) | Odds ratio (95% confidence interval) |
|----------|------------------------|------------------|------------------------------------------------------------------------------------------|-------------------------------------|
| Pavia    | Positive               | 321 (8.5)        | 7 (2.18)                                                                                 | 0.33 (0.15-0.69)                   |
|          | Negative               | 3441 (91.5)      | 219 (6.36)                                                                               |                                     |
| Piacenza | Positive               | 965 (29.1)       | 14 (1.45)                                                                                | 0.24 (0.14-0.42)                   |
|          | Negative               | 2356 (70.9)      | 136 (5.77)                                                                               |                                     |
| Lecco    | Positive               | 174 (6.9)        | 5 (2.87)                                                                                 | 0.35 (0.15-0.81)                   |
|          | Negative               | 2353 (93.1)      | 185 (7.86)                                                                               |                                     |
| Total    | Positive               | 1460 (15.2)      | 26 (1.78)                                                                                | 0.26 (0.17-0.38)                   |
|          | Negative               | 8150 (74.8)      | 540 (6.63)                                                                               |                                     |
Figure 1 legend. Anti-SARS-CoV-2 S1/S2 IgG antibody serum levels (A) and neutralizing serum titers (B) in SARS-CoV-2-seropositive subjects with no SARS-CoV-2 secondary infection (n=313 for anti- SARS-CoV-2 S1/S2 IgG antibody and n=13 for neutralizing antibody) or with secondary infection (n=7). Median levels (and interquartile range for subjects with no secondary infection) are shown. Dotted horizontal lines represent cutoff levels for positive results (≥15 arbitrary units (AU)/ml for anti-SARS-CoV-2 S1/S2 IgG antibody and ≥1:10 neutralizing serum titer). Each symbol represents an individual.