Antibody Seronegativity in COVID-19 RT-PCR–Positive Children

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Abstract: This substudy of a prospective case-ascertained household transmission study investigated severe acute respiratory syndrome coronavirus 2 reverse transcription polymerase chain reaction–positive individuals without antibody development and factors associated with nonseroconversion. Approximately 1 of 8 individuals with coronavirus disease 2019 did not seroconvert. Children, particularly the youngest, were approximately half as likely to seroconvert compared with adults. Apart from the absence of fever/chills, individual symptoms did not strongly predict nonseroconversion.

Key Words: COVID-19, SARS-CoV-2, seroconversion, seronegative, children

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The standard for diagnosing acute symptomatic and asymptomatic coronavirus disease 2019 (COVID-19) infection is severe acute respiratory syndrome coronavirus (SARS-CoV-2) gene detection via nucleic acid amplification testing, such as reverse transcription polymerase chain reaction (RT-PCR). A humoral immune response consisting of SARS-CoV-2–specific antibodies (seroconversion) is often detectable 5 days postsymptom onset (IgM) and can remain detectable 12 months postinfection (IgG).[1] However, not all infected individuals seroconvert; disease severity, symptoms, and viral load may affect antibody response, and the response may differ between children and adults.[2–6] This study investigated SARS-CoV-2 RT-PCR–positive individuals without antibody development and factors associated with nonseroconversion.

MATERIALS AND METHODS

We conducted a secondary analysis of a prospective case-ascertained study of household COVID-19 transmission in Ottawa, Canada, from September 2020 to March 2021. All participating households had at least 1 member with RT-PCR–confirmed COVID-19 infection and where at least 1 participating member was a child (<18 years). Participants with a positive COVID-19 RT-PCR test were included in this substudy; vaccinated individuals were excluded. Participants underwent phlebotomy for SARS-CoV-2–specific antibody measurement at least 2 weeks after diagnosis (no maximum postinfection duration). Automated chemiluminescent enzyme-linked immunosorbent assay (ELISA) assays evaluated SARS-CoV-2–specific IgA, IgM and IgG against the spike-trimer and nucleocapsid protein (Langlois Laboratory, University of Ottawa). The validated serology platform used in the Langlois Laboratory has a sensitivity and specificity of >98%, and is comparable to 10 other commercial platforms.[7,8] Samples were considered isotype positive for an individual isotype (IgG, IgA or IgM) when both antispike and antinucleocapsid antibodies were detected above cutoff values (S/CO ≥ 1). Samples were considered SARS-CoV-2-antibody–positive (as a result of infection) when IgG was positive, or if both IgA and IgM were positive. The primary outcome was the proportion of participants who did not seroconvert (SARS-CoV-2-antibody–negative). Factors associated with nonseroconversion were examined. Univariable and multivariable logistic regressions were fitted with estimation of robust (Huber-White) standard errors applying household as the clustering unit to examine factors related to nonseroconversion. The Research Ethics Boards of CHEO (20/81/X), The Ottawa Hospital (20200673-01K) and University of Ottawa (20200358) approved this study.

RESULTS

Three hundred thirty RT-PCR–positive participants [162 children, median age 8.9 years (IQR 5.6–13.1) and 168 adults, median age 40.7 years (IQR 36.5–46.8)] completed blood sampling for SARS-CoV-2 antibodies. Forty-three [13%; 95% confidence interval (CI): 9.7–17.0] did not seroconvert, 63% (27/43) of whom were children (Table 1). All hospitalized participants (10/330, 3%) seroconverted. Individuals who were asymptomatic at time of RT-PCR testing were no more or less likely to seroconvert [odds ratio (OR) = 0.4; 95% CI: 0.1–1.2]. Seroconversion was not associated with time since infection (≤30 vs >30 days; OR = 0.9; 95% CI: 0.4–1.8).

Predictors of Nonseroconversion

Multivariable analysis revealed children 0–4 years of age had lower odds of seroconversion than older children (5–11 years: OR = 0.2; 95% CI: 0.1–0.7 and 12–17 years: OR = 0.1; 95% CI: 0.0–0.5) and adults (18–49 years: OR = 0.1; 95% CI: 0.1–0.4 and >50 years: OR = 0.1; 95% CI: 0.0–0.4). Odds of seroconversion decreased with decreasing age. Symptom count (1, 2 symptoms) was not associated with seroconversion (OR = 1.7; 95% CI: 0.2–14.3 and OR = 1.0; 95% CI: 0.1–8.2, respectively). The presence of fever/chills was associated with increased seroconversion

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TABLE 1. Clinical and Demographical Characteristics of COVID-19 Patients According to Seroconversion Status (Overall Call)

	Overall (n = 330) No. (%)	Seroconverters (n = 287) No. (%)	Nonseroconverters (n = 43) No. (%)
Age (yr), median (IQR)	19.3 (9.0–41.0)	30.3 (9.6–41.4)	12.0 (5.3–36.5)
Age group			
Preschool (0–4 yr)	34 (10.3)	24 (8.4)	10 (23.3)
School age (5–11 yr)	81 (24.5)	70 (24.4))	11 (25.6)
Adolescent (12–17 yr)	47 (14.2)	41 (14.3)	6 (14.0)
Adult (18–49 yr)	145 (43.9)	130 (45.3)	15 (34.9)
Older adult (>50 yr)	23 (7.0)	22 (7.7)	1 (2.3)
Female	169 (51.5)	149 (52.3)	20 (46.5)
Race			
Indigenous identity	4 (1.2)	4 (1.4)	0 (0.0)
Black	37 (11.2)	36 (12.5)	1 (2.3)
Asian	6 (1.8)	6 (2.1)	0 (0.0)
South Asian	2 (0.6)	2 (0.7)	0 (0.0)
West Asian	38 (11.5)	34 (11.8)	4 (9.3)
Latin American	8 (2.4)	7 (2.4)	1 (2.3)
White	256 (77.6)	218 (76.0)	38 (88.4)
Other*	2 (0.6)	2 (0.7)	0 (0.0)
1+ underlying comorbidities	69 (20.9)	60 (20.9)	9 (20.9)
Symptom burden			
Asymptomatic	61 (18.4)	57 (19.7)	4 (9.3)
Mild (no hospitalization)	261 (78.6)	222 (76.8)	39 (90.7)
Moderate (hospitalized, no ICU)	7 (2.1)	7 (2.4)	0 (0.0)
Severe/critical (ICU)	3 (0.9)	3 (1.0)	0 (0.0)
Symptoms			
Fever (≥38) or chills	145 (44.8)	131 (46.5)	14 (33.3)
Sore throat	132 (41.4)	110 (39.3)	22 (56.4)
Runny nose as the only symptom	20 (6.1)	15 (5.2)	5 (11.9)
Cough/SOB	169 (51.4)	142 (49.7)	27 (62.8)
Vomiting or diarrhea	73 (22.3)	65 (22.8)	8 (18.6)
Nausea	55 (16.9)	44 (15.4)	11 (26.8)
Headache	172 (53.6)	156 (55.1)	16 (42.1)
Rash	16 (4.9)	15 (5.3)	1 (2.3)
Conjunctivitis	13 (3.9)	12 (4.2)	1 (2.3)
Muscle aches	125 (38.6)	110 (38.7)	15 (37.5)
Joint aches	89 (27.5)	78 (27.5)	11 (27.5)
Loss of appetite	101 (30.8)	90 (31.6)	11 (25.6)
Loss of smell or taste	115 (35.7)	108 (38.3)	7 (17.5)

*Nonspecified selection of more than 1 category.

COVID indicates coronavirus disease; ICU, intensive care unit; IQR, interquartile range; SOB, shortness-of-breath.

(OR = 0.4; 95% CI: 0.2–0.9). There was no demonstrable association between nonseroconversion and the presence of cough/shortness-of-breath (OR = 2.1; 95% CI: 0.8–5.7), rhinorrhea when it was the only symptom (OR = 3.1; 95% CI: 0.6–15.2) and the presence of ≥3 symptoms (OR = 4.5; 95% CI: 0.9–23.9).

DISCUSSION

In this study, approximately 1 of 8 individuals with COVID-19 did not seroconvert. Children, particularly the youngest, were approximately half as likely to seroconvert compared with adults. Apart from the absence of fever/chills, individual symptoms did not strongly predict nonseroconversion. Although young children and adults have been found to have similar respiratory SARS-CoV-2 viral loads, children's failure to seroconvert could be due to robust mucosal immunity or lower expression of angiotensin converting enzyme-2-receptors in the nasal epithelium.[9,10] In a recent study by Toh et al,[6] 61% of children with RT-PCR–confirmed SARS-CoV-2 infection did not seroconvert. This proportion is significantly higher than we observed, possibly due to their younger cohort [median age 4 years (IQR 2–10) vs. 9 years (IQR 6–13)], as we observed decreased odds of seroconversion with decreasing age. In this study, lower viral load was associated with nonseroconversion; it is possible that a greater proportion of children in this cohort had a low viral load leading to the high rate of nonseroconversion.

Our study has limitations. Because of noncentralized RT-PCR testing in our region, we could not obtain Ct values as a proxy for viral load. Highly sensitive RT-PCR testing has been associated with nonseroconversion.[4] We did not standardize time of antibody testing in relation to confirmed infection date. However, seroconversion did not differ between those tested ≤30 and >30 days from infection. Some symptoms (eg, loss of taste or smell) may be difficult to discern in infants and young children.

Our findings support emerging evidence of uneven antibody responses to SARS-CoV-2 infection, especially in children. Therefore, seroprevalence studies in children should be interpreted with caution, as they may underestimate prior infection. It is not known whether previously infected individuals who do not seroconvert are protected from subsequent infection; theoretically, memory B-cells and T-cells may be contributing to protection even in the absence of circulating antibodies. Nevertheless, natural infection should not diminish the recommendation for widespread vaccination as we continue to learn about the longevity of natural antibody protection, antibody neutralization and the interplay between natural and vaccine immunity. Public health messaging should inform populations that seroconversion, and possibly immunity, cannot be assumed after a symptomatic infection.

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