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Nasopharyngeal Viral Load Is the Major Driver of Incident Antibody Immune Response to SARS-CoV-2 Infection

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Background. Virologic determinants of seroconversion to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection were defined in a post hoc analysis of prospectively studied vaccine- and infection-naïve individuals at high risk for coronavirus disease 2019 (COVID-19).

Methods. This phase 3 COVID-19 prevention trial (NCT04452318) with casirivimab and imdevimab was conducted in July 2020–February 2021, before widespread vaccine availability. Placebo-treated participants who were uninfected (SARS-CoV-2 quantitative reverse transcription polymerase chain reaction [RT-qPCR] negative) and seronegative were assessed weekly for 28 days (efficacy assessment period [EAP]) for COVID-19 symptoms and SARS-CoV-2 infection by RT-qPCR of nasopharyngeal swab samples and for serostatus by antinucleocapsid immunoglobulin (Ig) G. Regression-based modeling, including causal mediation analysis, estimated the effects of viral load on seroconversion.

Results. Of 157/1069 (14.7%) uninfected and seronegative (for antispike IgG, antispike IgA, and antinucleocapsid IgG) participants who became infected during the EAP, 105 (65%) seroconverted. The mean (SD) maximum viral load of seroconverters was 7.23 (1.68) $\log_{10}$ copies/mL vs 4.8 (2.2) $\log_{10}$ copies/mL in those who remained seronegative; viral loads of ~6.0 $\log_{10}$ copies/mL better predicted seroconversion. The mean of the maximum viral load was 7.11 $\log_{10}$ copies/mL in symptomatic participants vs 5.58 $\log_{10}$ copies/mL in asymptomatic participants. The mean duration of detectable viral load was longer in seroconverted vs seronegative participants: 3.24 vs 1.63 weeks.

Conclusions. Maximum SARS-CoV-2 viral load is a major driver of seroconversion and symptomatic COVID-19, with high viral loads (~6.0 $\log_{10}$ copies/mL) better predicting seroconversion. Serology underestimates infection rates, incidence, and prevalence of SARS-CoV-2 infection.

Keywords. antibody immune responses; causal mediation analysis; COVID-19; neutralizing monoclonal antibodies; SARS-CoV-2.

As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants emerge despite widespread vaccination, an improved understanding of the natural history of protective immune responses to SARS-CoV-2 may inform future prevention strategies. Estimates of the prevalence, durability, and function of antibody immune responses to SARS-CoV-2 have been previously reported [1–6]; however, the virologic determinants of incident antibody immune responses to SARS-CoV-2 are not well described. Cross-sectional studies have demonstrated that seroconversion rates are higher with symptomatic infection but lower with asymptomatic infection, including asymptomatic infection in those who were vaccinated [7, 8], demonstrating that the development of antibody immune responses is not universal with SARS-CoV-2 infection. Quantitation of the nasopharyngeal (NP) viral load that elicits antibody immune responses during infection may inform our understanding of SARS-CoV-2 host immunity and the relative length of time of potential infectiousness/ability to transmit virus while asymptomatic.

This is a post hoc exploratory analysis of a randomized, placebo-controlled phase 3 coronavirus disease 2019 (COVID-19) prevention trial (NCT04452318) with casirivimab and imdevimab, which was initiated in July 2020 during the onset of the COVID-19 pandemic, and enrollment was completed in February 2021, before widespread vaccine availability [9]. Here, we describe the results from placebo-treated participants who had never been infected or vaccinated (naïve) and were at high risk for infection due to a close contact exposure to COVID-19, who were assessed for COVID-19 symptoms,

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SARS-CoV-2 infection, and serostatus over 28 days. Seroconversion was expected within 28 days in association with infection due to household exposure [10–14].

METHODS

Study Population

The results of the phase 3 COVID-19 prevention trial (NCT04452318) have been previously described [9]. The study was conducted between July 2020 and February 2021, before the emergence of the Omicron variants. Study participants were randomized (1:1) to receive a single administration of subcutaneous casirivimab and imdevimab 1200 mg or placebo. The trial consisted of a 1-day screening/baseline period, a 28-day efficacy assessment period (EAP), and a 7-month follow-up period. During the EAP, participants underwent active surveillance with weekly NP swab sampling for SARS-CoV-2 by quantitative reverse transcription polymerase chain reaction (RT-qPCR) and were interviewed weekly for the development of symptoms related to COVID-19.

Serology Assays

Serologic testing was performed at a central laboratory (ICON Central Laboratories, Farmingdale, NY, USA). Antispike (anti-S) IgA (EUROIMMUN, Lübeck, Germany), anti-S IgG (EUROIMMUN, Lübeck, Germany), and anti-nucleocapsid (anti-N) IgG (Abbott, Abbott Park, IL, USA) were tested at baseline, as previously described [15]. A participant was categorized as seronegative at baseline if all available serologic tests were negative and as seropositive if any serologic test was positive. Participants were classified as sero-unknown if data were missing or if they were tested outside of the EAP. At baseline, anti-SARS-CoV-2 serology testing was performed for anti-S IgG, anti-S IgA, and anti-N IgG. Serologic testing for the occurrence of SARS-CoV-2 infection and seroconversion postbaseline was assessed by detection of anti-N IgG antibodies in blood collected at days 29, 57, 85, 113, 141, 169, 197, and 225 (end of study), with a ±3-day window allowed. Anti-N IgG, not anti-S IgA or IgG, was employed in the COV-2069 study postbaseline to monitor seroconversion, as anti-N IgG discriminates between infection and vaccination.

Virologic Assays and Assessments

NP swabs were collected for SARS-CoV-2 RT-qPCR at baseline, before study drug administration, weekly during the EAP, and weekly during follow-up if positive during the EAP (until they tested negative twice, where the limit of detection was 299 copies/mL). NP swabs were used to determine viral load at a central laboratory (Viracor Eurofins Clinical Diagnostics, Lenexa, KS, USA), as previously described [9]. Viral load was described as the maximum detected (or measured). Viral load, measured in $\log_{10}$ copies/mL vs cycle threshold value, is shown in Supplementary Table 1.

All participants in the study underwent NP testing for SARS-CoV-2 RT-qPCR at baseline and weekly throughout the EAP. For individuals who tested positive during the EAP, weekly swabs were collected.

Clinical Assessments

Data on the type and severity of signs and symptoms of COVID-19 were collected by investigator-led interviews weekly during the EAP, or more often as needed, as well as weekly until symptoms resolved. Duration of symptoms was defined as the time from the first day of any COVID-19 symptom to the last day of any COVID-19-associated symptom.

Statistical Analyses

All statistical analyses were conducted using R Statistical Software (version 4.1.2; Vienna, Austria). The *cutpointr* package (version 1.1.2) was used to account for the optimal cutoff viral load for seroconversion. Association analyses and optimal cutoffs are described in the Supplementary Methods. For causal mediation analysis, we extended an analytical framework [16] with a continuous exposure and 2 different (binary and continuous) types of mediators (Supplementary Figure 1; Supplementary Methods).

Patient Consent

This trial was conducted in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonization Good Clinical Practice guidelines, and all applicable regulatory requirements. The central or local institutional review board or ethics committee at each study center oversaw trial conduct and documentation. All participants provided written informed consent before participating in the trial.

RESULTS

Summary of Incident Infection and Seroconversion

Of the 1069 uninfected, placebo-treated participants who were seronegative at baseline (naïve), 157 (14.7%) became infected during the 28-day EAP, as evidenced by a positive RT-qPCR test result and/or anti-N seropositivity (Supplementary Figure 2). Of the 157 patients infected, 102 (65.0%) seroconverted by day 32. Overall, 144 out of the 157 (91.7%) who were infected had a positive RT-qPCR test result, and only 13 participants who seroconverted did not have evidence of a positive RT-qPCR test result, indicating that weekly NP sampling for RT-qPCR captured the majority of infections.

Of the 144 participants with a positive SARS-CoV-2 RT-qPCR test result during the EAP, 89 (61.8%) seroconverted, compared with 43 (29.9%) who remained seronegative (Supplementary Table 2). Baseline characteristics and demographics by seroconversion by day 32 are shown in Table 1.

Of the 144 participants with a positive RT-qPCR test result during the EAP, 106 (73.6%) seroconverted by day 60

Table 1. Baseline Characteristics and Demographics

Variable	Overall (N = 144)	Seroconversion by EAP		
		Yes (n = 89)	No (n = 43)	Unknown (n = 12)
Age, mean (SD), y	43.9 (15.3)	45.2 (14.7)	42.0 (16.0)	41.2 (17.6)
≥50 y, No. (%)	56.0 (38.9)	39.0 (43.8)	13.0 (30.2)	4.0 (33.3)
Sex, No. (%)				
Female	80.0 (55.6)	53.0 (59.6)	21.0 (48.8)	6.0 (50.0)
Male	64.0 (44.4)	36.0 (40.4)	22.0 (51.2)	6.0 (50.0)
Race, No. (%)				
Asian	3.0 (2.1)	0.0 (0.0)	1.0 (2.3)	2.0 (16.7)
Black or African American	13.0 (9.0)	6.0 (6.7)	7.0 (16.3)	0.0 (0.0)
Native Hawaiian or other Pacific Islander	1.0 (0.7)	1.0 (1.1)	0.0 (0.0)	0.0 (0.0)
White	121.0 (84.0)	81.0 (91.0)	33.0 (76.7)	7.0 (58.3)
Other	6.0 (4.2)	1.0 (1.1)	2.0 (4.7)	3.0 (25.0)
Ethnicity, No. (%)				
Hispanic or Latino	50.0 (34.7)	26.0 (29.2)	21.0 (48.8)	3.0 (25.0)
Not Hispanic or Latino	93.0 (64.6)	63.0 (70.8)	22.0 (51.2)	8.0 (66.7)
Not reported	1.0 (0.7)	0.0 (0.0)	0.0 (0.0)	1.0 (8.3)
Baseline weight, mean (SD), kg	83.8 (20.3)	84.0 (20.8)	81.4 (19.4)	90.1 (19.5)
Missing data	1.0	0.0	1.0	0.0
Baseline BMI, mean (SD), kg/m^2	29.3 (6.3)	29.5 (6.7)	28.4 (5.7)	30.7 (5.8)
Missing data	1.0	0.0	1.0	0.0
Any high-risk factor, No. (%)	51.0 (35.4)	33.0 (37.1)	11.0 (25.6)	7.0 (58.3)
Age ≥65 y	11.0 (7.6)	7.0 (7.9)	4.0 (9.3)	0.0 (0.0)
BMI ≥35 kg/m^2	28.0 (19.4)	20.0 (22.5)	5.0 (11.6)	3.0 (25.0)
Chronic kidney disease	4.0 (2.8)	1.0 (1.1)	3.0 (7.0)	0.0 (0.0)
Diabetes	12.0 (8.3)	6.0 (6.7)	3.0 (7.0)	3.0 (25.0)
Immunosuppressive treatment	2.0 (1.4)	1.0 (1.1)	0.0 (0.0)	1.0 (8.3)
Age ≥55 y with CVD, hypertension, or COPD	18.0 (12.5)	13.0 (14.6)	2.0 (4.7)	3.0 (25.0)

Seroconversion was assessed by the detection of anti-N IgG antibodies.

Abbreviations: anti-N, antinucleocapsid; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; EAP, efficacy assessment period; Ig, immunoglobulin.

Table 2. Virologic and Clinical Characteristics During the EAP by Seroconversion Status

Variable	Seroconversion by EAP		
	Yes (n = 89)	No (n = 43)	Unknown (n = 12)
Maximum viral load, $\log_{10}$ copies/mL			
Mean (SD)	7.2 (1.7)	4.8 (2.2)	6.2 (2.3)
Median (Q1, Q3)	7.6 (6.7, 8.5)	3.9 (3.0, 6.8)	6.4 (4.1, 8.2)
Symptomatic, No. (%)			
Yes	58 (65)	15 (35)	5 (42)
No	31 (35)	28 (65)	7 (58)
Duration of RT-qPCR positivity, wk			
Mean (SD)	3.2 (1.9)	1.6 (1.1)	2.3 (1.7)
Median (Q1, Q3)	3 (2, 4)	1 (1, 2)	2 (1, 2.5)

Seroconversion was assessed by the detection of anti-N IgG antibodies. Missingness is not reported in this table.

Abbreviations: anti-N, antinucleocapsid; EAP, efficacy assessment period; Ig, immunoglobulin; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

(Supplementary Table 2). An additional 17 participants seroconverted after the EAP. Baseline characteristics and demographics by seroconversion by day 60 are shown in Supplementary Table 3.

Of the 106 seropositive participants by day 60, 38 remained seropositive and 64 were seronegative by the end of the study (Supplementary Table 4). The mean (SD) maximum viral load was 6.93 (1.68) $\log_{10}$ copies/mL in those who were seropositive by the end of the study compared with 7.18 (1.60) $\log_{10}$ copies/mL in those who were seropositive by day 60.

A higher mean maximum viral load was associated with symptomatic disease or a longer duration of viral load

Table 3. Results of the Logistic Regression of Seroconversion on Symptomatic Infection, Duration of RT-qPCR+, and Maximum Viral Load Separately During the EAP

	Logistic Regression								
	Seroconversion								
Dependent Variables	Log Odds	SE	*P*	Log Odds	SE	*P*	Log Odds	SE	*P*
Intercept	−1.00	1.80	5.807e−01	0.12	2.02	9.527e−01	−3.29	2.19	1.332e−01
Maximum viral load, copies/mL	…	…	…	…	…	…	0.63***	0.14	4.176e−06
Duration of RT-qPCR+ (log_2), wk	…	…	…	1.65***	0.36	5.121e−06	…	…	
Symptomatic infection	1.48**	0.52	4.275e−03	…	…		…	…	
Age, y	0.01	0.02	5.973e−01	0.02	0.02	4.652e−01	0.03	0.02	2.039e−01
Sex: male	−1.13	0.67	9.259e−02	−1.72*	0.79	3.068e−02	−1.68*	0.78	3.136e−02
Race: Asian	−37.13	6679.29	9.956e−01	−35.71	6648.61	9.957e−01	−34.23	6679.97	9.959e−01
Race: Black or African American	−1.39	0.73	5.626e−02	−0.47	0.96	6.260e−01	−0.24	0.91	7.913e−01
Race: Native Hawaiian or other Pacific Islander	17.69	6522.64	9.978e−01	19.88	6522.64	9.976e−01	21.00	6522.64	9.974e−01
Race: other	−1.23	1.35	3.634e−01	−0.47	1.42	7.393e−01	−1.08	1.37	4.294e−01
Ethnic: Hispanic or Latino	−0.33	0.50	5.109e−01	−0.79	0.56	1.585e−01	−0.21	0.55	6.964e−01
Baseline weight, kg	0.05	0.04	1.414e−01	0.08	0.04	5.082e−02	0.07	0.04	8.495e−02
Baseline BMI, kg/m^2	−0.11	0.12	3.753e−01	−0.26	0.14	7.481e−02	−0.21	0.14	1.360e−01
RF: age ≥65 y	−3.45*	1.69	4.056e−02	−3.67*	1.74	3.481e−02	−4.37*	1.90	2.137e−02
RF: BMI ≥35 kg/m^2	0.46	1.10	6.781e−01	1.12	1.18	3.425e−01	1.16	1.19	3.291e−01
RF: chronic kidney disease	−19.35	1438.09	9.893e−01	−19.75	1288.10	9.878e−01	−19.16	1441.26	9.894e−01
RF: diabetes	1.74	1.27	1.708e−01	2.36	1.30	6.953e−02	2.04	1.44	1.562e−01
RF: immunosuppressive treatment	18.34	6522.64	9.978e−01	15.72	6522.64	9.981e−01	17.08	6522.64	9.979e−01
RF: age ≥55 y with CVD, hypertension, or COPD	20.13	1438.09	9.888e−01	20.89	1288.10	9.871e−01	19.90	1441.26	9.890e−01
Observation	131	131	130	…	…	…	…	…	…
R^2	0.303	0.458	0.453	…	…	…	…	…	…

Seroconversion was assessed by the detection of anti-N IgG antibodies.

Abbreviations: anti-N, antinucleocapsid; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; EAP, efficacy assessment period; Ig, immunoglobulin; RF, risk factor; RT-qPCR+, quantitative reverse transcription polymerase chain reaction positivity; SE, standard error.

*$P < .05$; **$P < .01$; ***$P < .001$.

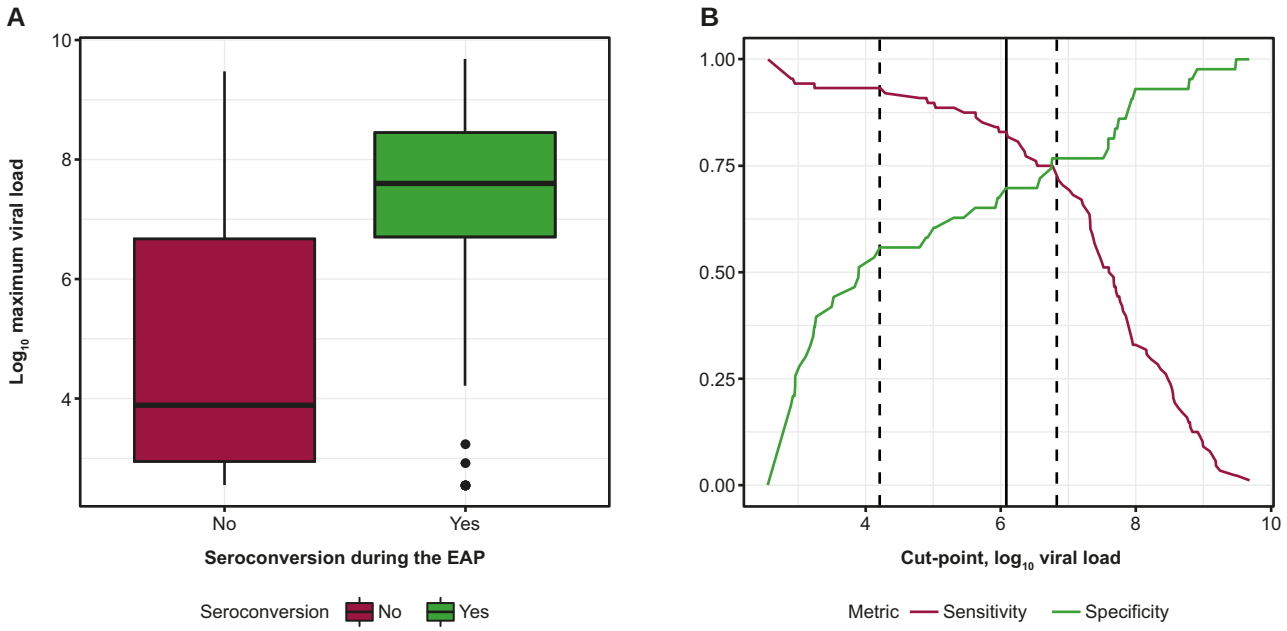

Figure 1. *A*, Threshold SARS-CoV-2 viral load required to predict seroconversion in the EAP. *B*, Sensitivity and specificity plot (Youden's index). Sensitivity: the proportion of seroconverted individuals being classified as seroconverted based on viral load threshold; specificity: the proportion of nonseroconverted individuals being classified as nonseroconverted based on viral load threshold. Seroconversion was assessed by the detection of anti-N IgG antibodies. Abbreviations: anti-N, antinucleocapsid; EAP, efficacy assessment period; Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(Tables 2 and 3; Supplementary Figure 3). Participants with symptomatic disease had a higher maximum viral load vs asymptomatic patients (Supplementary Table 5). Moreover, an increase in viral load was associated with increased odds of symptomatic disease (Supplementary Table 6)

Association of Symptomatic Disease and Seroconversion

Of the 157 participants who became infected, defined as a positive RT-qPCR result and/or anti-N seropositivity during the 28-day EAP, 78 developed symptomatic infection and 79 had asymptomatic infection. A total of 58/78 (74%) symptomatic patients seroconverted by day 32 (Fisher's test $P = .0014$). Of the 79 participants with asymptomatic infection, 44 (56%) seroconverted by day 32. A total of 58/89 (65%) seroconverted patients had symptomatic COVID-19, while 15/43 (35%) of those who did not seroconvert had symptomatic COVID-19 (Table 2). For symptomatic patients compared with asymptomatic patients, the odds ratio of seroconversion was 4.39 (log odds = 1.48; $P = .0013$) when controlled for covariates (Table 3). Of participants who were symptomatic (n = 78), duration of symptoms did not appear to be a significant indication of seroconversion (Supplementary Table 7). In nonseroconverted patients, the median (range) duration of symptoms was 2.57 (1.64–3.93) weeks compared with 2.86 (1.43–3.82) weeks in seroconverted patients (Supplementary Table 8). A total of 69/78 (88.5%) participants who had symptomatic laboratory-confirmed PCR+ infection during the EAP became seropositive by day 60. Further data on seroconversion and symptomatic disease at day 60 are summarized in Supplementary Table 9.

Association of Viral Load and Seroconversion

A higher viral load was associated with seroconversion (Table 2). The mean (SD) of the maximum viral load in those who seroconverted by day 32 was 7.23 (1.68) $\log_{10}$ copies/mL, compared with 4.8 (2.2) $\log_{10}$ copies/mL in those who remained seronegative and 6.2 (2.3) $\log_{10}$ copies/mL in those with unknown seroconversion status. With every $\log_{10}$ increase in the mean maximum viral load, the odds of seroconversion increased by a factor of 1.88 (log odds = 0.63; $P < .0001$) when controlled for covariates (Table 3).

Based on Youden's index, a mean viral load of 6.08 $\log_{10}$ copies/mL (95% CI, 4.21–6.83) accurately predicted seroconversion at day 32 (Figure 1). Youden's index was maximized at 6.08 $\log_{10}$ copies/mL, with a sensitivity of 85% and a specificity of 67%. Based on the cutoff viral load, the maximum viral load in the low–viral load group was 3.76 $\log_{10}$ copies/mL, and the maximum viral load in the high–viral load group was 7.84 $\log_{10}$ copies/mL. The proportion of patients who were symptomatic in the low–viral load group was 31% compared with 67% in the high–viral load group (Supplementary Table 10).

In addition, longer duration of detectable viral load indicated a higher chance of seroconversion postinfection. The mean (SD) duration of detectable viral load in seroconverted patients was 3.24 (1.85) weeks compared with 1.63 (1.09) weeks for

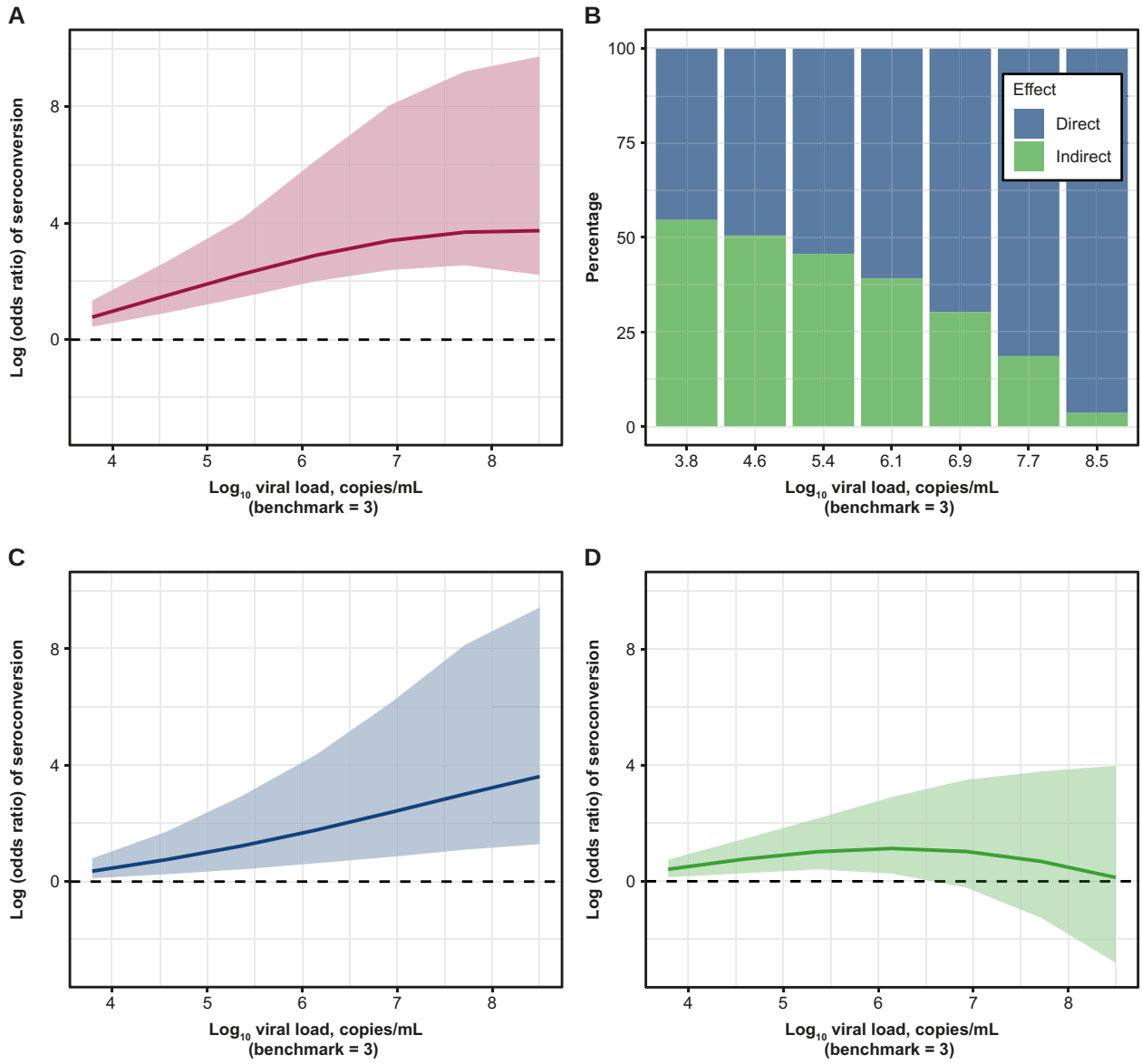

Figure 2. Results of causal mediation analysis on (*A*) total effect, (*B*) proportion of indirect vs direct effects, (*C*) direct effects, and (*D*) indirect effects of SARS-CoV-2 viral load on seroconversion. Seroconversion was assessed by the detection of anti-N IgG antibodies. Abbreviations: anti-N, antinucleocapsid; Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

patients who remained seronegative (Table 2). With every $\log_2$ week increase in the duration of detectable viral load, the odds of seroconversion increased by a factor of 5.21 (log odds = 1.65; $P < .0001$) when controlled for covariates (Table 3). Data on seroconversion, viral load, and duration of detectable viral load at day 60 are summarized in Supplementary Table 9.

Causal Mediation Analysis

We performed a causal mediation analysis to analyze the direct and indirect effects of viral load on seroconversion mediated by duration of infection and/or symptomatic infection (Supplementary Figure 1). When viral load increased from 3.8 to 8.5 $\log_{10}$ copies/mL, compared with 3 $\log_{10}$ copies/mL as a benchmark, the total effect log odds ratio of an antibody response significantly increased from 0.76 (95% CI, 0.42–1.33; $P < .001$) to 3.73 (95% CI, 2.21–9.73; $P < .001$) (Figure 2*A*). The direct effect dominated at higher viral loads; the direct effect log odds ratio of an antibody response increased from 0.34 (95% CI, 0.10–0.78; $P = .008$) to 3.60 (95% CI, 1.27–9.43; $P = .012$) (Figure 2*B* and *C*), and the contribution ranged from 45.4% to 96.5%. Therefore, with a higher viral load, there was more impact of the direct effect of viral load on seroconversion. With the indirect effect (the pathway from viral load to antibody response via duration of RT-qPCR

positivity and/or presence of symptoms), there was a nonlinear influence on seroconversion as viral load increased (Figure 2*D*); the indirect effect log odds ratio increased from 0.41 (95% CI, 0.14–0.75; $P = .02$) to 1.13 (95% CI, 0.27–2.90; $P = .004$) when the viral load was lower than ~6 $\log_{10}$ copies/mL, but it decreased to 0.13 (95% CI, −2.80 to 3.98; $P = .904$) the when viral load was greater than ~6 $\log_{10}$ copies/mL.

Adopting a cutoff value of 6.08 $\log_{10}$ copies/mL, we dichotomized the continuous viral load into binary exposure, that is, high (≥6.08 $\log_{10}$ copies/mL) and low (<6.08 $\log_{10}$ copies/mL) viral load. The total effect log odds ratio of seroconversion when comparing high and low viral load was 2.93 (95% CI, 1.68–7.59; $P < .001$). The direct effect log odds ratio was 2.23 (95% CI, 0.65–6.66; $P = .012$), the proportion of which was 76.3%, whereas the indirect effect log odds ratio was 0.70 (95% CI, −0.77 to 2.84; $P = .33$), which contributed to 23.7% of the total effect. High viral load was therefore the major driver of seroconversion.

DISCUSSION

Despite widespread vaccination, serious SARS-CoV-2 infections remain a societal burden [17], and adults aged ≥65 years and persons with multiple underlying medical conditions remain at increased risk of severe COVID-19 illness and death [18]. Development of antibody responses in seronegative individuals following infection with SARS-CoV-2 is not universal, with variations in the type, potency, and duration of individuals' immune responses, which may vary depending on disease severity and clinical presentation [13, 19, 20]. Therefore, new information about the virological and clinical characteristics of SARS-CoV-2 that determine natural immune responses, notably seroconversion and seroprevalence, may inform public health strategies.

This is the first analysis to prospectively assess the determinants of antibody immune responses during primary SARS-CoV-2 infection and before vaccination, with weekly RT-qPCR NP testing and symptom collection after a high-risk exposure. Other studies have prospectively assessed for symptomatic infection and seroconversion but have not assessed asymptomatic infection by frequent RT-qPCR NP testing [21], or have cross-sectionally examined serostatus in relation to COVID-19 symptomatology and severity [13, 22–28]. Previous studies have shown that a high viral load, symptomatic infection, and a longer duration of infection are associated with seroconversion [29–34], but the primary driver of seroconversion has not been clearly demonstrated. Causal mediation analysis allows for an understanding of whether, and to what degree, the effect of an exposure (in this case, viral load on an outcome of seroconversion) involves changing mediators such as symptomatic infection and duration of infection.

The findings of this analysis in participants who were uninfected and seronegative at baseline and who were closely monitored for infection after a close contact exposure to COVID-19 conclusively demonstrate that maximum NP viral load, rather than duration of infection or presence of symptomatic infection, is a major driver of seroconversion within 32 days of COVID-19 infection, with high viral loads >~6.0 $\log_{10}$ copies/mL being most associated with seroconversion, consistent with other reports [21]. Rates of seroconversion vary across the published literature, likely due to differences in the study design, populations studied, and time points when seroconversion was tested. The seroconversion rate of 65% at day 32, which included both symptomatic and asymptomatic infected participants, was lower than rates reported in other studies; however, other studies determined seroconversion rates at later time points after infection. In Dobano et al., seropositivity rates of 80.3%–86.6% were tested at 5–9 months after symptomatic infection [23]. In Follman et al., placebo-treated participants in the mRNA 1273 vaccine trial had seroconversion rates of 93% when tested ~2 months after infection [21]. These higher rates are consistent with seroconversion rates in our study, where participants with symptomatic infection had seroconversion rates at day 60 of 88.5% (69/78). The Youden metric with bootstrapping sampling further demonstrated that individuals with a low maximum viral load (which was associated with asymptomatic disease) were less likely to mount antibody immune responses. These data are consistent with the overall findings in the COV-2069 clinical trial, where participants treated with the potent antiviral monoclonal antibodies casirivimab and imdevimab were significantly less likely than placebo-treated participants to seroconvert (as measured by anti-N IgG) [35]. Our data also confirmed that serology underestimates infection rates, incidence, and prevalence of SARS-CoV-2 infection.

Of participants who seroconverted by day 60, a more durable antibody immune response was observed in a proportion of participants who remained seropositive by the end of the study (Supplementary Table 4). Interestingly, participants who were seropositive at the end of the study had a lower mean maximum viral load cutoff than those who seroconverted during the EAP, which increased the odds of remaining seropositive for longer. While we did not explore this further, it may reflect a stronger immune response during the acute infection period in these participants with better virologic control, and thus a higher chance of staying seropositive for longer. These virologic data are consistent with previously described clinical findings that early immune responses, as evidenced by the presence of antibodies during symptomatic infection, are associated with milder disease [36, 37].

The limitations of the study include weekly, rather than more frequent, testing of NP swab viral load by RT-qPCR; the peak maximum viral load may therefore have been missed during this time. It has previously been shown that SARS-CoV-2 viral shedding can range between 1 day and >7 days [38]. While 91.7%

of participants in the current study who became seropositive also had a positive RT-qPCR test result, indicating that weekly NP swab sampling for RT-qPCR captured the majority of infections, daily viral load assessments may have identified infected study participants with a shorter duration of viral RNA shedding and without seroconversion. During this study, only anti-N IgG was tested due to the study design, as the treatment group included casirivimab and imdevimab, which interfere with anti-S IgG assays. The sensitivity of anti-N IgG and anti-S IgG serological assays in detecting antibody responses against SARS-CoV-2 has been shown to be comparable. However, while seroconversion occurs within 3 weeks after onset of COVID-19 symptoms with anti-N IgG and anti-S IgG assays, seroconversion on average occurs 2 days earlier for assays detecting anti-N IgG than anti-S IgG [39]. Notably, anti-S IgG titers stabilize and persist over time while anti-N IgG titers decline sharply 7–9 months post–COVID-19 infection [40]. Although this raises the potential for underestimating seroprevalence, anti-N IgG assays are often preferred, as anti-N IgG response is elicited only by natural infection, unlike the anti-S IgG response, which is elicited by natural infection and vaccination.

In terms of the causal mediation analysis that was performed, viral load, duration of infection, and presence/absence of symptomatic infection were included as mediators; however, the analysis did not assume unobserved mediators. The study was conducted in the United States, Romania, and Moldova in 2020–2021, before the widespread use of vaccines and before the emergence of the Omicron (B.1.1.529) and Omicron-lineage variants; immune responsiveness in the current study therefore represents a first exposure to SARS-CoV-2, and the results may not extrapolate to those patients with prior exposure to the virus or prior vaccination. Finally, while 1069 participants were included in this analysis, only 89 had both a positive RT-qPCR test and seroconversion at day 32, which may have impacted the results.

In conclusion, an analysis of prospectively collected NP viral load, serology, and COVID-19 symptoms data in a large, prospective clinical trial of seronegative naïve study participants at high risk of SARS-CoV-2 infection demonstrated that maximum viral load is the major determinant of seroconversion and that low–viral load infections may not stimulate antibody immune responses. Although these findings relate to primary SARS-CoV-2 infections in seronegative individuals and may not apply to those who have already had a previous infection or been vaccinated, these data may inform public health considerations, as SARS-CoV-2 infections with low viral loads may not lead to protective immunity, and serology underestimates infection rates, incidence, and prevalence of SARS-CoV-2 infection.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Data availability. Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing once the indication has been approved by a regulatory body, if there is legal authority to share the data and there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/.

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