Long-term persistence of SARS-CoV-2 neutralizing antibody responses after infection and estimates of the duration of protection

Eric HY Lau,a David SC Hui,b Owen TY Tsang,b Wai-Hung Chan,a Mike YW Kwan,a Susan S Chiu,b Samuel MS Cheng,a Ronald LW Ko,d John KC Li,a Sara Chaoothai,a Chi H Tsang,a Leo LM Poon,1,9 and Malik Peiris,a,g*

aSchool of Public Health, The University of Hong Kong, Special Administrative Region of Hong Kong, China
bDepartment of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, China
cInfectious Diseases Centre, Princess Margaret Hospital, Hospital Authority of Hong Kong, Special Administrative Region of Hong Kong, China
dDepartment of Paediatrics, Queen Elizabeth Hospital, Hospital Authority of Hong Kong, Special Administrative Region of Hong Kong, China
eDepartment of Paediatric and Adolescent Medicine, Princess Margaret Hospital, Hospital Authority of Hong Kong, Special Administrative Region of Hong Kong, China
fDepartment of Paediatric and Adolescent Medicine, The University of Hong Kong and Queen Mary Hospital, Hospital Authority of Hong Kong, Special Administrative Region of Hong Kong, China
gHKU-Pasteur Research Pole, The University of Hong Kong, Special Administrative Region of Hong Kong, China

Abstract

Background The duration of immunity in SARS-CoV-2 infected people remains unclear. Neutralizing antibody responses are the best available correlate of protection against re-infection. Recent studies estimated that the correlate of 50% protection from re-infection was 20% of the mean convalescent neutralizing antibody titre.

Methods We collected sera from a cohort of 124 individuals with RT-PCR confirmed SARS-CoV-2 infections from Prince of Wales Hospital, Princess Margaret Hospital, Queen Elizabeth Hospital and Queen Mary Hospitals of the Hospital Authority of Hong Kong, for periods up to 386 days after symptom onset and tested these for antibody to SARS-CoV-2 using 50% virus plaque reduction neutralization tests (PRNT50), surrogate neutralization tests and spike receptor binding domain (RBD) binding antibody. Patients were recruited from 21 January 2020 to 16 February 2021 and follow-up samples were collected until 9th March 2021.

Findings Because the rate of antibody waning slows with time, we fitted lines of decay to 115 sera from 62 patients collected beyond 90 days after symptom onset and estimate that PRNT50 antibody will remain detectable for around 1,717 days after symptom onset and that levels conferring 50% protection will be maintained for around 990 days post-symptom onset, in symptomatic patients. This would potentially be affected by emerging virus variants. PRNT titres wane faster in children. There was a high level of correlation between PRNT50 antibody titers and the % of inhibition in surrogate virus neutralization tests.

Interpretation The data suggest that symptomatic COVID-19 disease is followed by relatively long-lived protection from re-infection by antigenically similar viruses.

Funding Health and Medical Research Fund, Commissioned research on Novel Coronavirus Disease (COVID-19) (Reference Nos. COVID190126 and COVID1903003) from the Food and Health Bureau and the Theme-based Research Scheme project no. T11–712/19-N, the University Grants Committee of the Hong Kong SAR Government.

Copyright © 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Keywords: COVID-19; SARS-CoV-2; Coronavirus; Neutralizing antibody; Kinetics; Protection; Immunity, duration

Introduction Duration of immunity to virus infections can vary, ranging from lifelong immunity with measles to transient protection to seasonal coronaviruses.1,2 Antibody
Neutralizing antibody responses wane over time. Some of the early reports of the kinetics of pseudotype neutralization antibody responses reported very rapid antibody waning suggesting that 1/3rd of patients had lost detectable virus pseudotype neutralizing antibody by 1–2 months of illness. Antibody waning is more rapid in the first 2-3 months after infection and slower waning thereafter. This change in rate of antibody waning is because the first phase of antibody production is driven by short lived plasma cells but the longer-term antibody responses are maintained by antigen-specific long-lived plasma cells in the bone marrow. More recent reports have suggested that neutralizing antibody is likely to be more long lasted but most of them have been based on the first 4–6 months of convalescence, which does not allow the implications of the slower waning phase of the antibody response to be assessed. Longer term kinetics of neutralizing antibody titres beyond 5 months of convalescence is needed. More data from children is also needed.

We have a cohort of RT-PCR confirmed adults and children that is being longitudinally followed up during convalescence. We previously reported on the neutralizing antibody kinetics of this cohort over the early convalescent phase up to day 209 post onset of symptoms and we concluded that 50% plaque reduction neutralizing antibody titres were likely to remain detectable for at least 1 year in mild and severely ill patients although durability of antibody was likely to be shorter in asymptomatic infections. But this study did not capture sufficient data to assess the slower waning phase of the antibody response. We have now followed up this cohort for a longer period of time, up to day 386 post symptom onset with 119 additional sera. Focussing on individuals from whom we have multiple sera and on the period 90 to 386 days post symptom onset, we now demonstrate that neutralizing antibody is likely to persist for much longer than original estimates. We use recent data suggesting that a 50% protection from infection is conferred by a neutralizing antibody titre that is approximately 1/3rd of the mean of convalescent titres after natural infection to estimate the duration of protection from re-infection in our cohort.

Methods
A cohort of 124 individuals with symptomatic or asymptomatic RT-PCR confirmed SARS-CoV-2 infections were followed up at the Prince of Wales Hospital, Princess Margaret Hospital, Queen Elizabeth Hospital and Queen Mary Hospitals of the Hospital Authority of Hong Kong, for periods up to 386 days after onset of symptoms or initial RT-PCR confirmation of infection. Patients were recruited from 21 January 2020 to 16 February 2021 and follow-up samples were collected until 9th March 2021. Clinical management was based on the standard of care as recommended by the Central
Plaque reduction neutralization test (PRNT): Vero E6 cells (ATCC CRL-1586) were maintained in Dulbecco’s Modified Eagle Medium (DMEM) medium supplemented with 10% fetal bovine serum (FBS) and 100 U/mL of penicillin-streptomycin. The assay was performed in duplicate using 24-well tissue culture plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland) in a biosafety level 3 facility. Serial dilutions of each serum sample was incubated with 30–40 plaque-forming units of the virus isolate BetaCoV/Hong Kong/VM20001061/2020 for 1 h at 37°C. The virus–serum mixtures were added onto pre-formed Vero E6 cell monolayers and incubated for 1 h at 37°C in a 5% CO2 incubator. The cell monolayer was then overlaid with 1% agarose in cell culture medium and incubated for 3 days, at which time the plates were fixed and stained. Antibody titres were defined as the highest serum dilution that resulted in ≥ 90% (PRNT90) or ≥ 50% (PRNT50) reduction in the number of virus plaques. This method has been extensively validated on SARS-CoV-2 infected and control sera previously.18,19

Surrogate neutralization test: SARS-CoV-2 surrogate virus neutralization test (sVNT) kits were obtained from GeneScript USA, Inc, New Jersey and the tests carried out according to the manufacturer’s instructions. The test sera (10 µL) positive and negative controls were diluted at 1:10 and mixed with an equal volume of horseradish peroxidase (HRP) conjugated to SARS-CoV-2 spike receptor binding domain (RBD) (6 ng) and incubated for 30 min at 37°C. 100 µL of each mix was added to each well on the microtiter plate coated with ACE-2 receptor. The plate was sealed and incubated at room temperature for 15 min at 37°C. Plates were then washed with wash-solution, tapped dry, and 100 µL of 3,3',5,5'-Tetramethylbenzidine (TMB) solution was added to each well and incubated in the dark at room temperature for 15 min. Reaction was stopped by addition of 50 µL of Stop Solution to each well and the absorbance read at 450 nm in an ELISA microplate reader. The assay validity was based on optical density (OD)450 values for positive and negative with falling within recommended values. The estimated values and variance-covariance matrix of the parameters, with 1000 resamples. Based on the fitted line, we extrapolated to the time when PRNT titers reach 1:10, ELISA reaches 0.5 and sVNT inhibition reaches 50%, the time when PRNT titers reach 50% correlates of protection (CoP) and half-life (T1/2) (a drop of 50%). We calculated the 50% CoP as 20% geometric mean PRNT90 and PRNT50 antibody titers from 30 to 60 days after symptom onset of the mild and severe COVID-19 cases. The decay in the antibody response is not statistically significant, only the lower 95% confidence bound will be provided as more than 2.5% of the fitted lines from the bootstrap resamples would not reach these thresholds. Antibody titers which were censored at ≥ 1:320 to antibody titers from 1:320 to 1:160, and applied the fitted distribution to redistribute the samples with antibody titers ≥ 1:320 to antibody titers from 1:320 to 1:2560. The fitted line for PRNT50 and PRNT90, 50% CoP and 95% CI were calculated based on 1000 randomly imputed samples.
We calculated the spearman correlation between PRNT\textsubscript{50} and PRNT\textsubscript{90} titers, ELISA OD values versus sVNT inhibition (%) when paired data were available.

Ethics statement: Written informed consent was obtained from the participants or their parents (when the participant was a child) and the studies were approved by the institutional review boards of the respective hospitals, viz. Kowloon West Cluster (KW/EX-20–039 (144–27)), Kowloon Central / Kowloon East cluster (KC/KE-20–0154/ER2) and HKU/HA Hong Kong West Cluster (UW 20–273).

Role of the funding source: The funding sources had no role in study design, data collection, data analysis and interpretation, writing of the manuscript or decision to submit the manuscript for publication. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
A cohort of 124 patients from whom more than one serial serum sample was available were studied and the demographic characteristics of the patients shown in Supplementary Table 1. All sera were collected prior to any of these individuals receiving any COVID-19 vaccine. Patients with severe illness were those requiring over 3 L of supplemental oxygen per minute while mild illness were those not oxygen dependent or required ≤ 3 L of supplemental oxygen per minute, but were still symptomatic. Asymptomatic infections were those who did not manifest symptoms throughout the course of infection.

The number of sera tested by PRNT\textsubscript{50}, PRNT\textsubscript{90}, and spike RBD ELISA from these 124 patients were 329, 329 and 334, respectively. Two hundred and fifty-one cases tested by spike RBD ELISA from these 124 patients were 329, 334, respectively. Two hundred and fifty-one cases tested by PRNT\textsubscript{50}, PRNT\textsubscript{90} and ELISA (with effective degree of freedom < 2), but could be less reliable for sVNT (Supplementary Figure 1). Assuming a linear trend of decay since day 90, we predicted that it takes on average 1717 (95% CI lower bound: 951, decline not statistically significant) days for the PRNT\textsubscript{50} titers of symptomatic patients to drop to 1:10; 1574 (95% CI lower bound: 877, decline not statistically significant) and 2709 (95% CI lower bound: 1328, decline not statistically significant) days for mild and severe patient groups, respectively. Since the slope of decline for PRNT\textsubscript{90} titers was not statistically significant for symptomatic patients it is not certain that antibody titres were materially reducing beyond day 90 and the estimates of remaining seropositive (at titers ≥1:10) may be under-estimates. Similarly, it takes 731 days (95% CI: 486–2157) days for PRNT\textsubscript{90} titers to drop to 1:10 for symptomatic patients; 665 (95% CI: 418–2248) and 1053 (95% CI lower bound: 526, decline not statistically significant) days for mild and severe patients respectively.

Recent studies have estimated that the correlate of 50% protection from re-infection was 20% of the convalescent neutralizing antibody titre.\textsuperscript{10} From the PRNT\textsubscript{50} and PRNT\textsubscript{90} antibody titres in symptomatic COVID-19 cases between 30 and 60 days after illness onset in this study, (163 samples from 74 cases), we estimated the geometric mean antibody titres (GMT) and then, estimated 20% of the GMT, which represents the 50% correlate of protection (Supplementary Figure 2). The threshold for 50% protection from re-infection for PRNT\textsubscript{50} and PRNT\textsubscript{90} were 1:25.9 (95% CI: 1:24.7–1:27.6) and 1:8.9 (95% CI: 1:8.6–1:9.4) respectively. It was estimated that PRNT\textsubscript{50} will drop to this threshold 990 (95% CI lower bound 441, decline not statistically significant) days after symptom onset in symptomatic patients. The comparable estimate for PRNT\textsubscript{90} was 701 (95% CI: 405–2442) days after symptom onset.

It is estimated that sVNT inhibition will drop to the threshold of detection in 377 days (95% CI: 463–769) days for symptomatic patients; 530 (95% CI: 432–715) days in mild and 1098 (95% CI lower bound: 655), decline not statistically significant) days for more severely ill patients, respectively. RBD ELISA optical density is estimated to drop to the cut off of 0.5 in 529 days (95% CI: 457–648) for symptomatic patients; 490 (95% CI: 421–589) in mild infections and 1068 (95% CI lower bound: 677, decline not statistically significant) days in severe infection.

Only 9 asymptomatic infections had available sera beyond 90 days of illness onset and it was not possible to have a conclusion of their antibody kinetics.

The half-life (T\textsubscript{1/2}) (a drop of 50%) of levels of antibody waning was on average, 469 days for mild and severe patients respectively.
Fig. 1. Antibody responses in COVID-19 cases by days after illness onset and severity, Hong Kong. Sera tested were as follows: 329 samples from 124 cases tested for 90% plaque reduction neutralization test (PRNT90) (A) and PRNT50 titres (B), 334 samples from 124 cases tested for receptor binding domain binding antibody by ELISA optical density 450 nm OD450 (C) and 251 sample from 99 cases tested for % inhibition in surrogate virus neutralization (sVNT) (D). Small random noises were added to the PRNT90 and PRNT50 titers for better presentation. The fitted lines were based on 105 samples from 53 symptomatic cases for PRNT90 and PRNT50, 110 samples from 57 cases for ELISA and 96 samples from 51 cases for sVNT (samples indicated by triangles, other samples indicated by circles). The dashed horizontal lines showed the 50% correlate of protection for PRNT90 and PRNT50, and negative cutoff values for ELISA and sVNT.
(95% CI: 268–1884) and 614 (95% CI lower bound: 282, decline not statistically significant) days for PRNT₉₀ titers, 970 (95% CI lower bound: 470, decline not statistically significant) and 1560 (95% CI lower bound: 453, decline not statistically significant) days for PRNT₅₀ titers, 278 (95% CI: 231–352) and 638 (95% CI lower bound: 379, decline not statistically significant) days for ELISA, and 393 (95% CI: 310–555) and 739 (95% CI lower bound: 404) days for sVNT. When all symptomatic infections are considered together, the T₁/₂ was 500 (95% CI: 307–1663) days, 303 (95% CI: 253–386) days and 423 (95% CI: 330–585) days for PRNT₉₀ titers, ELISA and sVNT respectively.

We carried out a subset analysis of antibody waning in symptomatic children with SARS-CoV-2 infection aged ≤15y (Fig. 2). In mild disease (none of the children had severe disease), we estimated that it takes on average 257 (95% CI: 217–329) days and 216 (95% CI: 176–267) days for PRNT₉₀ titers, 201 (95% CI: 144–275) days and 163 (95% CI: 114–230) days for PRNT₅₀ titers, 226 (95% CI: 170–301) days and 181 (95% CI: 127–251) days for ELISA, and 353 (95% CI: 290–432) days and 307 (95% CI: 238–395) days for sVNT.

---

**Table 1:** Patient characteristics for those with samples ≥ 90 days after symptom onset/confirmation.

* May not add up to 1 due to rounding.

| Age (y)                  | No. samples | N (%) | No. samples | N (%) | No. samples | N (%) |
|--------------------------|-------------|-------|-------------|-------|-------------|-------|
| ≤ 15                      |             | 15     | 24%         | 19    | 29%         | 32%   |
| 16–60                     |             | 33     | 53%         | 33    | 50%         | 48%   |
| > 60                      |             | 14     | 23%         | 14    | 21%         | 20%   |
| Male                      |             | 35     | 56%         | 38    | 58%         | 58%   |
| With underlying conditions |             | 20     | 32%         | 20    | 30%         | 30%   |
| Antiviral treatment       |             | 40     | 65%         | 41    | 62%         | 63%   |
| Corticosteroid treatment  |             | 4      | 8%          | 4     | 8%          | 5%    |

| Worst condition type      | No. samples | N (%) | No. samples | N (%) | No. samples | N (%) |
|---------------------------|-------------|-------|-------------|-------|-------------|-------|
| Severe                    | 7           | 11%   | 7           | 11%   | 5           | 8%    |
| Mild                      | 46          | 74%   | 50          | 76%   | 46          | 77%   |
| Asymptomatic              | 9           | 14%   | 9           | 14%   | 9           | 15%   |

**Table 2:** Detection of antibody by PRNT₅₀, PRNT₉₀, sVNT and spike RBD ELISA at day 90 to 386 post onset of symptoms or first positive RT-PCR result in asymptomatic individuals.

* The number of sera and % of sera positive at a cutoff of 20% or 30% inhibition in sVNT is indicated.
Fig. 2. Antibody responses in pediatric COVID-19 cases (≤ 15y) by days after illness onset/confirmation and severity, Hong Kong (71 samples from 28 cases tested by 90% plaque reduction neutralization tests (PRNT$_{90}$) (A) and PRNT$_{50}$ (B), 76 samples from 28 cases tested for receptor binding domain binding antibody by ELISA (optical density 450 nm) (C) by ELISA and% inhibition in surrogate neutralization tests (sVNT) (D). All pediatric patients were mild or asymptomatic. Small random noises were added to the PRNT$_{90}$ and PRNT$_{50}$ titers for better presentation. The fitted lines were based on 14 samples from 8 symptomatic cases for PRNT$_{90}$ and PRNT$_{50}$ and 18 samples from 12 symptomatic cases for ELISA and sVNT (samples indicated by triangles, other samples indicated by circles).
−293) days for PRNT_{50} and PRNT_{90} and titers to drop to 1:10. Compared to adults (Supplementary Figure 3), children had significantly faster waning of antibody titers for both PRNT_{50} (p < 0.001) and PRNT_{90} (p = 0.004). The difference in peak (between 30 and 60 days) PRNT titers did not differ significantly between children and adults with mild disease. The half-life (a drop of 50%) of levels of antibody waning assessed after day 90 after onset of symptoms of infection in children was on average 192 (95% CI: 168–238) days for PRNT_{50} titers, 183 (95% CI: 156–247) days for PRNT_{90} titers.

It takes on average 365 (95% CI: 290–467) days for ELISA OD values to drop to 0.5, and takes 524 (95% CI lower bound: 338) days for sVNT dropping to limit of detection. The corresponding half-lives were 263 (95% CI: 220–335) days for ELISA, and 442 (95% CI lower bound: 286, decline not statistically significant) days for sVNT. Among mild cases, children had significantly higher ELISA OD values (p = 0.013) compared to adults at day 90 after onset of symptoms but OD values dropped significantly faster than in adults (p = 0.014).

Correlations between sVNT inhibition versus PRNT_{50}, PRNT_{90} and ELISA were high (all correlations ≥ 0.77, Fig. 3).

Discussion

The proportion of COVID-19 cases who were children was 6.8% in Hong Kong for the study period. Our study had an interest in the pediatric population and hence they were over-sampled. Other than that, all patients were invited to the study without any specific selection criteria and hence should not introduce any bias on the persistence of antibody responses. Our results indicate that sera collected from 201 to 386 days post infection have detectable PRNT_{50} and PRNT_{90} antibodies in 100% and 92.3%, respectively; and detectable by spike RBD ELISA assays in 92.6% of sera. The sVNT was positive in 100% of sera collected from day 90–150 post infection, dropped to 73.1% using the revised 30% inhibition cut-off but 96.2% positive using the previous cut-off of 20% inhibition, which we previously found to have acceptable specificity.

In symptomatic COVID-19 patients, it is estimated that PRNT_{90} antibody would remain detectable for 1717 days post-infection, 1574 days for mild infections and 2709 days for severe infections. However, because the slope of decline was not significant for symptomatic patients, these may be under-estimates. The finding that 97.5% of individuals remain positive in spike RBD ELISA assays for over 200 days has implications for sero-epidemiology and suggest that waning of antibody is unlikely to be a major issue for spike RBD ELISA, sVNT or PRNT assays.

These findings of a slow decay of neutralizing antibody are in agreement with data from recent studies suggesting the presence of spike RBD specific bone
mucosal specific plasma cells late in convalescence which are known to be those responsible for long lasted antibody responses. RBD-specific memory B cells remain unchanged or even increased over the first six to eight months. After a new infection, short-lived plasmablasts are an early source of antibodies. But these cells recede soon after a virus is cleared from the body and memory B cells patrol the blood for reinfection, while plasma cells resident in bone marrow continue to secrete lower levels of antibodies for decades. Furthermore, even if protection from re-infection may wane beyond two years after infection, immune memory for both B and T cell compartments are likely to remain and will lead to rapid increase in neutralizing antibody upon re-infection, thus conferring even longer protection from severe disease. SARS-CoV-2-specific CD4+ T cells and CD8+ T cells declined with a half-life of 3 to 5 months respectively and are also likely to contribute to modulation of disease severity.

The rate of decline of PRNT50 antibodies in children appears significantly faster than that observed in adults although peak PRNT titres did not differ. It may be speculated that because of multiple infections of seasonal coronaviruses, adults may have with cross-reactive CD4+ helper T-cells that may boost antibody response to both cross-reactive and SARS-CoV-2 specific epitopes. This may lead to a more sustained neutralizing antibody response in adults.

We had too few sera from asymptomatic infections followed up beyond 90 days for us to make reliable assessments of duration of immunity in this group of individuals. Others have reported that milder disease is associated with more rapid waning of neutralizing antibody responses.

Protection from re-infection is unlikely to be absolute. In large scale population-based studies, the protection elicited by infection against re-infection was assessed to be 60.5%, reducing to 47.1% in those older than 65 years of age. There was no increase in rates of re-infection with longer follow up periods after the initial infection. Some of these re-infections occurred following asymptomatic infection and in those who failed to develop detectable neutralizing antibody response.

Out data shows considerable heterogeneity of neutralizing antibody responses in convalescence. Assessing the correlates of protection from infection with SARS-CoV-2 is a major challenge. Neutralizing antibody is clearly one major correlate of protection, but the titres associated with protection from re-infection are not precisely defined. Even if they are defined, standardization of neutralizing antibody titres between laboratories poses a major challenge. The recent study demonstrating that approximately 20% of the mean convalescent antibody levels correlates with the titres associated with protection of 50% of the individuals from re-infection, while imprecise, has the merit that it affords a means for internally standardize the methods used, provided the investigators use the same methods to compare their cohort data with a panel of convalescent sera. Using this approach, we estimate that 50% of patients who recover from symptomatic SARS-CoV would be protected from re-infection for 701 (95% CI 405–2242) days based on PRNT90 titres or 990 days (95% CI lower bound 441, decline not statistically significant) as estimated by PRNT50 titres. As a sensitivity analysis, the duration of protection was estimated to be 519–871 days based on PRNT90 titres or 714–1190 days based on PRNT50 titres, by using the 95% CI of 14.4–28.4% of the mean convalescent antibody levels as a correlate of 50% protection.

The duration of protection resulting from natural symptomatic infection may not be directly extrapolated to results from immunization. On the one hand, RNA vaccines elicit neutralizing antibody titres markedly higher that those obtained from natural infection but vaccines differ in immunogenicity. The generation of memory B cells and bone marrow plasma cells resident in bone marrow following vaccination may differ markedly with that arising from natural infection and this may be reflected in differences in rates of antibody waning and also in the correlates of protection. The half-life (time to a two-fold decline) of PRNT50 titre among mildly ill patients in our cohort was 469 days (95% confidence interval 268–1884). Although direct comparisons are difficult because of differences in methods used to estimate these parameters, the half-life of neutralizing antibodies after mRNA-1273 vaccine was 202 days (95% confidence interval 159–272). More data on waning of neutralizing antibody after natural infection and vaccination is needed.

Some variants of concern (VOC) such as the B.1.351 (Beta), P1 (Gamma) or B.1.617.2 (Delta) variants may show reduced susceptibility to neutralizing antibody in convalescent and post vaccine sera. Convalescent sera of those infected with early pandemic viruses show 2.9 fold reduction of neutralization titres for B.1.1.7; 13.3 fold to B.1.351 and 2.6 fold for B.1.617.2. Variants which have >8-fold reduction of neutralization titres would fall below the 50% protection threshold by day 90 post infection while those with 2-fold reduction will remain above the 50% protection threshold for 474 (95% CI lower bound: 242) days. By assessing the fold reduction in PRNT50 reported with a particular VOC, it may be possible to model the adjusted duration of protection from past infections. This remains to be validated in future studies. Interestingly, the breadth of cross-neutralization appears to increase over time following natural infections but whether then same will occur after vaccination remains to be understood.

While PRNT assays require handling of live SARS-CoV-2 in Biosafety level (BSL) 3 facilities and takes around 5 days to complete, sVNT can be done within a few hours in BSL2 containment. The good quantitative correlation between PRNT50 and sVNT suggests that %
sVNT inhibition can be used to semi-quantitatively predict PRNT50 titres in a serum within the range of titers of 1:10 to 1:320. Others have reported good correlation between sVNT and neutralization results but not a linear correlation with PRNT50 titres as we have found.

There were a number of limitations in our study. The sample size of sera collected beyond day 90 post infection is a limitation of our study, as is the lack of adequate data from asymptomatic infections. It is important to assess the duration of T cell responses following natural infection because such responses may modulate severe outcomes following infection. The assumed linear decrease in the antibodies may require further confirmation with longer observations, especially for sVNT.

In summary, our findings suggest that neutralizing antibody mediated protection from re-infection against the original strains of SARS-CoV-2 is likely to remain for 700 days or more after symptomatic COVID-19 infection but variants of concern may lead to an erosion, but not abrogation, of this protection.

Data sharing statement
Upon publication, the anonymised original data will be available from the corresponding author upon request.

Funding
The study was supported by the Health and Medical Research Fund, Commissioned research on Novel Coronavirus Disease (COVID-19) (Reference nos COVID190126 and COVID1903003) from the Food and Health Bureau, Hong Kong SAR Government and the Theme-based Research Scheme project no. T11-712/19-N, the University Grants Committee of the Hong Kong Government.

Contributors
All the authors met the ICMJE criteria for authorship. EHYL, DSCH, OTYT, MYEK, WHC, SSC, LLMP, MP conceived and designed the study, DSCH, OTYT, MYEK, WHC, SSC recruited the study participants and collected clinical specimens, SMSC, RLWK, JKCL, SC, CHT carried out the laboratory work, EHYL, LLMP, MP analysed the data, EHYL and MP wrote the first draft of the manuscript and all authors provided critical comments and input on revision of the manuscript. EHYL and MP have verified all the data and data analysis.

Declaration of Competing Interest
Dr. Peiris reports grants from Health & Medical Research Fund, Hong Kong (COVID190126), grants from Health & Medical Research Fund, Hong Kong (COVID1903003), grants from University Grants Committee, Hong Kong (T11-712/19-N), during the conduct of the study; Dr. Hui reports grants from Health & Medical Research Fund COVID-19, 1,903,003, HKSAR government, during the conduct of the study; all the other authors reports no conflicts.

Acknowledgements
We thank Leo LH Luk, Yonna WY Leung and Zacary YH Chai for technical assistance.

Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.101174.

References
1. Amanna IJ, Carlsen NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med 2007;357:1903-14.
2. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med. 2020. https://doi.org/10.1038/s41591-020-0785-y. Epub ahead of print.
3. Liu J, Xie J, Sun J, et al. Longitudinal profiles of immunoglobulin G antibodies against severe acute respiratory syndrome coronavirus components and neutralizing activities in recovered patients. J Infect Dis 2021;33:135-41.
4. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Natur 2020;584:67-62.
5. Lumley SF, O’Donnell D, Stoeßer NE, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. N Engl J Med 2021;384:333-40.
6. Kim JH, Marks F, Clemens JD. Looking beyond COVID-19 vaccine phase 3 trials. Nat Med 2021;27:205-11.
7. Tan AT, Linster M, Tan CW, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Rep 2021;34:10828.
8. Valkenburg SA, Leung NHL, Bull MB, et al. The hurdles from bench to bedside in the realization and implementation of a universal influenza vaccine. Front Immunol 2019;10:1479.
9. Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with high attack rate. J Clin Microbiol 2020;58:e00207-20.
10. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021. https://doi.org/10.1038/s41591-021-01377-8. May 17Epub ahead of print.
11. Holohan D, Curty RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond) 1972;79:567-77.
12. Cohet KB, Nason MC, Flach B, et al. Immune correlates of protection by mRNA-1273 immunization against SARS-CoV-2 infection in nonhuman primates. bioRxiv 2021. https://doi.org/10.1101/2021.04.20.440647. [Preprint] Apr 23:2021.04.20.440647.
13. Robbins DF, Gazzler C, Muecksch F, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 2020;584:437-42.
14. Wheatley AK, Juno JA, Wang JJ, et al. Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. Nat Commun 2021;12:1162.
15. Fairfax KA, Kallies A, Nutt SL, Tarlinton DM. Plasma cell development: from B-cell subsets to long-term survival niches. Semin Immunol 2020;40:40-58.
16. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371 (6529):eabf4063. https://doi.org/10.1126/science.abf4063. Epub 2021 Jan 6.
Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. Nature 2021;591(7853):639–44.

Lau EHY, Tsang OTY, Hui DSC, et al. Neutralizing antibody titres in SARS-CoV-2 infections. Nat Commun 2021;12:63. https://doi.org/10.1038/s41467-020-20247-4.

Perera RA, Mok CK, Tsang OT, et al. Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Euro Surveill 2020;25(16):2000421.

Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockade of ACE2-spike protein-protein interaction. Nat Biotechnol 2020;38:1023–8.

Perera RAPM, Ko R, Tsang OTY, et al. Evaluation of a SARS-CoV-2 surrogate virus neutralization test for detection of antibody in human, canine, cat, and hamster sera. J Clin Microbiol 2021;59(2):e02504–20.

Turner JS, Kim W, Kalaidina E, et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. Nature 2021. https://doi.org/10.1038/s41586-021-03650-7. Epub ahead of print.

Chia WN, Zhu F, Ong SWX, et al. Dynamics of SARS-CoV-2 neutralizing antibody responses and duration of immunity: a longitudinal study. Lancet Microbe 2021;2(6):e240–9.

Boitsjak B, Stein SC, Willenzon S, et al. Low serum neutralizing anti-SARS-CoV-2 S antibody levels in mildly affected COVID-19 convalescent patients revealed by two different detection methods. Cell Mol Immunol 2021;18:936–44.

Choe PG, Kim KH, Kang CK, et al. Antibody responses 8 months after asymptomatic or mild SARS-CoV-2 infection. Emerg Infect Dis 2021;27:928–31.

Hansen CH, Michlmayr D, Guðbóls SM, et al. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. Lancet 2021;397:1204–12.

Chan PKS, Lui G, Hachim A, et al. Serologic responses in healthy adult with SARS-CoV-2 reinfection, Hong Kong, 2020. Emerg Infect Dis 2020;26:3076–8.

Walsh EE, Frenck Jr RW, Falsey AR, et al. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. N Engl J Med 2020;383:2410–50.

Doria-Rose N, Suthar MS, Makowski M, et al. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for COVID-19. N Engl J Med 2021;384:2259–61.

Zani A, Caccuri F, Messali S, et al. Serosurvey in BNT162b2 vaccine-elicited neutralizing antibodies against authentic B.1, B.1.1.7, B.1.351, B.1.525 and P.1 SARS-CoV-2 variants. Emerg Microbes Infect 2021;10:1241–1.

Liu C, Gino HM, Dejnirattisai W, et al. GR. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell 2021;184:4230–36.

Wang Z, Muecksch F, Schaefer-Bahajew D, et al. Naturally enhanced neutralizing breadth to SARS-CoV-2 after one year. bioRxiv 2021. https://doi.org/10.1101/2021.05.07.443175. [Preprint]Sun 23/2021-05.07.443175.

Sahin U, Muik A, Vogler I, et al. BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. Nature 2021. https://doi.org/10.1038/s41586-021-03653-6. Epub ahead of print.