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Research paper

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

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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 ($PRNT_{50}$), 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 $PRNT_{50}$ 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 $PRNT_{50}$ 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.
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1. Introduction

Duration of immunity to virus infections can vary, ranging from lifelong immunity with measles to transient protection to seasonal coronaviruses [1,2]. Antibody responses to SARS in 2003 remained detectable for around 3 years [3] but T cell responses proved much more durable [4]. Prior infection and vaccination are associated with high levels of protection against re-infection with SARS-CoV-2 [5,6]. Both humoral and T-cell mediated immunity are likely to contribute to protection from infection and disease from COVID-19 as is the case with other respiratory virus infections [7,8]. Virus neutralizing antibodies correlate with protection from COVID-19 infection and in

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Research in context

Evidence before this study

We searched PubMed on 29th June 2021 with no restrictions using the terms "SARS-CoV-2" OR COVID-19 AND "neutralizing antibody" AND duration AND protection. Our search revealed 10 published papers which were assessed individually. One relevant research paper was identified which reported antibody beyond the acute stage of infection. They found rapid decline of pseudotyped virus neutralizing antibody activity over a 6 month follow up period. Another study identified patient groups with rapid or slow waning of neutralizing antibody. No previous publications attempted to relate long term persistence of live virus neutralizing antibody to antibody levels now know to be associated with protection

Added value of this study

We used 50% plaque reduction neutralization test (PRNT) antibody titre data from 115 sera collected longitudinally from 90 to 386 days after onset of symptoms or first RT-PCR confirmation from 62 RT-PCR confirmed SARS-CoV infected individuals, to estimate that PRNT antibody will remain detectable for around 1,717 days after symptom onset and that a threshold for 50% protection from re-infection will be maintained for around 990 days post-symptom onset, in symptomatic patients. PRNT titres in mildly symptomatic children wane faster than in adults.

Implications of all the available evidence

Our results are in agreement with other recent studies reporting the persistence of SARS-CoV-2 specific long-term plasma cells resident in bone-marrow following natural infection and immunization. There is need for more data on asymptomatic infections and children.

diseased humans [9,10] and in experimental animal models [11]. Precise correlates of protection comparable to those defined for influenza [12] are not yet available for SARS-CoV-2 infection and disease. However, recent studies have reported that the level of neutralizing antibody providing 50% protection from re-infection was approximately 20% of the mean level of antibody found in COVID-19 convalescent sera [10].

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 [13]. Antibody waning is more rapid in the first 2-3 months after infection and slower waning thereafter [14]. 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 [15]. 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 [14,16,17]. 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 [18]. 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 1/5th of the mean of convalescent titres after natural infection [10] to estimate the duration of protection from re-infection in our cohort.

2. 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 [18]. 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 Committee on Infectious Diseases and Emergency Response (CCIDER) of the Hospital Authority of Hong Kong, as revised periodically since February 2020, and decided by the attending clinicians. The patients were followed up at intervals following discharge from hospital and venous blood collected for serology testing. We included patients with multiple longitudinally sampled sera for this analysis.

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% CO_2 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 $\geq$ 90% ($PRNT_{90}$) or $\geq$ 50% ($PRNT_{50}$) 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 falling within recommended values. Assuming the positive and negative controls gave the recommended OD450 values, the % inhibition of each serum was calculated as Inhibition (%) = (1 - Sample OD value/Negative Control OD value) x 100. Inhibition (%) of $\geq$ 20% was initially regarded as a positive result while that $<$ 20% is negative [20,21]. More recently, the manufacturer has recommended the use of $\geq$ 30% inhibition as the cutoff for a positive result. We have therefore used $\geq$ 30% inhibition for the waning assessments.

SARS-CoV-2 spike receptor binding domain ELISA: 96-well ELISA plates (Nunc MaxiSorp, Thermo Fisher Scientific) were coated overnight with 100 ng per well of the purified recombinant RBD protein in PBS buffer. The plates were then blocked with 100 μl of Chonblock blocking/sample dilution ELISA buffer (Chondrex Inc, Redmon, US) and incubated at room temperature for 2 h. Each serum or plasma sample was tested in duplicate at a dilution of 1:100 in Chonblock blocking/sample dilution ELISA buffer and 100 μL was added to the wells of each plate for 2 h incubation at 37 °C. After extensive washing with PBS containing 0.1% Tween 20, horseradish peroxidase (HRP)-conjugated goat anti-human IgG (1:5000, GE Healthcare) was added for 1 h at 37 °C. The ELISA plates were then washed five times with PBS containing 0.1% Tween 20. Subsequently, 100 μL of HRP substrate (Ncm TMB One; New Cell and Molecular Biotech Co. Ltd, Suzhou, China) was added into each well. After 15 min incubation, the reaction was stopped by adding 50 μL of 2 M H2SO4 solution and analysed on a Sunrise (Tecan, Männedorf, Switzerland) absorbance microplate reader at 450 nm wavelength. The method was previously validated and reported [19].

Statistical analysis: We presented $PRNT_{50}$, $PRNT_{90}$ log titers, ELISA OD values and sVNT inhibition (%) stratified as asymptomatic and symptomatic (mild and severe) confirmed COVID-19 infections. A time trend was fitted to the antibody response from 90 days after symptom onset to model the decay over time using linear mixed effects model. The 95% confidence intervals were constructed using parametric bootstrap based on 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 30%, the time when PRNT titers reach 50% correlates of protection (CoP) and half-life ($T_{1/2}$) (a drop of 50%). We calculated the 50% CoP as 20% of geometric mean $PRNT_{90}$ and $PRNT_{50}$ antibody titers from 30 to 60 days after symptom onset of the mild and severe COVID-19 cases [10]. If 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 $\geq$1:320 were imputed by fitting a Poisson distribution to the titers from $<$1:10 to 1:160, and applied the fitted distribution to redistribute the samples with antibody titers $\geq$ 1:320 to antibody titers from 1:320 to 1:2560. The fitted line for $PRNT_{50\ and}$ $PRNT_{90}$, 50% CoP and 95% CI were calculated based on 1000 randomly imputed samples.

We calculated the spearman correlation between $PRNT_{50}$ and $PRNT_{90}$ 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.

3. 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 $\leq$ 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 $PRNT_{50}$, $PRNT_{90}$ and spike RBD ELISA from these 124 patients were 329, 329 and 334, respectively. Two hundred and fifty-one sera from 99 patients were tested by sVNT. The kinetics of the antibody responses are shown in Fig. 1.

The rate of antibody waning was more rapid during the first three months and progressively slower thereafter. Thus, we focused our analysis on the subset of sera collected 90 or more days after onset. The characteristics of this sub-set of patients and sera tested is shown in Table 1, which were similar to those of the 124 patients in the full cohort (Supplementary Table 1). Those with antibody detectable by $PRNT_{50}$, $PRNT_{90}$ and RBD ELISA from day 90 to 386 was 99.1%, 91.3% and 97.5% respectively; and in the subset of sera collected 201 to 386 days after onset, was 100.0%, 92.3% and 92.6% respectively (Table 2). Positivity by sVNT at cutoff of 20% or 30% inhibition was 100% at day 90−150; 96.2 and 73.1% at day 201−386.

We fitted lines of decay on those sera collected 90 or more days after onset (Fig. 1). These include 115 sera from 62 patients tested by PRNT, 120 sera from 66 cases tested by spike RBD ELISA and 106 sera from 60 cases tested by sVNT assays. The patient characteristics of this subset is shown in Table 1. We also fitted Generalized Additive Mixed Models for the full cohort and found that the linearity assumption was valid for $PRNT_{50}$, $PRNT_{90}$ 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 $PRNT_{50}$ titers of symptomatic patients to drop to 1:10; 1574 (95% CI lower bound: 857, 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 $PRNT_{50}$ 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 $\geq$1:10) may be under-estimates. Similarly, it takes 731 days (95% CI: 486−2137) days for $PRNT_{90}$ 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 [10]. From the $PRNT_{50}$ and $PRNT_{90}$ 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 titers (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 $PRNT_{50}$ and $PRNT_{90}$ 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 $PRNT_{50}$ 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 $PRNT_{90}$ was 701 (95% CI: 405−2442) days after symptom onset.

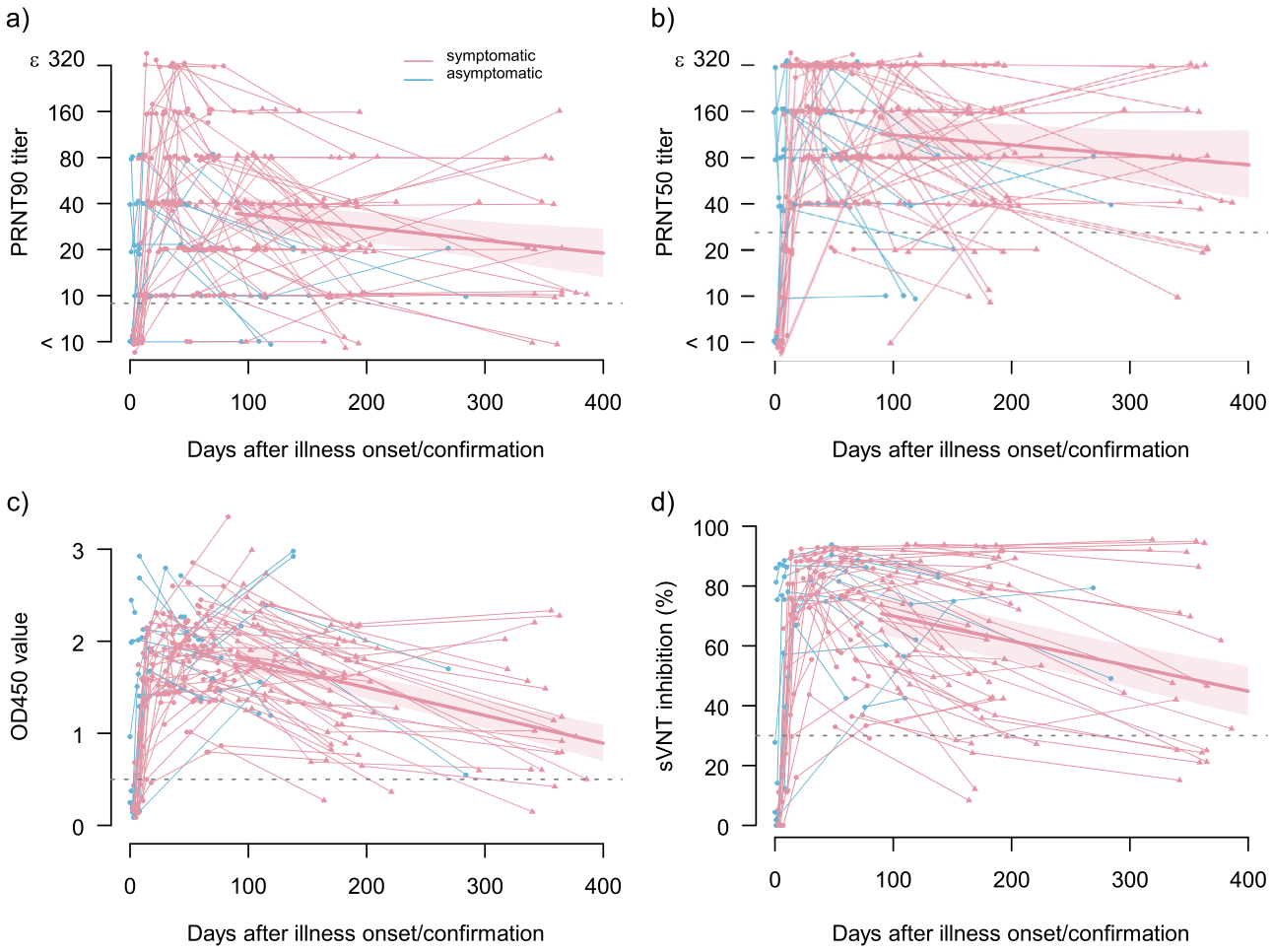

Fig. 1. Antibody responses in COVID-19 cases by days after illness onset and severity, Hong Kong. Sera tested were as follows: 329 samples from 124 cases tested for 90% plaque reduction neutralization test ($PRNT_{90}$) (A) and $PRNT_{50}$ titres (B), 334 samples from 124 cases tested for receptor binding 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 $PRNT_{90}$ and $PRNT_{50}$ titers for better presentation. The fitted lines were based on 105 samples from 53 symptomatic cases for $PRNT_{90}$ and $PRNT_{50}$, 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 horizonal lines showed the 50% correlate of protection for PRNT90 and PRNT50, and negative cutoff values for ELISA and sVNT.

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

	PRNT (n = 62)		ELISA (n = 66)		sVNT (n = 60)	
	N	(%)*	N	(%)*	N	(%)
Age (y)						
≤ 15	15	(24%)	19	(29%)	19	(32%)
16–60	33	(53%)	33	(50%)	29	(48%)
> 60	14	(23%)	14	(21%)	12	(20%)
Male	35	(56%)	38	(58%)	35	(58%)
With underlying conditions	20	(32%)	20	(30%)	18	(30%)
Antiviral treatment	40	(65%)	41	(62%)	38	(63%)
Corticosteroid treatment	4	(6%)	4	(6%)	3	(5%)
Worst condition						
Severe	7	(11%)	7	(11%)	5	(8%)
Mild	46	(74%)	50	(76%)	46	(77%)
Asymptomatic	9	(14%)	9	(14%)	9	(15%)
No. samples	115		120		106	

* May not add up to 1 due to rounding.

It is estimated that sVNT inhibition will drop to the threshold of detection in 577 days (95% CI: 463–765) 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

Table 2
Detection of antibody by $PRNT_{50}$, $PRNT_{90}$, sVNT and spike RBD ELISA at day 90 to 386 post onset of symptoms or
first positive RT-PCR result in asymptomatic individuals.

Serology test	Days after symptom onset or first RT-PCR positive result			
	90 to 150	151 to 200	201 to 386	Total
PRNT50				
No tested	50	39	26	115
No positive	49	39	26	114
% positive	98.0	100.0	100.0	99.1
PRNT90				
No tested	50	39	26	115
No positive	46	35	24	105
% positive	92.0	89.7	92.3	91.3
RBD ELISA				
No tested	52	41	27	120
No positive	52	40	25	117
% positive	100.0	97.6	92.6	97.5
Surrogate neutralization test				
No tested	41	39	26	106
No positive (>20%)*	41	37	25	103
% positive*	100.0	94.9	96.2	97.2
No positive (>30%)*	41	33	19	103
% positive*	100.0	84.6	73.1	90.3

* The number of sera and % of sera positive at a cutoff of 20% or 30% inhibition in sVNT is indicated.

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 ($T_{1/2}$) (a drop of 50%) of levels of antibody waning assessed after day 90 after onset of symptoms in mild and severe patients was on average, 469 (95% CI: 268–1884) and 634 (95% CI lower bound: 282, decline not statistically significant) days for $PRNT_{90}$ titers, 970 (95% CI lower bound: 470, decline not statistically significant) and 1560 (95% CI lower bound: 453, decline not statistically significant) days for $PRNT_{50}$ 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 $T_{1/2}$ was 500 (95% CI: 307–1663) days, 303 (95% CI: 253–386) days and 423 (95% CI: 330–585) days for $PRNT_{90}$ 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: 176–293) 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 ($p = 0.034$).

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

4. 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

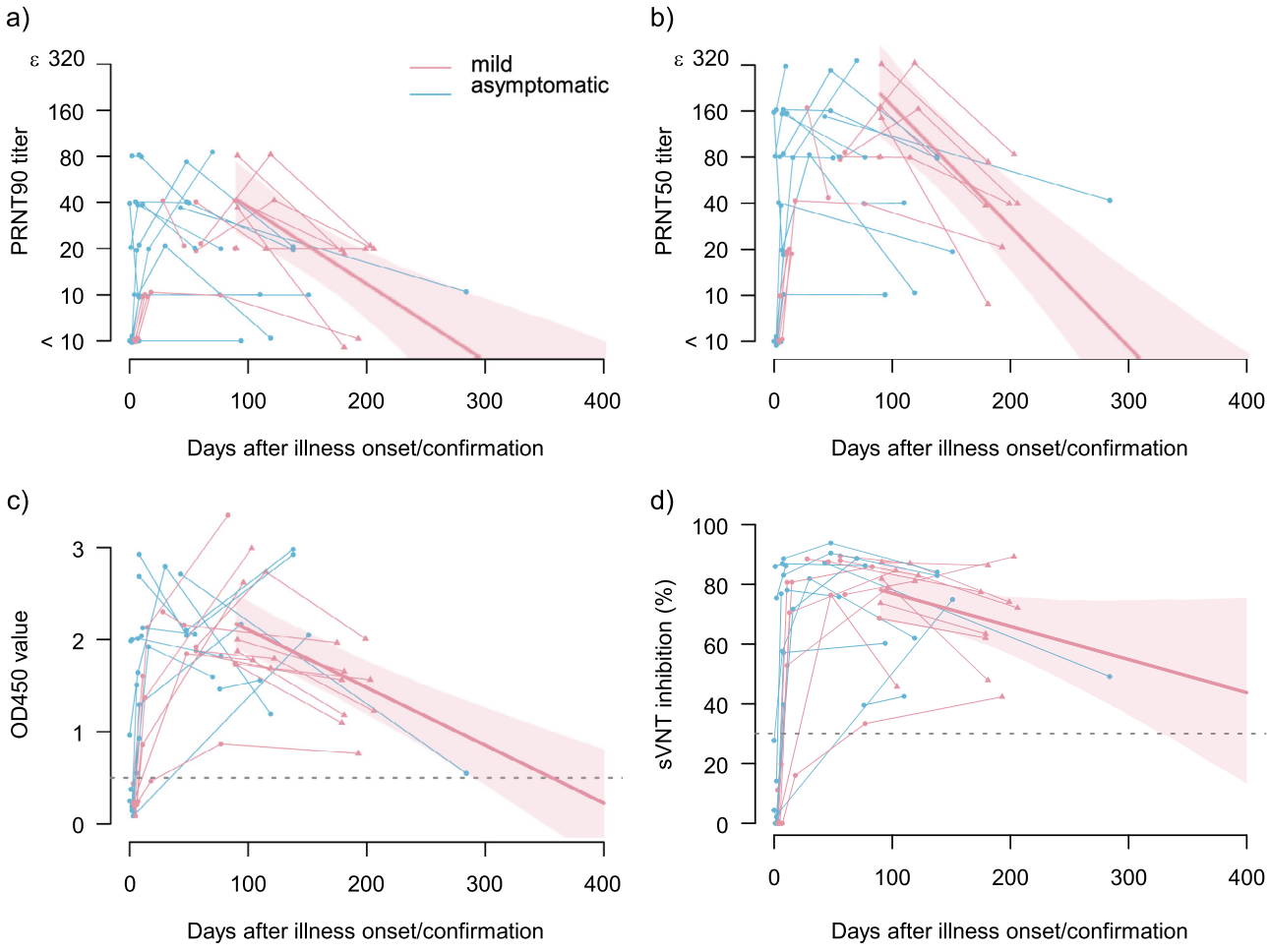

Fig. 2. Antibody responses in pediatric COVID-19 cases (≤ 15y) by days after illness onset/confirmation and severity, Hong Kong (71 samples from 28 cases tested by 90% plaque reduction neutralization tests ($PRNT_{90}$) (A) and $PRNT_{50}$ (B), 76 samples from 28 cases tested for receptor binding domain 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).

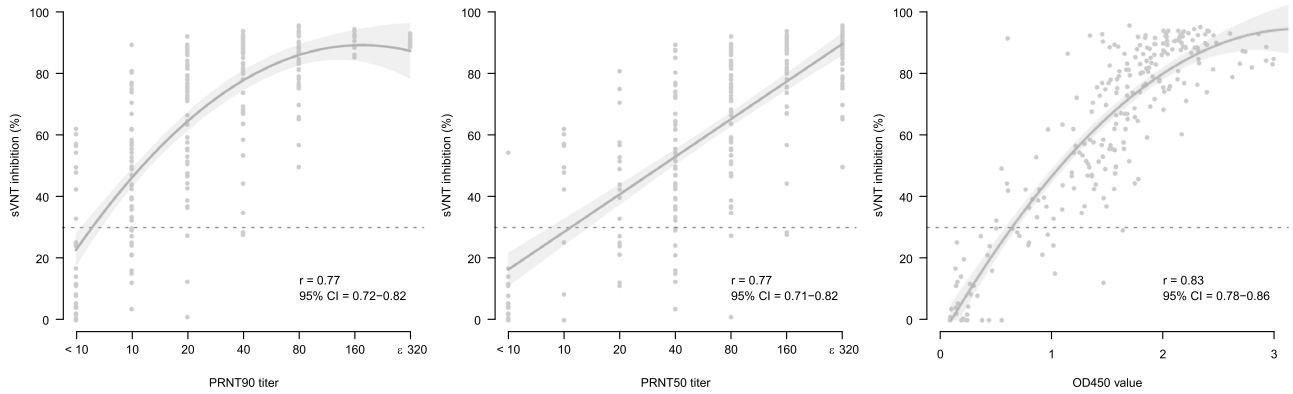

Fig. 3. Correlation between% inhibition in surrogate neutralization tests (sVNT) and 90% plaque reduction neutralization tests (PRNT90) (240 samples from 97 cases) (A), PRNT50 (240 samples from 97 cases) (B) and receptor binding domain antibody by ELISA (optical density 450 nm) (251 samples from 99 cases) (C) in COVID-19 cases. The gray area represents the 95% confidence intervals.

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 cutoff of 20% inhibition, which we previously found to have acceptable specificity [21].

In symptomatic COVID-19 patients, it is estimated that $PRNT_{50}$ 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 marrow specific plasma cells late in convalescence which are known to be those responsible for long lasted antibody responses [22]. RBD-specific memory B cells remain unchanged or even increased over the first six to eight months [16,17]. 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 [16] and these are also likely to contribute to modulation of disease severity.

The rate of decline of $PRNT_{50}$ 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 [23− 25].

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 80.5%, reducing to 47.1% in those older than 65 years of age [26]. 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 [27]. Our 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 [9,10], 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 [10], 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 $PRNT_{90}$ titers or 990 days (95% CI lower bound 441, decline not statistically significant) as estimated by $PRNT_{50}$ titers. As a sensitivity analysis, the duration of protection was estimated to be 519−871 days based on $PRNT_{90}$ titers or 714−1190 days based on $PRNT_{50}$ titers, by using the 95% CI of 14.4−28.4% of the mean convalescent antibody levels as a correlate of 50% protection [10].

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 [28] 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 $PRNT_{90}$ titres amoung 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) [29]. 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 B1.617.2 (Delta) variants may show reduced

susceptibility to neutralizing antibody in convalescent and post vaccine sera [30,31]. 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 $PRNT_{50}$ reported with a particular VOC, it may be possible to model the adjusted duration of protection from past infections [10]. This remains to be validated in future studies. Interestingly, the breadth of cross-neutralization appears to increase over time following natural infections [32] 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 $PRNT_{50}$ and sVNT suggests that % sVNT inhibition can be used to semi-quantitatively predict $PRNT_{50}$ 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 [24] but not a linear correlation with $PRNT_{50}$ 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 [7,33]. 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.

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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

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Supplementary materials

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

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