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MAJOR ARTICLE

Association Between Cycle Threshold Value and Vaccination Status Among Severe Acute Respiratory Syndrome Coronavirus 2 Omicron Variant Cases in Ontario, Canada, in December 2021

Saranyah Ravindran,[1] Jonathan B. Gubbay,[2,3] Kirby Cronin,[2] Ashleigh Sullivan,[2] Austin Zygmunt,[1,3,4] Karen Johnson,[1] Sarah A. Buchan,[1,5,6,7,a] and Alyssa S. Parpia[1,7,a]

[1]Health Protection, Public Health Ontario, Toronto, Ontario, Canada, [2]Public Health Ontario Laboratory, Public Health Ontario, Toronto, Ontario, Canada, [3]Department of Laboratory Medicine and Pathobiology and Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada, [4]School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada, [5]Centre for Vaccine Preventable Diseases, University of Toronto, Toronto, Ontario, Canada, [6]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada, and [7]Institute of Health Policy, Management, and Evaluation, University of Toronto, Toronto, Ontario, Canada

Background. Increased immune evasion by emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants and occurrence of breakthrough infections raise questions about whether coronavirus disease 2019 vaccination status affects SARS-CoV-2 viral load among those infected. This study examined the relationship between cycle threshold (Ct) value, which is inversely associated with viral load, and vaccination status at the onset of the Omicron wave onset in Ontario, Canada.

Methods. Using linked provincial databases, we compared median Ct values across vaccination status among polymerase chain reaction–confirmed Omicron variant SARS-CoV-2 cases (sublineages B.1.1.529, BA.1, and BA.1.1) between 6 and 30 December 2021. Cases were presumed to be Omicron based on S-gene target failure. We estimated the relationship between vaccination status and Ct values using multiple linear regression, adjusting for age group, sex, and symptom status.

Results. Of the 27 029 presumed Omicron cases in Ontario, the majority were in individuals who had received a complete vaccine series (87.7%), followed by unvaccinated individuals (8.1%), and those who had received a booster dose (4.2%). The median Ct value for post–booster dose individuals (18.3 [interquartile range, 15.4–22.3]) was significantly higher than that for unvaccinated (17.9 [15.2–21.6]; $P = .02$) and post–vaccine series individuals (17.8 [15.3–21.5]; $P = .005$). Post–booster dose cases remained associated with a significantly higher median Ct value than cases in unvaccinated individuals ($P \leq .001$), after adjustment for covariates. Compared with values in persons aged 18–29 years, Ct values were significantly lower among most age groups >50 years.

Conclusions. While slightly lower Ct values were observed among unvaccinated individuals infected with Omicron compared with post–booster dose cases, further research is required to determine whether a significant difference in secondary transmission exists between these groups.

Keywords. COVID-19; COVID-19 testing; SARS-CoV-2; public health; vaccination.

Breakthrough severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections among those vaccinated have occurred, especially alongside the emergence of new variants [1–4] including Omicron. Real-time reverse-transcription polymerase chain reaction (PCR) testing, which is used to detect SARS-CoV-2 infection, generates cycle threshold (Ct) values. Ct values represent the number of amplification cycles required to detect viral RNA but do not provide a quantitative assessment of the amount of virus in a sample. Samples with higher relative viral RNA concentration will have lower Ct values with fewer amplification cycles needed to detect the viral RNA, compared with samples with lower viral RNA concentrations (ie, high Ct values). Ct values can be associated with factors such as the PCR test used, time of sample collection relative to infection, and symptom status [5, 6], as well as individual characteristics, such as age [5, 7]. Studies have shown that a high SARS-CoV-2 viral load (ie, low Ct value) may be associated with increased transmission probability [8–11] and higher risk of severe disease [12]. With the emergence of new variants of concern (VOC) [1–4], SARS-CoV-2 infections among those vaccinated against coronavirus disease 2019 (COVID-19) have been occurring to a greater degree, highlighting the need to

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Correspondence: Sarah A. Buchan, Public Health Ontario, 661 University Ave, Floor 17, Toronto, ON M5G 1M1, Canada (sarah.buchan@oahpp.ca); Alyssa S. Parpia, Public Health Ontario, 480 University Ave, Toronto, ON M5G 1V2, Canada (alyssa.parpia@oahpp.ca).

[a]S. A. B. and A. S. P. contributed equally to this work.

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understand the association between viral load and COVID-19 vaccination status.

In earlier waves of the pandemic, specimens from SARS-CoV-2–positive individuals who were vaccinated had higher Ct values (ie, lower relative viral RNA concentration) than unvaccinated individuals [13, 14] suggesting that they may be less infectious [15]. During the Delta variant wave, the relationship between vaccination status and Ct values for those positive for SARS-CoV-2 became less clear [16, 17] with similar Ct values observed regardless of vaccination status. While studies by Woodbridge et al [18] and Hirotsu et al [3] found similar viral loads between Omicron-infected individuals with 3 vaccine doses and those who were unvaccinated, compared with the Delta-dominant period, a smaller body of literature that has examined the relationship between vaccination status and Ct values for Omicron-infected individuals [3, 18]. There is high 2-dose vaccination coverage in Ontario adults (approximately 87.0% at the time of this study) [19], but booster dose coverage among adults was low because rollout of the booster dose program had just begun during our study period, and bivalent vaccines were not yet available [20].

Because Omicron is the current dominant variant in Canada [21] and other jurisdictions at the time of writing [22], it is necessary to examine the relationship between vaccination status and Ct values of Omicron cases [3, 23] to help understand transmission potential [10]. At the population level, low Ct values could provide an early indication of transmission-related epidemic trends [24] and may be used for monitoring severity. In the current study, our objective was to examine the association between COVID-19 vaccination status and Ct values of Omicron cases in adults from 6 to 30 December 2021 in Ontario, Canada.

METHODS

Data Sources

Individuals with confirmed SARS-CoV-2 infections were identified using provincial reportable disease data from the Public Health Case and Contact Management Solution (CCM). Vaccination status and dose administration date(s) were identified through the centralized provincial COVID-19 vaccine information system (COVaxON). Laboratory data extracted from the Public Health Ontario (PHO) Laboratory Information Management System (LIMS) were used to identify SARS-CoV-2 specimens determined to be the Omicron variant based on S-gene target failure (SGTF) detection, a reliable proxy for Omicron B.1.1.529 lineage identification [25, 26]. We linked data from CCM to COVaxON using a combination of health card number, name, date of birth, sex, and postal code. Subsequently, LIMS data were linked using health card number if available or first name, last name, and date of birth if the number was not available.

Study Population

Our study included PCR-confirmed SARS-CoV-2 cases extracted from CCM on 24 January 2022 that were aged ≥18 years with SGTF detection in Ontario. We included cases wherein a real-time reverse-transcription PCR test was conducted using the Thermo Fisher TaqPath assay and SGTF was detected for samples collected between 6 and 30 December 2021. During this period, all individuals in Ontario were eligible for SARS-CoV-2 testing; after 30 December 2021, testing was restricted to higher-risk individuals [27].

TaqPathCOVID-19 PCR is the assay used to test the majority of SARS-CoV-2 samples at the PHO Laboratory, the provincial reference laboratory that is one of several completing COVID-19 PCR testing. All samples were tested for *ORF1ab* and N genes using TaqPath. During this period, all samples at PHO that were positive for SARS-CoV-2 and had a Ct value ≤35 for either the *ORF1ab* or the N gene were included in universal screening for SGTF, a reliable proxy (98.9% sensitivity, 99.9% specificity, 99.5% positive predictive value, and 99.7% negative predictive value) [25] for distinguishing the Omicron variant and select sublineages (B.1.1.529, BA.1 and BA.1.1) from cocirculating Delta variant (B.1.617.2) cases. In this study, Omicron variant samples refer primarily to sublineages B.1.1.529, BA.1 and BA.1.1, because very few other sublineages (eg, BA.2) were observed during our study period. For cases with multiple positive samples, the sample with the earliest collection date between 6 and 30 December 2021 was included. If the sample collection date was missing, the date the sample was received at PHO was used when aligning sample and case information.

For this alignment, we excluded cases (3.9%) with >2 days between the earliest positive sample collection date from CCM and the earliest sample date from LIMS. We also excluded samples for cases in which sex was not specified, a non–Health Canada authorized vaccine(s) was used for any dose, onset occurred after initial vaccination but without completion of the primary series, a second booster dose was received (which was not yet offered in Ontario during this period), or SGTF results were unknown or inconclusive (owing to low viral load).

COVID-19 Vaccination Status

Cases were classified by vaccination status through linkage to COVaxON data extracted on 24 January 2022; this included classification based on timing of their most recent vaccine dose relative to symptom onset. Cases were grouped into the following vaccination status categories: unvaccinated, post–vaccine series (ie, cases with a symptom onset date ≥14 days after receipt of the first dose of a 1-dose series or the second of a 2-dose series vaccine or <14 days after receipt of a booster dose following the primary series) and post–booster dose (ie, cases with a symptom onset date ≥14 days after receipt of a booster

dose). Cases in which a vaccine event was not documented in COVaxON at the time of linkage and those considered not yet protected from vaccination (symptom onset <14 days after a first vaccine dose) were classified as unvaccinated.

Ct Values

SGTF cases with specimens tested by TaqPath were identified using laboratory data extracted on 26 January 2022. The TaqPath assay targets the N, *ORF1ab*, and S genes of SARS-CoV-2. We defined SGTF as the failure to detect (ie, with Ct <37) the S-gene target while detecting both the *ORF1ab* and N-gene targets, with ≥1 detected target having a Ct ≤30. The Ct value for the *ORF1ab* gene was used for all Ct analyses as there was no significant difference in Ct values between the 2 genes, with more results available for the *ORF1ab* gene. Of the samples that met our inclusion criteria, 94.6% of samples had an *ORF1ab* gene Ct value.

Covariates

Information obtained from CCM included age group (in decades), sex, symptom status, severity (ie, outcome of hospitalization or death), and whether cases were linked to a confirmed outbreak and risk factors such as being a long-term care home resident or a healthcare worker. Individuals with unknown age were excluded from age-specific analyses. Symptomatic cases were defined as those with a reported symptom onset date irrespective of the symptom. Asymptomatic cases were defined as those in which the asymptomatic field in CCM was reported as "yes" and no symptom information was reported. Owing to changes in case follow-up guidance given the surge of cases during the Omicron wave, symptom information may have been incomplete or not completed for cases reported after 18 December 2021. Time since vaccination was calculated as the difference in days from sample collection date and dose administration date and categorized into 3 time periods (≤3, >3 and ≤6, or >6 months).

Statistical Analysis

We calculated the frequencies of baseline characteristics as well as median Ct and interquartile ranges (IQRs) stratified by vaccination status. Differences in median Ct values were assessed using the nonparametric Mann-Whitney *U* test for pairwise comparisons. A multiple linear regression model was fitted to estimate the relationship of vaccination status on Ct values after adjustment for age group, sex, and symptom status. Long-term care home resident risk factor was not included in the model because these residents made up a very small percentage (<1.0%) of cases in the sample. Time since vaccination was also not included in the model as the covariate would not be applicable to unvaccinated cases in the model. In addition, we further stratified and compared Ct values by vaccination status, age group, time since vaccination, as and both vaccination and symptom status. A Kruskal-Wallis test was performed to test for differences in Ct value by time since vaccination among post–vaccine series cases. A Mann-Whitney *U* test was subsequently performed for pairwise comparisons across time since vaccination categories, with false discovery rate (FDR) adjustment applied to pairwise results to account for multiple comparisons. All analyses were conducted using SAS software, version 9.4 (SAS Institute).

Ethics

This project did not require research ethics committee approval as the activities described herein were conducted in fulfillment of PHO's legislated mandate and are therefore considered public health practice, not research.

RESULTS

Overall, 1832 individuals (1832 of 28 861 [6.3%]) were excluded from our study due to having both N-gene and *ORF1ab* Ct values >30 after application of other exclusion criteria. We identified 27 029 SARS-CoV-2 cases demonstrating SGTF (ie, presumed Omicron variant) in testing completed at PHO Laboratory with a sample collection date between 6 and 30 December 2021 that met our inclusion criteria for analysis. The majority of cases were post–vaccine series (87.7%), followed by cases in unvaccinated (8.1%) and post–booster dose (4.2%) individuals. Among samples from individuals who were vaccinated (after series completion or after booster dose), 91.3% (22 669 of 24 835) of individuals received a messenger RNA vaccine for all applicable doses, 7.2% (1783 of 24 835) received a mix of messenger RNA and viral vector–based vaccines, and 1.5% (383 of 24 835) received viral vector–based vaccine doses for their primary series (ie, no viral vector–based vaccine doses were administered as a booster among this sample). Women accounted for 52.1% of cases, and 38.1% of the study sample was 18–29 years old, although the distribution of ages varied by vaccination status. While 18–29-year-olds represented the highest proportions of unvaccinated (46.2%) and post–vaccine series (37.9%) cases, ≥ 80-year-olds reported the highest proportion of post–booster dose cases (18.7%) (Table 1). Most cases had symptom information missing (57.5%), and the rest reported symptoms (37.0%) or were asymptomatic (5.5%). Among symptomatic cases (n = 10 005), the median time from symptom onset to sample collection date was 2 days (IQR, 1–3 days). Among those with recorded symptom onset dates, samples were collected a median (IQR) of 1.0 (0.0–3.0), 2.0 (1.0–3.0), and 2.0 (1.00–4.0) days after symptom onset for those who were post–booster dose, post–vaccine series, or unvaccinated, respectively. The median times from symptom onset to sample collection were found to be similar across vaccination status groups, and we therefore do not expect impacts to our results.

Table 1. Key Demographic Variables in S-Gene Target Failure–Detected Severe Acute Respiratory Syndrome Coronavirus 2 Cases With Samples Collected 6–30 December 2021 in Ontario, Canada, Stratified by Vaccination Status

Variable	SARS-CoV-2 Cases by Vaccination Status, No. (%)		
	Unvaccinated (n = 2194)	After Complete Vaccine Series (n = 23 691)	After Booster Dose (n = 1144)
Sex			
Female	1095 (49.9)	12 286 (51.9)	694 (60.7)
Male	1099 (50.1)	11 405 (48.1)	450 (39.3)
Age group, y[a]			
18–29	1014 (46.2)	8974 (37.9)	132 (11.5)
30–39	562 (25.6)	4970 (21.0)	140 (12.2)
40–49	299 (13.6)	4181 (17.6)	141 (12.3)
50–59	191 (8.7)	3404 (14.4)	182 (15.9)
60–69	79 (3.6)	1553 (6.6)	190 (16.6)
70–79	22 (1.0)	398 (1.7)	145 (12.7)
≥80	26 (1.2)	209 (0.9)	214 (18.7)
Outbreak related	162 (7.4)	1106 (4.7)	314 (27.4)
Long-term care home resident	25 (1.1)	75 (0.3)	149 (13.0)
Healthcare worker	33 (1.5)	269 (1.1)	41 (3.6)
Disease severity			
Ever hospitalized	10 (0.5)	36 (0.2)	12 (1.0)
Death	3 (0.1)	8 (<0.1)	12 (1.0)
Vaccine type			
mRNA doses only	NA	21 659 (91.4)	1010 (88.3)
Viral vector doses only	NA	383 (1.6)	0 (0.0)
Viral vector primary series + mRNA booster	NA	NA	88 (7.7)
Mixed primary series	NA	1649 (7.0)	NA
Mixed primary series + mRNA booster	NA	NA	46 (4.0)
Time since last vaccination, mo			
≤3	NA	1156 (4.9)	961 (84.0)
>3 and ≤6	NA	13 567 (57.3)	183 (16.0)
>6	NA	8968 (37.9)	0 (0.0)
Symptom status			
Asymptomatic	148 (6.7)	1242 (5.2)	90 (7.9)
Symptomatic	700 (31.9)	8907 (37.6)	398 (34.8)
Missing symptom information	1346 (61.3)	13 542 (57.2)	656 (57.3)

Abbreviations: mRNA, messenger RNA; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Numbers may not add up to totals because of missing age data in some cases.

During the short duration of our study, hospitalization occurred in 0.2% of all cases and death in 0.1%.

Overall, the median Ct value for post–booster dose cases (18.3 [IQR, 15.4–22.3]) was significantly higher than that for unvaccinated (17.9 [15.2–21.6]; $P = .02$) and post–vaccine series (17.8 [15.3–21.5]; $P = .005$) cases (Figure 1). After adjustment for age group, sex, and symptom status (Table 2), cases classified as post–booster dose were associated with a significantly higher Ct value (lower viral load) than those in unvaccinated individuals (difference 0.64; $P \leq .001$). After adjustment, there was no significant difference in Ct values between cases classified as post–booster dose versus post–vaccine series (difference, 0.10; $P = .32$). In the adjusted model, lower Ct values were significantly associated with increasing age groups, symptomatic status ($P = .001$), and male sex ($P = .001$) (Table 2).

We further stratified Ct values by time since vaccination within each vaccination status (Figure 2) and found that among post–vaccine series cases, there was a significant difference in Ct values across time since vaccination ($P = .04$), but this difference was no longer observed after false discover rate adjustment for pairwise Mann-Whitney *U* test results. For post–vaccine series cases, the median Ct value was highest (18.3 [IQR, 5.6–21.7]) in those who received their last vaccination ≤3 months before their infection (Figure 2 and Supplementary Table 1). For post–booster dose cases, the median Ct value was highest in those who received their last vaccine 3–6 months before their infection (18.5 [IQR, 14.6–22.6]). Among post–vaccine series cases, higher median Ct values were reported across all time since vaccination categories for those aged 18–49 years, compared with the 50–79-year and ≥80-year age groups (Supplementary Table 1); however, this trend did not hold for post–booster dose cases. In addition, when comparing Ct values by symptom status (asymptomatic and symptomatic) within each vaccination status (Supplementary Figure 1), we found that Ct values were lower among symptomatic than among asymptomatic cases across all 3 vaccination status categories.

DISCUSSION

In the current study, we examined Ct values of SARS-CoV-2–positive specimens with SGTF by vaccination status and time since last vaccination at the start of the Omicron wave in Ontario in December 2021. After adjusting for age group, sex, and symptom status, we observed that median Ct values among cases were low (ie, <20) regardless of the number of vaccine doses received (ie, vaccination status) or time since the last dose. While we found that those who received a booster dose had slightly higher median Ct values than unvaccinated individuals (Ct value difference, 0.5), caution should be heeded when interpreting Ct value data in light of the numerous factors that play a role in infectiousness at the pathogen and host level, as well as the impact of laboratory-specific standards and individuals' health-seeking behavior [18]. Furthermore, while it is unclear to what degree the observed differences in Ct values are clinically significant or have broader impact on the population level, some work has identified low correlation between RNA genome copies and infectious viral titers [11]. Ct values were similar for individuals classified as unvaccinated or

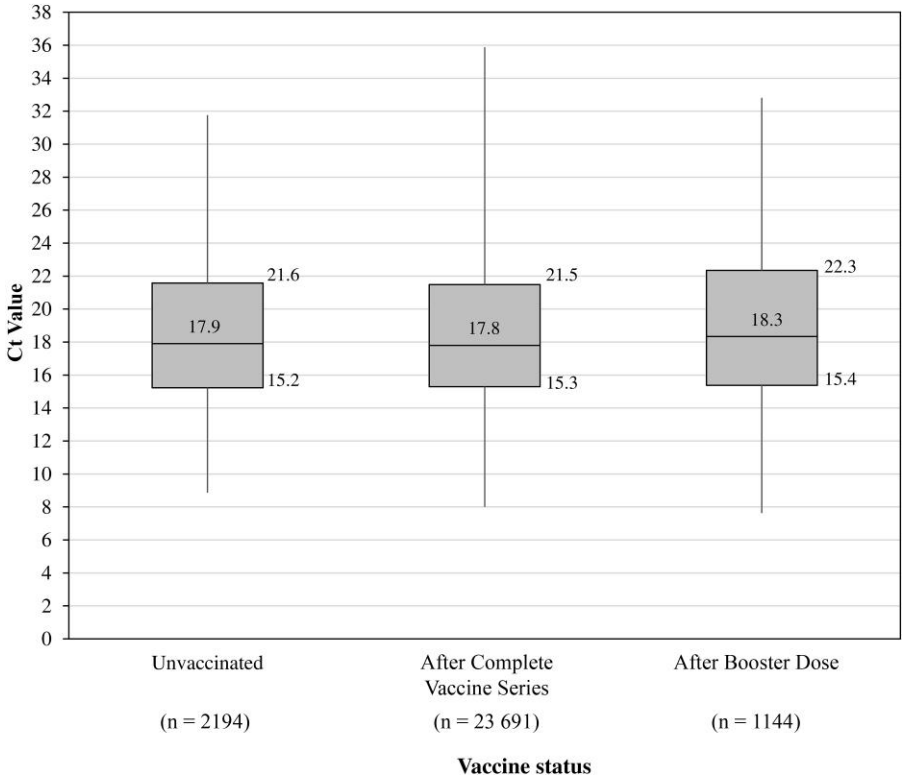

Figure 1. Box plots of median cycle threshold (Ct) values (with interquartile range) for S-gene target failure–detected cases of severe acute respiratory syndrome coronavirus 2, from samples collected 6–30 December 2021 in Ontario, Canada, stratified by vaccination status.

Table 2. Parameter Estimates for Variables Included in the Model of Cycle Threshold Values in S-Gene Target Failure–Detected SARS-CoV-2 Cases With Samples Collected from December 6-30, 2021 in Ontario, Canada

Variable	Adjusted Estimate (Standard Error)[a]	*P* Value
Vaccination status		
Unvaccinated	Reference	…
After complete vaccine series	0.10 (0.10)	.32
After booster dose	0.64 (0.17)	<.001
Age group, y		
18–29	Reference	…
30–39	0.07 (0.07)	.37
40–49	−0.07 (0.08)	.36
50–59	−0.17 (0.09)	.048
60–69	−0.29 (0.12)	.01
70–79	0.37 (0.20)	.06
≥80	−0.59 (0.23)	.01
Sex		
Female	Reference	…
Male	−0.32 (0.06)	<.001
Symptom status		
Asymptomatic	Reference	…
Symptomatic	−0.88 (0.13)	<.001
Missing symptom information	−0.35 (0.12)	.005

[a]Estimates were adjusted for age group, sex, and symptom status.

post–vaccine series, indicating similar viral load concentrations. Throughout the COVID-19 pandemic, studies have found that lower Ct values are associated with higher viral loads [28] and risk of SARS-CoV-2 transmission [29, 30]. Further research is required to determine whether SARS-CoV-2–infected individuals who have received a booster dose are less likely to transmit to others than unvaccinated individuals with SARS-CoV-2 infection.

Consistent with other studies, we found that lower Ct values were associated with older age [31, 32]. This may be due to slower viral clearance in older individuals with SARS-CoV-2 infection [32] or due to presence of comorbid conditions that affect the clinical course of disease [12]. In addition, among those hospitalized for COVID-19, higher rates of severe disease and mortality have been observed among those with lower Ct values [12, 33]. Booster doses in older populations have been shown to reduce clinical severity [34]; however, further investigation may be needed to examine the relationship between viral load, vaccination status, and clinical outcomes among older age groups.

While differences in Ct values were observed by vaccination status early in the pandemic, in the Delta wave Ct values among unvaccinated and those that were vaccinated with 2 doses (eg, post–vaccine series) were similar [35]. Our study found similar

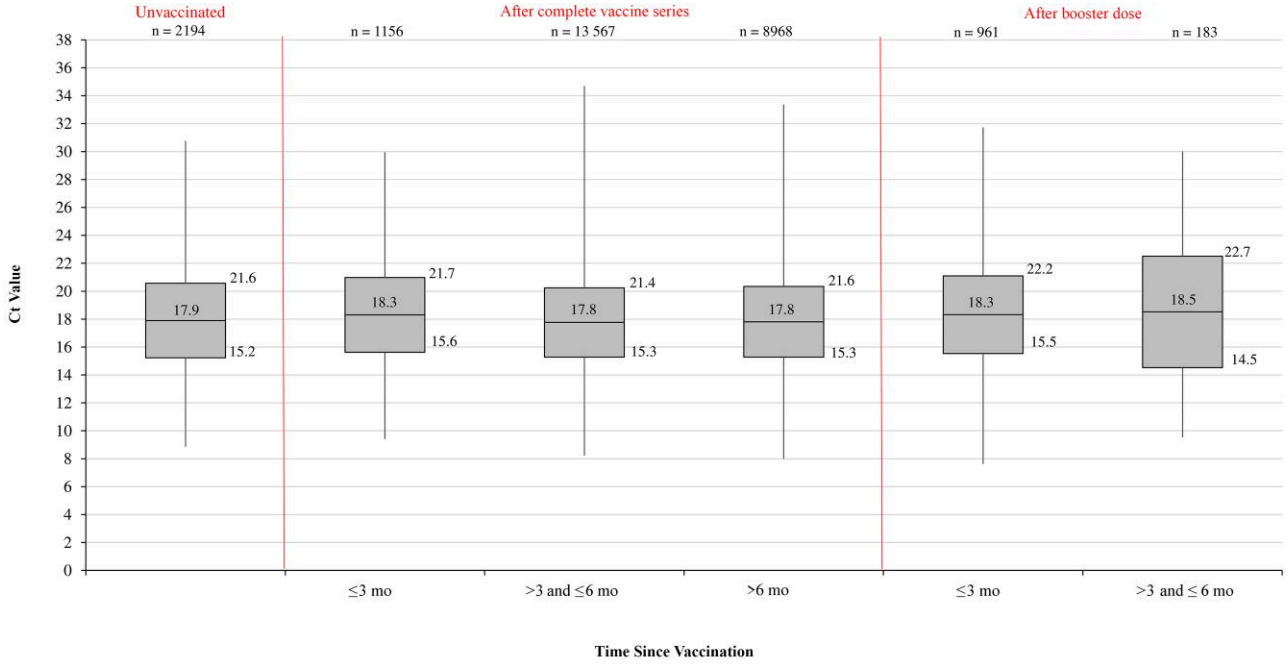

Figure 2. Box plots of median of cycle threshold (Ct) values (with interquartile range) for S-gene target failure–detected cases of severe acute respiratory syndrome coronavirus 2, from samples collected between 6–30 December 2021 in Ontario, Canada, stratified by vaccination status and time since vaccination. Given the timing of the booster dose program rollout, there were no reported cases during the study period with a vaccination status of post–booster dose and >6 months since the last vaccination, because those eligible for a booster dose could not have received that dose >6 months earlier. Cases in unvaccinated individuals were not further stratified because time since vaccination was not applicable to that status.

median Ct values between unvaccinated and post–vaccine series individuals. This finding may be due to an inability of ancestral vaccines to prevent infection or reduce viral load from an infection with a new VOC such as Omicron [36–38]. During the Delta variant period in Israel, booster dose effectiveness in protecting against infection and reducing viral loads declined within months of vaccination, similar to the waning observed after second doses, suggesting that a similar trend could occur among post–booster dose Omicron cases [39]. In Ontario, while no significant protection against symptomatic Omicron infection was observed after a second dose, vaccine effectiveness was 61% more than a week after receiving a first booster dose [40]. While our study found that time since vaccination did not result in lower median Ct values among post–booster dose cases, this could be attributable to the Ontario booster dose program beginning during our study period. During our study period, a limited number of individuals (16.0%) had received their booster dose >3 months earlier, so our findings may not accurately reflect the true relationship between median Ct value and time since booster dose receipt. Given suboptimal coverage of bivalent boosters [41], our results may still be applicable to a large proportion of the population who have yet to receive a bivalent booster dose.

Our study had certain limitations. Our SGTF definition requires an *ORF1ab* or N-gene Ct ≤ 30. In addition, previously positive SARS-CoV-2 samples referred for SGTF testing must have a Ct value ≤ 35. Our exclusion of high-Ct samples therefore biases our results toward lower Ct values, which may have influenced our comparison of median Ct values across age or vaccination status strata. Given our study time frame, we used SGTF as a proxy for Omicron variant cases, yet this method was found to be highly accurate [26]. While BA.2 is S-gene target positive, the prevalence of this variant was low during our study period, so we expect that its exclusion from our analysis is unlikely to bias findings. A study by Qassim et al [32] in Qatar found that, among those with a prior infection, Ct values were 1.30 (95% confidence interval, 1.20–1.39) cycles higher than in those without prior infection. We were unable to account for prior SARS-CoV-2 infection owing to a lack of data, which may have biased results toward higher Ct values.

The age of eligibility for COVID-19 vaccines during our study may have affected the characteristics of individuals in each of the vaccination groups (ie, older adults in the post–booster dose category). Post–booster dose cases were not necessarily representative of those eligible, as our analysis was conducted early in the provincial booster dose program: eligibility was restricted to specific high-risk groups in mid-September 2021 and expanded to individuals aged ≥50 years on 13 December 2021 [20]. Thus, individuals who received a booster dose during our study may be different from

the general population for reasons such as their health status (eg, immunocompromised) or health-seeking behavior. Owing to difficulties in identifying immunocompromised individuals in our data, it was not possible to account for a 3-dose primary series in our analysis, and some of these individuals may have been included in the post–booster dose category. Furthermore, because data on immunocompromised status were not available, we were unable to adjust for this status in our investigation of Ct values with respect to vaccination status.

Our study also had some strengths. Ct values are influenced by numerous factors, and we were able to account for significant ones including the time of sample collection relative to infection, PCR test used, symptom status [5, 6] and age [5, 7]. Furthermore, we assessed trends in time since vaccination via a descriptive summary. While eligibility for PCR testing in Ontario was severely restricted on 31 December 2021 [42], this restriction occurred after our study period, rendering our findings representative of Ontario.

Despite not providing direct estimates of transmission likelihood, population-level relative Ct values can be indicative of broad trends in outbreak expansion [24], disease severity [43, 44], or waning vaccine effectiveness. As an early warning indicator, low population-level Ct values could provide insight about changes to COVID-19 epidemiology [24]. Our results from the beginning of the Omicron wave in Ontario found low population-level Ct values (<20) regardless of age group, sex, vaccination status, and time from vaccination and further support findings from the beginning of other VOC waves [16, 17]. Population-level Ct values could act as a marker for new wave or VOC emergence and trigger enhanced laboratory surveillance and public health communication (eg, promoting booster dose uptake).

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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