SARS-CoV-2 outbreak in a synagogue community: longevity and strength of anti-SARS-CoV-2 IgG responses

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Abstract

SARS-CoV-2 pandemic is still ongoing along with the global vaccination efforts against it. Here we aimed to understand the longevity and strength of anti-SARS-CoV-2 IgG responses in a small community (n=283) six months following local SARS-COV-2 outbreak in March 2020. Three serological assays were compared and neutralization capability was also determined. Overall 16.6% (47/283) of the participants were seropositive and 89.4% (42/47) of the IgG positives had neutralizing antibodies. Most of the symptomatic individuals confirmed as PCR positive during the outbreak were seropositive (30/32, 93.8%) and 33.3% of the individuals who quarantined with a PCR confirmed patient had antibodies. Serological assays comparison revealed that Architect (Abbott) targeting the N protein LIASON®
(DiaSorin) targeting the S protein and ELISA targeting RBD detected 9.5% (27/283), 17.3% (49/283) and 17% (48/283), respectively, as IgG positives. The latter two assays highly agreed (kappa=0.89) between each other. In addition, 95%, (19/20, by ELISA) and 90.9% (20/22, with LIASON) and only 71.4% (15/21, by Architect) of individuals that were seropositive in May 2020 were found positive also in September. The unexpected low rate of overall immunity indicates absence of un-noticed, asymptomatic infections. Lack of overall high correlation between the assays is attributed mainly to target-mediated antibody responses and suggests that using a single serological assay may be misleading.

**Key words:** SARS-CoV-2, herd immunity, sero-prevalence, IgG antibodies, Neutralizing antibodies.

**Introduction**

Seropositivity combined with neutralizing capability of IgG antibodies is the ultimate humoral measure of the immune system against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission. With the initiation of vaccination efforts, understanding the efficacy and persistence of natural immunity is critical when deciding how to allocate a limited number of vaccines, as well as determining when herd immunity is achieved. Yet, data on the duration of immune responses following natural infection, the persistence of IgG antibodies and their viral neutralizing capacity, is still limited.

Seroconversion, determined by positive IgG result in serological assays, occurs usually within 7-14 days following diagnosis. Asymptomatic individuals are considered to have a weaker immune response to SARS-CoV-2 infection compared to those who were clinically ill [1]. However, the durability of IgG response over time is not clear yet; some groups have reported reduction in IgG and neutralizing antibody levels in the early convalescent phase [1],[2], whereas others [3] showed that IgG remained stable in convalescent for at least 31 weeks [4]. The different sensitivities and specificities of the
serological assays used in these studies, may explain these contradictory reports. Indeed, since a high positive predictive value could not currently be assured using a single test, CDC recommended to use an orthogonal testing algorithm and confirm a positive first assay result with a secondary assay [5]. Virus neutralization assays which are usually not applicable for high-throughput screening, remain the gold standard approach for determining antibody efficacy and their results could confirm serological assays results. However, it is important to keep in mind that because neutralizations are functional assays they are often less sensitive than other serological tests which only measure the existence of specific antibodies regardless of their neutralization capacity. Neutralization assays that utilize SARS-CoV-2 require BSL3 conditions and are therefore difficult to use [6]. Recently, tests which use recombinant pseudoviruses that incorporate the S protein of SARS-CoV-2 and could be used under regular laboratory conditions were demonstrated to efficiently identify neutralizing antibodies in patient samples [7].

Two of the first commercial assays approved in April 2020 via the emergency use authorization (EUA) are the Architect SARS-CoV-2 IgG (Abbott Laboratories) detecting IgG antibodies against the viral N antigen, and the LIASON® SARS-CoV-2 S1/S2 IgG, (DiaSorin) identifying IgG antibodies against the viral S1 and S2 proteins. While S protein is a surface protein essential for viral entry, the N protein is a structural protein that binds to the coronavirus RNA genome and is known to be the most abundantly expressed protein of the SARS-CoV-2 [8]. Recently we have reported 84.7% sensitivity and 99.5% specificity with Architect and 82.4% sensitivity and 98.7% specificity with LIASON for the detection of anti SARS-CoV-2 antibodies [9]. In addition, using a new RBD-ELISA assay identifying IgG antibodies against the viral receptor binding domain within the S protein (RBD-ELISA), we determined 88% sensitivity and 98% specificity [10].
One of the earliest local-outbreaks in Israel occurred in the centrally located city of Modiin, within a synagogue congregation of approximately 600 individuals. The source of the exposure was eventually identified as an individual, who was infected by a returning traveler from the USA. It should be noted that during the time of this outbreak, national health policy did not require any social distancing measures, and that PCR testing was not available per request.

Here, we aim to describe the immunity among this outbreak participants and to understand the longevity and strength of the anti-SARS-CoV-2 IgG responses using different serological assays from samples taken 6 months following the exposure. Together with the progress of worldwide anti- SARS-CoV-2 vaccination efforts, these data, among similar studies, are highly important.

**Methods**

**Study participants**

All members of the Hoshen synagogue community (approximately 600 individuals, adults and children) that were potentially exposed to SARS-CoV-2 outbreak in mid-March 2020, were approached 180 days later, in September 2020. Two hundred and eighty-three individuals agreed to participate in the study. An explanation of the methods and aims of the study and a link to an online questionnaire were provided. Demographics, data on exposure to COVID-19 positive individuals during and after the local outbreak, symptoms consistent with COVID-19, quarantine (yes/no), SARS-CoV-2 PCR testing results, previous SARS-CoV-2 IgG antibody results and pre-existing medical conditions were requested. Clinical symptoms- fever, cough, throat pain, dyspnea, anosmia/ageusia, other respiratory symptoms, and other constitutional symptoms (malaise, myalgia, headache and gastrointestinal symptoms) were recorded.
This study was approved by the Israeli Ministry of Health and individuals were exempted from signing an informed consent.

**Sample collection and assessment of anti SARS-CoV-2 antibodies**

Blood samples from 283 individuals that agreed to participate in this study were collected in September 2020, six months after the local outbreak. Before this study was initiated, and upon request of the family members, blood samples from 33 of the study participants who were PCR positive or quarantined with a positive PCR patient during the outbreak, were also collected in May 2020 (two months following exposure). Each sample was tested once with the following assays: Architect anti SARS-CoV-2 IgG test (Abbott Laboratories, Abbott Park, IL), LIASON IgG test (DiaSorin, Centralino, Italy) and a RBD-ELISA. The commercial assays were performed according to the instructions using the cutoff values reported by the manufacturers’ s (Architect <1.40 is considered negative; LIASON < 12.0 is negative, 12.0 – 15.0 is equivocal and > 15.0 is positive). The RBD-ELISA assay was performed as previously described [10] and the results were interpreted based on the CDC recommendations [5], for a population with a SARS-CoV-2 prevalence of 5%, whereby all results under index value of 1.83 were considered negative [9].

All samples from IgG positive individuals were tested for the presence of viral neutralizing antibodies (psNUT). A green fluorescent protein (GFP) reporter-based pseudotyped virus neutralization assay with a non-replicative vesicular stomatitis virus (VSV) backbone coated with SARS-CoV-2 spike (S) protein was generously obtained from Dr. Gert Zimmer (Institute of Virology and Immunology, Mittelhäusern, Switzerland). psNUT assay was technically performed as described [11]. Sera not capable of reducing viral replication by 50% at 1/16 dilution were considered non-neutralizing [12, 13].

**Results interpretation**
A person was determined seropositive if a positive IgG result was obtained in at least two of the three serological tests. However, for individuals found previously to be SARS-CoV-2 positive by PCR, a single positive antibody test result together with the positive PCR assay results were sufficient to determine positive sero-status. Equivocal results (observed in LIASON test only) were considered positive when either Architect or RBD-ELISA were positive for the same participant.

BioVenn [14] was used to compare the positive IgG results of the different assays and was visualized using area-proportional Venn diagram. The agreement between any two serological assays was assessed using the Cohen’s kappa (κ) statistic.

**Results**

**Outbreak description and characteristics of study participants**

The outbreak occurred during the week of Purim, a Jewish holiday, March 6-14, 2020. The index patient was infected by a returning traveler from USA. Exposures in the community occurred at the synagogue, at individuals’ homes, as well as during youth group events. Table 1 summarizes basic information on the 283 study participants, members of the Hoshen synagogue community that agreed to participate in this study. More males were included (male/female 1.24); median age was 37 years, (IQR 16-47). Although the majority of all community members (82.7%) were asymptomatic, most of them (89%) were put into quarantine. Seventy of the 283 individuals that agreed to participate were tested by PCR at the time of the outbreak; 36 of them were found positive. The proportion of these confirmed PCR positive individuals was similar in both sexes; 6.7% of children below the age of 10 were PCR positive, 10.8% of those between 11 and 19 years and 14% of adults >19 years were PCR positive.

**Comparison between IgG and PCR results**
The total number of IgG positive samples was 27, 48 and 49 for the Architect, RBD-ELISA and LIASON, respectively. LIASON and RBD-ELISA shared the highest number of positive results as observed by Venn Diagram (Figure 1a). Indeed, the highest agreement between assays (kappa=0.89) was obtained for LIASON and the RBD-ELISA assays. Moderate agreement was found between Architect and LIASON (kappa=0.58) and between Architect and RBD-ELISA, (kappa=0.56, Figure 1b).

When PCR results were also considered, the proportion of those confirmed to be positive by PCR among those with positive IgG results in each of the assays was between 65%-77% (Figure 2a). Finally, for an individual to be seropositive, at least two assays had to be positive as described in methods section. Accordingly, 47 individuals were hereby considered to be positive for anti SARS-CoV-2 IgG antibodies (Figure 2b). As only 36 individuals were confirmed as PCR positive during the outbreak, the likely proportion of unrecognized infections was 23.4% (11/47). All individual IgG, PCR and final serological verdict is presented in supplementary Figure 1.

**Correlation between antibody responses, PCR results, clinical and epidemiological data**

Presence of anti SARS-CoV-2 IgG antibodies and viral neutralization capability classified by clinical and epidemiological data is summarized in Table 2. Overall, 89.4% (42/47) of the participants who were considered to have positive IgG results (47/283, 16.6%) had neutralizing antibodies.

More symptomatic individuals with PCR positive results were considered seropositive (30/32, 93.8%) compared to those who did not have positive PCR results (7/17, 41.2%). In addition, more individuals who quarantined with a PCR confirmed patient had antibodies (5/15, 33.3%) compared to those who quarantined with none-PCR -confirmed
patient (9/203, 4.4%). Positive antibody results were obtained both for young children 0-10 years old (2/15, 13.3%) and teenagers, 11-19 years old (15/83, 18.1%). Also, 16.2% of those >19 years of age were IgG positive.

**Antibody durability**

Thirty-three of the participants had antibodies tested in blood drawn during May 2020, two months after the outbreak, 23 of which were found IgG positive. This serology evaluation early after the infection, was performed for these participants upon their request, as they were all either PCR positive or quarantined with a confirmed patient. Figure 3 compares IgG results in samples taken two and six months following exposure. Loss of antibodies was mainly observed with Architect assay, whereby 71.4% (15/21) of participants remained IgG positive. RBD-ELISA and LIASON results were positive for 95% (19/20) and 90.9% (20/22) of the samples, suggesting longer durability of these antibodies compared to the Architect. Importantly, neutralizing antibodies persisted in 88.9% (16/18) of the participants.

**Discussion**

This study assessed persistence of anti SARS-CoV-2 antibodies in a small and close community exposed to infection in an early time following the start of this global pandemic. Although most the individuals were exposed to the virus and many were quarantined, the overall sero-prevalence identified was 16.6%. In a nation-wide screening performed by the Ministry of Health at the same time a sero-prevalence of 5.5% was found. Similarly, during the same period (July-September 2020) low sero-prevalence of 3.1%-5% was reported in several other countries [15],[16],[17]. Although the seropositivity of this community was higher than in the general population, it was lower than initially expected. This may result from low assay sensitivity or from short durability of antibodies to this infection. On the other hand, it may suggest that although exposed, most community members were indeed not infected. As could be expected, most of the symptomatic individuals with PCR positive
results were seropositive (30/32, 93.8%). In addition, 33.3% of the individuals who quarantined with a PCR confirmed patient had antibodies.

To correctly determine seropositivity, three serological assays were compared and PCR data was also collected. We have shown that the degree of agreement between any two serological assays was different and that the Architect test failed to identify nearly 50% of the IgG positive individuals and was significantly less sensitive compared to the LIASON and the RBD-ELISA which were highly comparable (kappa=0.89). Moreover, our results demonstrate that persistence of antibodies directed to the viral nucleocapsid (N), the target identified by the Architect test, is inferior to that of the spike protein S1/S2, targeted by LIASON or the RBD. This conclusion is based on the reduced longevity of IgG antibodies detected by the Architect assay, exemplified by the lower prevalence of IgG positive results in samples taken six months compared to those taken two months following the SARS-CoV-2 exposure. Higher durability was observed for antibodies detected by LIASON and RBD-ELISA (90.9% and 95% durability in samples taken on May and on September 2020, respectively).

Similar observations were recently reported by others [18]. Indeed, it was already demonstrated that the antibody response to various viral proteins, including S, S1, S2, RBD, and N, varies and that assay sensitivity correlates with the abundance, conservation and antigenicity of the different viral proteins, as well as with the durability of the individual antibody response [19],[20].

Most of the seropositive individuals were capable to neutralize SARS-CoV-2 pseudo virus infection. This result is clinically important and may suggest long lasting immunity against such viral infection.

In this study, the RBD ELISA test-positive cutoff was calculated to give a sensitivity of 85% and specificity of 99.76%, according to the CDC recommendations, for a population with a SARS-CoV-2 prevalence of 5% [5, 9, 10]. However, our test results were slightly less
accurate (86.1% and 97.1%, respectively). The latter may be due to the higher seroprevalence of the study population when compared to the national average for which the cutoff was created.

SARS-CoV-2 is highly infective during incubation-period with rapid transmission in teenagers and children. Fast onset and various nonspecific atypical manifestations were reported in children [21]. Here, 35% of our cohort were below 19 years of age, and many of them participated in the youth events. However, no significant differences were found between the serological status of the young, below 19 years and those >19 years of age (17.3% and 16.2%, respectively). Thus, it seems that overall immunity in such exposed community, even among young population does not occur.

Despite being a small, volunteer-based, cohort, there are several advantages to this study. First, the relatively early occurrence of the outbreak in a close community allows for the investigation of antibody durability over six months. Moreover, previous serological results for a sub-cohort, allows to learn more about antibody longevity. Finally, personal details provided by the participants allowed to correlate with epidemiological, medical and demographical data.

This study demonstrates the paucity of overall immunity in an exposed population as well as the varying durability of different antibody responses. Therefore, serological investigations should bear in mind the characteristics of the population that is investigated as well as the technical limitations of serological assays and consider a multiple-and diverse test algorithm. Towards the coming global anti-SARS-CoV-2 vaccination, these data, as well as data from other studies are highly important.

**Data Availability Statement**

All data supporting the finding of this study are available upon request from the corresponding author.
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References

(1) Long QX, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nature Medicine 2020; 26(8): 1200-1204.
(2) Ibarrondo FJ, et al. Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. New England Journal of Medicine 2020; 383(11): 1085-1087.
(3) Wang Y, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. Journal of Clinical Investigation 2020; 130(10): 5235-5244.
(4) Anand SP, et al. Longitudinal analysis of humoral immunity against SARS-CoV-2 Spike in convalescent individuals up to 8 months post-symptom onset. bioRxiv 2021.
(5) CDC. Interim Guidelines for COVID-19 Antibody Testing. In, 2020.
(6) Tan CW, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nature Biotechnology 2020; 38(9): 1073-1078.
(7) Sahin U, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020; 586(7830): 594-599.
(8) Naaber P, et al. Evaluation of SARS-CoV-2 IgG antibody response in PCR positive patients: Comparison of nine tests in relation to clinical data. PLoS One 2020; 15(10).
(9) Oved K, et al. Multi-center nationwide comparison of seven serology assays reveals a SARS-CoV-2 non-responding seronegative subpopulation. eClinicalMedicine 2020; 29: 100651.
(10) Indenbaum V, et al. Testing IgG antibodies against the RBD of SARS-CoV-2 is sufficient and necessary for COVID-19 diagnosis. PLoS One 2020; 15(11): e0241164.
(11) Valdivia A, et al. Suitability of two rapid lateral flow immunochromatographic assays for predicting SARS-CoV-2 neutralizing activity of sera. Journal of Medical Virology 2020.
(12) Lustig Y, et al. Neutralizing Response against Variants after SARS-CoV-2 Infection and One Dose of BNT162b2. *New England Journal Medicine* 2021.
(13) Meyer B, et al. Validation of a commercially available SARS-CoV-2 serological immunoassay. *Clinical Microbiology and Infection* 2020; **26**(10): 1386-1394.
(14) Hulsen T, de Vlieg J, Alkema W. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC Genomics* 2008; **9**: 488.
(15) Hallal PC, et al. SARS-CoV-2 antibody prevalence in Brazil: results from two successive nationwide serological household surveys. *Lancet Global Health* 2020; **8**(11): e1390-e1398.
(16) Stringhini S, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. *Lancet* 2020; **396**(10247): 313-319.
(17) Pollan M, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020; **396**(10250): 535-544.
(18) Maine GN, et al. Longitudinal characterization of the IgM and IgG humoral response in symptomatic COVID-19 patients using the Abbott Architect. *Journal of Clinical Virolology* 2020; **133**: 104663.
(19) van Tol S, et al. Accurate serology for SARS-CoV-2 and common human coronaviruses using a multiplex approach. *Emerging Microbes and Infection* 2020; **9**(1): 1965-1973.
(20) Ripperger TJ, et al. Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low-Prevalence Communities and Reveal Durable Humoral Immunity. *Immunity* 2020; **53**(5): 925-933 e924.
(21) Huang L, et al. Rapid asymptomatic transmission of COVID-19 during the incubation period demonstrating strong infectivity in a cluster of youngsters aged 16-23 years outside Wuhan and characteristics of young patients with COVID-19: A prospective contact-tracing study. *Journal of Infection* 2020; **80**(6): e1-e13.

![Figure 1](https://www.cambridge.org/core/terms). https://doi.org/10.1017/S0950268821001369
Figure 2

Figure 3
| Parameter                  | n (%)   | PCR positive result, n (%) |
|----------------------------|---------|---------------------------|
| Sex                        |         |                           |
| Male                       | 157 (55.5) | 19 (12.1)                |
| Female                     | 126 (44.5) | 17 (13.5)                |
| Age                        |         |                           |
| 0-10                       | 15 (5.3)  | 1 (6.7)                   |
| 11-19                      | 83 (29.3) | 9 (10.8)                  |
| >19                        | 185 (65.4) | 26 (14)                  |
| Symptoms                   |         |                           |
| Any                        | 49 (17.3) | 32 (65.3)                 |
| None                       | 234 (82.7) | 4 (1.7)                   |
| Quarantine                 |         |                           |
| Yes                        | 254 (89.8) | 36 (14.2)                |
| No                         | 29 (10.2)  | 0                         |
| Pre-existing medical conditions* | |                           |
| Any                        | 56 (19.8)  | 8 (14.3)                  |
| None                       | 227 (80.2) | 28 (12.3)                 |

Table 1

| Age (years, y), N=283      | Positive IgG n (%) | psNUT n (%) |
|----------------------------|---------------------|-------------|
| 0-10, n=15                 | 2 (13.3)            | 2 (100)     |
| 11-19, n=83                | 15 (18.1)           | 13 (86.7)   |
| >19, n=185                 | 30 (16.2)           | 27 (90)     |
| Symptoms                   |         |                           |
| Any, n=49                  | Positive PCR, n=32  | 30 (93.8)   |
|                            | Negative PCR, n=8    | 6 (75)       |
|                            | Not tested/ no result PCR, n=9 | 1 (11.1) |
| None, n=234                | Positive PCR, n=4    | 4 (100)     |
|                            | Negative PCR, n=67   | 2 (3)        |
|                            | Not tested/ no result PCR, n=163 | 4 (2.5) |
| Quarantine*                |         |                           |
| n=247                      | With a confirmed patient, n=15 | 5 (33.3) |
|                            | Without a confirmed patient, n=203 | 9 (4.4) |
|                            | No, n=29             | 0            |
| Pre-existing medical conditions |         |                           |
| n=283                      | Yes, n=56            | 9 (16.1)    |
|                            | No, n=227            | 38 (16.7)   |

Table 2