Research paper

The impact of spike N501Y mutation on neutralizing activity and RBD binding of SARS-CoV-2 convalescent serum

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ABSTRACT

Background: Several SARS-CoV-2 lineages with spike receptor binding domain (RBD) N501Y mutation have spread globally. We evaluated the impact of N501Y on neutralizing activity of COVID-19 convalescent sera and on anti-RBD IgG assays.

Methods: The susceptibility to neutralization by COVID-19 patients' convalescent sera from Hong Kong were compared between two SARS-CoV-2 isolates (B117-1/B117-2) from the α variant with N501Y and 4 non-N501Y isolates. The effect of N501Y on antibody binding was assessed. The performance of commercially-available IgG assays was determined for patients infected with N501Y variants.

Findings: The microneutralization antibody (MN) titers of convalescent sera from 9 recovered COVID-19 patients against B117-1 (geometric mean titer[GMT];80; 95% CI, 47–136) were similar to those against the non-N501Y viruses. However, MN titer of these serum against B117-2 (GMT; 20; 95% CI, 11–36) was statistically significantly reduced when compared with non-N501Y viruses (P < 0.01; one-way ANOVA). The difference between B117-1 and B117-2 was confirmed by testing 60 additional convalescent sera. B117-1 and B117-2 differ by only 3 amino acids (nsp2-S512Y, nsp13-K460R, spike-A1056V). Enzyme immunoassay using 272 convalescent sera showed reduced binding of anti-RBD IgG to N501Y or N501Y-E484K-K417N when compared with that of wild-type RBD (mean difference: 0.1116 and 0.5613, respectively; one-way ANOVA). Of 7 anti-N-IgG positive sera from patients infected with N501Y variants (collected 9–14 days post symptom onset), 6 (85.7%) tested negative for a commercially-available anti-S1-IgG assay.

Interpretation: We highlighted the importance of using a panel of viruses within the same lineage to determine the impact of virus variants on neutralization. Furthermore, clinicians should be aware of the potential reduced sensitivity of anti-RBD IgG assays.

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1. Introduction

SARS-CoV-2 was first detected in humans during a cluster of pneumonia in China in December 2019 [1]. Efficient person-to-person transmission was demonstrated by the high attack rate in a familial outbreak [2]. Early SARS-CoV-2 virus isolates in December 2019 and January 2020 were already genetically diverse. In the first two months after the discovery of SARS-CoV-2, different genetic clades have emerged, including the V, S and L clade according to the GISAID nomenclature [3]. The first major mutation was the spike D614G, which first emerged in February 2020 and then dominated the world. Spike D614G affects the transmissibility of the virus [4], and SARS-CoV-2 with D614G mutation has been found in reinfection cases [5,6].

SARS-CoV-2 enters cells via the attachment of the spike protein receptor binding domain (RBD) to the host receptor angiotensin-converting enzyme 2 (ACE2) [7], although other host factors, such as heparan sulphate, has been shown to play a role in virus entry [8]. In addition to cell surface ACE2, the interaction between spike protein and soluble ACE2 has been shown to facilitate endocytosis [9]. The spike protein N-terminal domain, RBD and S2 are targets of neutralizing antibodies, with the RBD being the most immunogenic part [10–12]. Anti-RBD antibody has a high correlation with neutralizing antibody titers [13,14]. COVID-19 vaccines are designed to elicit antibody against the spike protein. Hence, SARS-CoV-2 variants with mutations in the RBD, especially those at positions that interacts with ACE2 [15,16], is of particular concern because these variants may escape natural infection or vaccine-induced humoral immunity.

Since November 2020, several variants bearing mutations in the spike RBD have rapidly spread in the United Kingdom (B.1.1.7; α variant), South Africa (B.1.351; β variant) and Brazil (P.1; γ variant). All three variants contain spike N501Y mutation. The B.1.351 and P.1 variants also contain mutation at the spike amino acid position 484 (E484K) and at position 417 (K417N for B.1.351; K417T for P.1). These mutations have led to heightened concern as epidemiological studies suggest that they are more transmissible [17,18], and a higher viral load is found in patients with the B.1.1.7 variant [19]. Furthermore, these variants may jeopardize vaccine efficacy [20]. These variants have also caused reinfections [21].

Although some studies have suggested that the convalescent sera neutralizing antibody titer is lower for these variants than for wild type, these studies were conducted in Europe or USA. Since there are geographical differences in the lineages in different parts of the world, results from Europe or USA patients may not represent those from others. Furthermore, the number of patients’ serum specimens in these studies are relatively small. In the current study, we compared the neutralizing antibody titers against different lineages of viruses with convalescent serum from patients initially infected with different lineages. Furthermore, we assessed the difference in antibody against wild type and N501Y mutant RBD with a large serum panel consisting of > 250 patients.

2. Methods

2.1. Study setting and clinical specimens

Archived posterior oropharyngeal saliva, nasopharyngeal swab and serum specimens from hospitalized COVID-19 patients in Hong Kong were retrieved for viral genome sequencing, viral culture or the determination of antibody titers. The SARS-CoV-2 virus isolates used for the live virus microneutralization antibody (MN) assay were isolated from clinical specimens between March 2020 and January 2021, while the serum specimens used in the antibody assays were collected between July 2020 and January 2021.

2.2. Ethics statement

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 13–372 and UW 20–292), the Hong Kong Polytechnic University (approval no. RSA20021), the Kowloon West Cluster REC (KW/EX-20–038[144-26]), and the Kowloon Central/Kowloon East Cluster REC (KC/KE-20–0140/ER-1). Since archived specimens were used, written informed consent was waived.

2.3. Next generation sequencing of clinical specimens

Library preparation, nanopore sequencing and bioinformatic analysis were performed as we described previously [3]. Briefly, nanopore sequencing was performed following the Nanopore protocol – PCR tiling of COVID-19 (Version: PTC_9096_v109_revH_06Feb2020)
according to the manufacturer’s instructions with modifications (Oxford Nanopore Technologies). Briefly, extracted RNA was first reverse transcribed to cDNA using SuperScript™ IV reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, USA; Cat#18090200). PCR amplification was then performed using the hCoV-2019/nCoV-2019 Version 3 Amplicon Set [Integrated DNA Technologies (IDT), Coralville, IA, USA; Cat#10006788]. End preparation and native barcode ligation were performed according to the PCR tiling of COVID-19 virus protocol (EXP-NBD196, Oxford Nanopore Technologies). Bar-coded and pooled libraries were then ligated to sequencing adapter and sequenced with the Oxford Nanopore MinION device using R9.4.1 flow cells for 24/70 h. For bioinformatics analysis, the recommended ARTIC bioinformatics workflow was used and minor modifications were applied as described previously [3] (Supplementary Method). The sequences of the SARS-CoV-2 isolates used in the MN assay have been deposited into the GISAID database (Supplementary Table S1).

2.4. Viral culture

Viral culture was performed using wild-type VeroE6 (ATCC Cat#CRL-1586; RRID:CVCL_0574) or TMPRSS2-expressing VeroE6 cells (JCRB Cat#JCRB1819; RRID:CVCL_YQ49) in a biosafety level-3 facility [22,23]. Briefly, cells were seeded with 1 mL of minimum essential medium (MEM) (Thermo Fisher Scientific, Gibco; Cat#11095) at 2 × 10^5 cells/mL in culture tubes and incubated at 37°C in a carbon dioxide incubator for 1–2 days until confluence for inoculation. Each tube was inoculated with 0.2 mL of specimen and was incubated in a slanted position so that the inoculum covered the monolayer for 60 min at 37°C. Then 1 mL of MEM was added and incubated in a roller apparatus at a speed 12 to 15 revolutions per hour. Virus-induced cytopathic effect was examined daily for up to 7 days. The cultures with more than 50% virus-induced cytopathic effect were expanded to large volume and the 50% tissue culture infective dose (TCID50) was determined.

2.5. Commercial IgG assays against N protein and spike protein S1 subunit

Anti-nucleocapsid (N) IgG was determined using Abbott SARS-CoV-2 IgG assay (Abbott, Abbott Park, Illinois, U.S.A). Anti-S1 IgG was determined using the Euroimmun anti-SARS-CoV-2 ELISA (IgG) (Euroimmun, Lubeck, Germany). The Euroimmun anti-S1 IgG assay is a semi-quantitative assay by calculating the ratio of the extinction coefficient of patient’s serum over the extinction coefficient of the calibrator.

2.6. Live virus MN assay

Live virus MN assay was performed as we described previously [23,24]. The MN antibody titer was the highest dilution with 50% inhibition of the cytopathic effect, and an MN antibody titer of ≥20 was considered positive. All dilutions were performed in duplicates.

### Table 1
Details of serum specimens used for the MN assay.

| Case no. | Month of diagnosis | Virus lineage/variant | Days after symptom onset | Anti-N IgG | Anti-RBD IgG |
|----------|-------------------|-----------------------|--------------------------|------------|-------------|
| N1       | March             | N/A                   | 32                       | Positive   | Positive    |
| N2       | April             | N/A                   | 18                       | Positive   | Positive    |
| N3       | April             | N/A                   | 31                       | Positive   | Positive    |
| N4       | August            | B.1.1.63              | 29                       | Positive   | Positive    |
| N5       | August            | B.1.2                 | 29                       | Positive   | Positive    |
| N6       | August            | N/A                   | 20                       | Positive   | Positive    |
| N7       | December          | B.1.36.27             | 31                       | Positive   | Positive    |
| N8       | December          | N/A                   | 33                       | Positive   | Positive    |
| N9       | November          | B.1.36.27             | 47                       | Positive   | Positive    |

N/A: Not available because sequencing not performed.

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Fig. 1. Phylogenetic tree showing the 6 different virus isolates used in the microneutralization assay in this study.
which contained the baculovirus was used to infect ExpiSf9 cell sus-
plectin and C-terminal 6 His tag. The constructs were fused with an N-terminal gp67
dual baculovirus expression vector (Thermo Fisher Scientific, cat#10712024). The constructs were fused with an N-terminal gp67
codon-optimized for baculovirus expression and cloned into pFast
RBD gene was transformed to DH10Bac to generate a recombinant
N501Y-E484K-K417N were expressed and puri-
spike protein from the reference sequence Wuhan-Hu-1 (GenBank ID
YP_009724390.1) (wild type) or with the mutations N501Y or
previously with modi-
Dunnett’s multiple comparison test was used for statistical analysis, with B117-1 as
the reference group. **, P < 0.01; ***, P < 0.001.

2.7. Expression and purification of RBD

Recombinant RBD (amino acid residues 306-543) of SARS-CoV-2
spike protein from the reference sequence Wuhan-Hu-1 (GenBank ID
YP_009724390.1) (wild type) or with the mutations N501Y or
N501Y-E484K-K417N were expressed and purified as we described
previously with modifications [25]. Briefly, RBD gene sequences were
codon-optimized for baculovirus expression and cloned into pFast
dual baculovirus expression vector (Thermo Fisher Scientific, Gibco; 
Cat#10712024). The constructs were fused with an N-terminal gp67
signal peptide and C-terminal 6xHis tag. The plasmid containing the
RBD gene was transformed to DH10Bac to generate a recombinant
bacmid DNA, which was used to transfect the Sf9 cells (ATCC Cat#CRL-1711; RRID:CVCL_0549) using Cellfectin II (Thermo Fisher Scientific, Gibco; Cat#10362100). After 72 h, the culture supernatant
which contained the baculovirus was used to infect ExpiSf9 cell sus-
pension culture (Thermo Fisher Scientific, Gibco; Cat#A35243) at a
multiplicity of infection of 1 to 10. Infected ExpiSf9 cells were incu-
bated at 27.5 °C with shaking at 125 r.p.m. for 96 h for protein expres-
sion. The supernatant was collected and then concentrated using a 10 kDa MW cutoff Labscale TFF System (Millipore). The RBD protein
was purified by Ni-NTA purification system and size exclusion chro-
matography. The concentration of purified RBD was determined by
using the Bradford Assay Kit (Bio-Rad; Cat#5000002) according to
the manufacturer’s instructions. The purity of recombinant RBD
mutants were verified by western blotting.

2.8. Anti-RBD assay for wild type, N501Y, and N501Y-E484K RBD

An in-house enzyme immunoassay coated with either wild type, N501Y, or N501Y-E484K RBD was used to determine the impact of
N501Y on RBD binding. Briefly, 96-well Nunc MaxiSorp™ flat-bottom
immunoplates (Thermo Fisher Scientific, Invitrogen, Denmark; 
Cat#44-2404) were coated with 100 μl/well (0.1 μg/well) of His-
tagged SARS-CoV-2 spike RBD with the wild type, N501Y or N501Y-
E484K-K417N RBD in 0.05 M NaHCO3 (pH 9.6) overnight at 4°C and
then followed by incubation with a blocking reagent. After blocking, 
100 μL heat-inactivated serum samples at 1:10 dilution or human
monoclonal antibody against SARS-CoV-2 RBD was added to the
wells and incubated at room temperature for 1 h. For normalization,
mouse monoclonal antibody against His-tag (Abclonal, Abclonal, Inc., 
Woburn, MA, USA; Cat# AE003; RRID:AB_2728734) was diluted in a
series of two-fold dilution from 1:12,000 to 1:6,14,000. The attached
human and mouse antibodies were detected using horseradish-per-
oxidase-conjugated goat anti-human IgG (Cat#A18811; RRID
AB_2535588) and anti-mouse IgG antibody (Cat#31430; RRID
AB_228307), respectively (Thermo Fisher Scientific, Invitrogen, Wal-
tham, MA, USA). The reaction was developed by adding diluted
3',3',5',5'-tetramethylbenzidine single solution (Thermo Fisher Scientific, 
Invitrogen; Cat#002023) and stopped with 0.3 N H2SO4. The
optical density (OD) was read at 450 and 620 nm.

For each run, we included two positive samples as positive con-
trol, and an archived anonymous sample from 2018 as negative con-
trol. For OD values greater than 4, a value of 4 is assigned.
Furthermore, we have compared the binding of WT, N501Y and
N501Y-E484K RBD with a SARS-CoV-2 human antibody which was
produced as previously described with modifications [26]. Briefly,
SARS-CoV-2 RBD-specific memory B cells were sorted by multi-laser
Ariall sorter (BD Biosciences, New Jersey, USA) from SARS-CoV-2-infected
individuals. The IgG heavy and light chain variable regions
were amplified independently by nested PCR. Full length IgG1 was
expressed by co-transfecting HEK-293T cells (ATCC Cat#CRL-3216; 
RRID:CVCL_0063) with equal amounts of paired heavy and light
chain plasmids based on the backbone of the pCI-neo mammalian
expression vector (Promega; Cat#E1841). Culture media were har-
vested six days after transfection and purified using protein A agarose
(Thermo Fisher Scientific; Cat#89931). The concentration of protein
was determined by using the Bradford Assay Kit (Bio-Rad; 
Cat#5000002) according to the manufacturer’s instructions.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 26.0 (IBM SPSS Statistics; 
RRID:SCR_019096) and GraphPad PRISM 9.1.1 (GraphPad Software, San Diego, CA, USA; RRID:SCR_002798). For the purpose of
statistical analysis, an MN titer of <20 was considered as 10. The
comparison of log-transformed MN titer was performed using one-
way ANOVA with Dunnett’s multiple comparisons test.

| Difference in microneutralization | No. of patients (%) (n=60) |
|----------------------------------|--------------------------|
| No difference                    | 4 (6.7)                  |
| B117-1 > B-117-2                 |                          |
| ≥2-fold                          | 56 (93.3)                |
| ≥4-fold                          | 35 (58.3)                |
| ≥8-fold                          | 9 (15)                   |

Fig. 2. Comparison of microneutralization titer between viruses from B.1.1.7 lineages (B117-1 and B117-2) and those from non-B.1.1.7 lineages (B1160-1, B13627-1, B1163-1, B1163-2). Data represent the geometric mean of convalescent serum specimens from 9 patients. Error bar indicates 95% confidence interval. One-way ANOVA with Dunnett’s multiple comparison test was used for statistical analysis, with B117-1 as the reference group. **, P < 0.01; ***, P < 0.001.
was determined using one-way ANOVA with Tukey’s multiple comparisons test. The EC50 of monoclonal anti-RBD IgG was determined with the 5-parameter dose-response curve.

2.10. Role of funding source

The funding source had no role in the study design, data collection, data analysis, interpretation, or writing of the manuscript.

3. Results

3.1. Identification of patients with N501Y variants at RBD

Since January 2020 till April 2021, we have sequenced a total of 858 specimens. We have identified 14 patients infected with B.1.1.7 lineage with N501Y alone, 4 patients infected with B.1.351 lineage with N501Y, E484K and K417N, and 1 patient infected with P.3 lineage with N501Y and E484K. All patients were imported cases.

3.2. Microneutralization antibody titers

We have performed MN assay using a total of 9 serum specimens and 6 viruses. The 9 serum specimens were collected from Hong Kong patients, including 3 from second wave (March-April, 2020); 3 from third wave (August 2020); and 3 from fourth wave (November-December 2020) (Tables 1 and Supplementary S2). The median age was 55 years (range 28 to 65 years), and their sera were collected at median of 30 days post-symptom onset (range 18–47 days). For the 6 viruses, 3 were isolated from patients returning from England, including 2 viruses in the B.1.1.7 lineage and 1 virus in the B.1.160 lineage (a non-N501Y lineage); 3 other viruses were isolated from patients who acquired the infection in Hong Kong, including 2 viruses in the B.1.63 lineage collected in July 2020, and 1 virus in the B.1.36.27 lineage collected in December 2020 (Fig. 1 and Supplementary Table S1).

There was no statistically significant difference in MN titer between B117-1 virus and viruses in non-N501Y lineages (Fig. 2). However, for B117-2 virus, there was a statistically significant decrease in MN titer when compared with all non-N501Y lineages. The MN titer of B117-1 (geometric mean titer [GMT], 80; 95% confidence interval [CI], 47–136) were similar to those of non-N501Y viruses, while the MN titer of B117-2 (GMT, 20; 95% CI, 11–36) was statistically significantly reduced when compared with non-N501Y viruses (P < 0.01; repeated measures one-way ANOVA with Dunnett’s multiple comparisons test).

To confirm the difference in the MN titer between the B117-1 and B117-2, we performed the MN assay against B117-1 and B117-2 for an additional 60 serum specimens collected. The MN titer against B117-1 was greater than B117-2 for 93.3% (56/60) of patients (Table 2).

Since there was a large difference in susceptibility to neutralization between the two B.1.1.7 lineage viruses, we compared the whole genome sequences of the two virus culture isolates. There were 3 amino acid differences between the two viruses, including nsp2 amino acid position 512 (B117-1: Y; B117-2: S), nsp13 amino acid position 460 (B117-1: K; B117-2: R) and spike amino acid position 1056 (B117-1: A; B117-2: V). To rule out the possibility of mutations that arose during virus culture, we have also compared the sequence from clinical specimen and from clinical culture isolate but there was no difference. In the GISAID database (as of 25th May 2021), nsp13 K460R was present in 23.7% (156257 of 658890) of sequences within the B.1.1.7 lineage (Supplementary Table S3), while spike A1056V and nsp2 S512Y mutation was present in 0.0127% (84/660147) and 0.0006% (4/657159) of sequences, respectively.

3.3. Comparison of anti-RBD IgG against wild type, N501Y, and N501Y-E484K RBD

Although the neutralizing antibody titers are mainly affected by mutations in the RBD, mutations outside the RBD may also affect the

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**Table 3**

Details of serum specimens from patients infected with SARS-CoV-2 with N501Y mutation.

| Case no. | Month of diagnosis | Virus lineage/variant | Days after symptom onset or hospital admission | Anti-N IgG | Anti-RBD IgG |
|----------|-------------------|-----------------------|-----------------------------------------------|------------|-------------|
| SA1      | December          | B.1.351               | 9                                             | Positive   | Negative    |
| SA2      | February          | B.1.351               | 1                                             | Positive   | Negative    |
| UK1      | December          | B.1.1.7               | 16                                            | Positive   | Positive    |
| UK2      | December          | B.1.1.7               | 10                                            | Negative   | Positive    |
| UK3      | December          | B.1.1.7               | 9                                             | Negative   | Positive    |
| UK4      | December          | B.1.1.7               | 14                                            | Positive   | Negative    |
| UK5      | December          | B.1.1.7               | 13                                            | Positive   | Negative    |

*All diagnosed in 2020.

*Days after hospital admission (patient was asymptomatic).*
neutralizing antibody titer [27]. To eliminate the effect from other mutations, we compared the levels of anti-RBD antibodies using recombinant RBD with or without N501Y mutation for 272 recovered COVID-19 patients’ sera. The normalized OD values for N501Y RBD (mean difference from wild type RBD, 0.1116; standard error [SE] of difference, 0.01405) and N501Y-E484K-K417N RBD (mean difference from wild type RBD, 0.5613; SE of difference, 0.02773) were statistically significantly lower than those from wild type RBD (P < 0.0001, repeated measures one-way ANOVA with Tukey’s multiple comparisons test) (Fig. 3). Furthermore, the normalized OD values for N501Y- E484K-K417N RBD was significantly lower than that of N501Y RBD (mean difference, 0.4497; SE of difference, 0.02648). We have also tested a human anti-RBD monoclonal IgG, but there was no significant difference in the OD between the wild type, N501Y and N501Y- E484K-K417N RBD (Supplementary Fig. S1).

3.4. Performance of commercially-available antibody assays for patients infected with N501Y variants

We assessed the anti-N and anti-spike S1 subunit (containing the RBD) for 7 patients infected with N501Y variant, including 5 patients with B.1.1.7 lineage and 2 patients with B.1.351 lineage. The serum specimens were collected between 9 and 14 days post symptom onset for symptomatic patients. All 7 patients tested positive for anti-N IgG, while only 1 of 7 patients (14.3%) tested anti-S1 IgG positive (Table 3). As controls, we have randomly tested 99 anti-N IgG, while only 1 of 7 patients (14.3%) tested anti-S1 IgG positive. Most early serum specimens from patients infected with non-N501Y viruses, that were collected between 9 and 14 days after symptom onset. Anti-S1 IgG was tested positive in 50% (49/98) of patients.

3.5. Correlation between commercially-available antibody assays and neutralizing antibody titer against B.1.1.7 and non-B.1.1.7 lineage viruses

For the serum specimens from the 9 patients infected with non-B.1.1.7 lineage virus, we performed the Euroimmun anti-S1 IgG assay. The correlation between the Euroimmun ratio and log MN titers was better for non-B.1.1.7 lineage viruses than those of B.1.1.7 lineage viruses (0.59–0.76 for non-B.1.1.7 lineage viruses; 0.16 and 0.37 for B.1.1.7 lineage viruses) (Fig. 4).

4. Discussions

Three SARS-CoV-2 variants with spike N501Y mutation, including B.1.1.7, B.1.351 and P.1, have been classified as “variants of concern” because of increased transmissibility, disease severity, or reduced susceptibility to neutralization by natural infection or vaccine-induced antibodies [28]. This study assessed the impact of N501Y variants on the neutralizing activity of convalescent sera from COVID-19 patients, and on anti-RBD immunoassays. Although one of the B.1.1.7 lineage virus (B117-1) did not exhibit a statistically significant difference with other non-B.1.1.7 lineage viruses, another B.1.1.7 lineage virus (B117-2) with 3 amino acid difference was much more resistant to neutralizing activity in convalescent sera, with > 4-fold reduction in MN titer for most serum specimens tested. The binding of antibody in convalescent sera were statistically significantly lower for N501Y RBD than for wild type RBD. Most early serum specimens from patients infected with B.1.1.7 or B.1.351 lineage viruses tested positive with antibody assay against the N protein but negative against the S1 subunit of the spike protein.

Most previous studies assessing the impact of N501Y variants on the antibody titer used only a single virus isolate in the variant lineage for the determination of neutralizing antibody titer [29,30]. There are conflicting results from these studies, with some showing slight reduction of antibody titers for B.1.1.7 lineage virus [31,32], while others showed no difference [33]. In the current study, we have shown a large difference between the two B.1.1.7 lineage viruses, although there was no difference in the RBD amino acid sequences of these two isolates. Therefore, it is important to select multiple viruses to ensure the generalizability of the results. The difference between these two strains were located at nsp2 (S512Y), nsp13 (K460R), and the spike protein (A1056V). A1056V is located in the S2 subunit, although there was no difference in the RBD amino acid sequences of these two isolates. Therefore, it is important to select multiple viruses to ensure the generalizability of the results. The difference between these two isolates was located at nsp2 (S512Y), nsp13 (K460R), and the spike protein (A1056V), A1056V is located in the S2 subunit, which is required for the fusion of host and viral membrane. It has been suggested S2 can be a target of the neutralizing antibody for SARS-CoV, MERS-CoV and SARS-CoV-2 [34,35]. Indeed, neutralizing antibodies targeting the S2 region have been found [36]. D796H mutation in the S2 region has been proposed to reduce neutralization susceptibility by convalescent plasma and is found in the B.1.1.318 [37,38]. D614G causes conformation change of the spike protein, and therefore mutations in the S2 subunit may affect neutralization by allosteric mechanism [39]. Nsp13 is a helicase and a potent interferon antagonist, while the function of nsp2 is currently unknown. It remains to be determined which of these mutations is responsible for the difference in MN titers.
Our current study showed that N501Y variants may affect the time-to-seropositivity for anti-RBD IgG with the commercial assay, as 6 of the 7 anti-N IgG positive specimens tested negative for anti-RBD IgG. One study showed that the anti-N IgG assay by Abbott had earlier seroconversion than the Euroimmun anti-s1 IgG assay, although the seropositive percentage was above 35% for samples collected 9-10 days after symptom onset and 70% for samples collected on days 13-14 [40]. Our previous study showed that anti-N and anti-RBD IgG appear near the same time [13]. Therefore, anti-RBD IgG induced by infection with N501Y variant may not bind well to wild type RBD. These results suggest that anti-RBD IgG immunoadsay should be modified for a better detection of anti-RBD antibodies. Furthermore, there is a possibility that second generation vaccines containing variant spike RBD may have reduced efficacy against non-N501Y strains.

Our study is specifically designed so that our findings are generalizable. Therefore, we have specifically chosen serum specimens from patients who were infected at different time periods, ranging from March to December 2020 (Table 1). Throughout this period, Hong Kong has experienced 4 major waves of COVID-19, and the virus lineages in each period varies [41]. Furthermore, to ensure that the virus culture isolates do not have mutations that arises during in vitro cell passage, we have sequenced the clinical specimen and the virus culture isolates.

There are several limitations in this study. First, we tested viruses from the B.1.1.7 lineage. As increasing number of variants are identified, testing on other variants are required. Second, the serum specimens were collected from patients in Hong Kong, although we have also selected travelers from other places. A global effort is required to understand how these variants affect the susceptibility of convalescent serum from different countries which may have been infected with unique lineages. Third, the number of patients infected with SARS-CoV-2 variants was small, and the anti-S1 antibody testing was performed with only a single commercial assay due to limited volume of the serum specimens. A larger cohort would be required to verify whether the variants will affect the performance of different serological assays.

Increasing number of variants with mutations at the spike RBD have been identified. The results from neutralization assays and anti-RBD antibody assays play a key role in the risk assessment of these variants. We demonstrated the large difference in neutralization antibody titer within the same variant lineage. Differences outside the RBD play an important role in neutralizing antibody titer. Therefore, it would be important to determine antigenic drift not only on variants with mutations in the RBD, but also with mutations outside RBD in the spike protein, or even outside the spike protein.

Contributors

LL and KKWT had roles in study design, data collection, data analysis, data interpretation, literature search and writing of the manuscript. DCL, ART, YSY, MYWK, WKT, OTYT, LLYL, VCC and IFNH had roles in recruitment, data collection, and/or clinical management. AWHC, RRZ, WMC, JDI, HWT, LLC, JPC, HY, TCI, RWSP, GKHS and L. Lu et al. / EBioMedicine 71 (2021) 103544 7

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2021.103544.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2021.103544.

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