THE LANCET
Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Kai-Wang To K, Tsang O T-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020; published online March 23. https://doi.org/10.1016/S1473-3099(20)30196-1.

APPENDIX

SUPPLEMENTARY METHODS

Nucleic acid extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for SARS-CoV-2

Total nucleic acid (TNA) extraction was performed using NUCLISENS® easyMAG® nucleic acid extraction system (bioMérieux, Marcy l'Etoile, France). An in-house real-time RT-qPCR targeting the SARS-CoV-2 RNA-dependent-RNA-polymerase (RdRp)-helicase gene region was performed using QuantiNova Probe RT-PCR Kit (QIAGEN, Hilden, Germany) as we described previously [1]. The sequences of primers and probe are: Forward primer (5'-CGCATACAGTCTTRCAGGCT -3'); reverse primer (5'-GTGTGATGTTGAWATGACATGGTC -3'); probe (5' FAM-TTAAGATGTGGTGCTTGCATACGTAGAC -IABkFQ 3'). The reagent mixture (20 μL) contained 10 μl of 2x QuantiNova Probe RT-PCR Master Mix, 0.2 μl of QN Probe RT-Mix, 1.6 μl of each 10 μM forward and reverse primer, 0.4 μl of 10 μM probe, 1.2 μl of RNase-free water and 5 μl of TNA as the template. The thermal cycling condition was 45 °C for 10 min (reverse transcription), 95 °C for 5 min (PCR initial activation), followed by 45 cycles of 95 °C for 5 s and 55 °C for 30 s. All reactions were performed using the LightCycler® 480 II Real-Time PCR System (Roche, Basel, Switzerland).

Cloning and purification of (His)6-tagged recombinant receptor binding domain (RBD) and nucleocapsid protein (NP) of SARS-CoV-2

The genes encoding the spike RBD (amino acid residues 306 to 543 of the spike protein) and full length NP of SARS-CoV-2 were codon-optimized, synthesized and cloned into the *NdeI*

site and *XhoI* site of expression vector pET-28b(+) (Novagen, Madison, WI, USA) in frame respective and upstream of the series of six histidine residues. The recombinant RBD and NP were expressed and purified using the $Ni2^{+}$-loaded HiTrap Chelating System (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions. The purity of RBD and NP was assessed by sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and western blotting (Figure 1). Nucleoprotein of severe fever with thrombocytopenia syndrome virus was used as a positive control [2]. The purified RBD and NP were separated electrophoretically in a 12% gel and transferred to a nitrocellulose membrane. After blocking with 3% BSA and 7% skim milk (Sigma-Aldrich), the membrane was incubated with anti-His monoclonal antibodies at a dilution of 1:4000 (Abcam, Cambridge, MA) for 1 hour at room temperature. After washing, the membrane was incubated with 1:4000 diluted goat anti-mouse horseradish peroxidase (Sigma-Aldrich) for 30 mins at room temperature, and developed by incubation with Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare, Fairfield, CT). The concentration of purified RBD and NP were determined by using the Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. Nucleoprotein of severe fever with thrombocytopenia syndrome virus was used as a positive control [2].

Enzyme immunoassay (EIA) for NP and spike RBD

Briefly, 96-well immunoplates (Nunc Immuno modules; Nunc, Denmark) were coated with 100 μl/well (0.1 μg/well) of SARS-CoV-2 NP or spike 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 1:100 dilution was added to the wells and incubated at room temperature for 1 h. For the determination of IgM, the serum was first mixed with an IgG

inactivation agent (Gullsorb) to inactivate serum IgG. The attached antibodies were detected using horseradish-peroxidase-conjugated goat anti-human IgG or IgM antibody (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The reaction was developed by adding diluted 3,3',5,5'-tetramethylbenzidine single solution and stopped with 0.3 N H_2SO_4. The optical density (OD) was read at 450 nm. A single positive sample was included in each run as positive control. An archived anonymous sample from 2018 used in our previous study was used as negative control [3]. To determine the cutoff value for positivity, the mean value of 93 anonymous archived serum specimens from 2018 plus 3 standard deviations was used as the cutoff. The cutoff values are: anti-NP IgG, 0.523; anti-NP IgM, 0.177; anti-RBD IgG, 0.108; and anti-RBD IgM, 0.085. The original Western blot film of Figure 1D is shown in Supplementary Figure S4.

For competitive EIA, serially-diluted heat-inactivated serum samples (from 1:100 to 1:12800) were mixed with 10 μg SARS-CoV-2 NP or spike RBD, and incubated at 37°C for 1 h before adding to 96-well immunoplates as in the EIA assay described above. The OD450 of serum samples that were pre-mixed with NP or RBD was compared with those that were not pre-mixed.

Microneutralization assay

Microneutralization (MN) assay and virus culture were performed as we described previously [4,5]. Briefly, serum samples were serially diluted in 2-folds with minimum essential medium from 1:10 to 1:320. Diluted sera were mixed with 100 $TCID_{50}$ of SARS-CoV-2 and incubated at 37°C for 1 hour. The mixture was added to VeroE6 cells and incubated at 37°C and 5% CO_2. Cytopathic effect was determined by examination under inversion microscopy after 72

hours of incubation. The microneutralization antibody titer was the highest dilution with 50% inhibition of cytopathic effect.

Whole genome sequencing and genome analysis by bioinformatics

Whole genome sequencing was performed using the Oxford Nanopore MinION device (Oxford Nanopore Technologies) as we described previously [6]. Briefly, to deplete host cells, respiratory specimens were centrifuged at 16,000 × g for 2 min, and the supernatant was used for subsequent RNA extraction. RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). DNase treatment (TURBO DNA-free Kit, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used in removing residual host DNA. Whole genome amplification of SARS-CoV-2 was performed using sequence-independent single-primer amplification (SISPA) approach. Bioinformatics analysis was performed using an in-house pipeline. The consensus sequences and raw reads have been deposited into GenBank (Accession number: MT114412-19) and Bioproject (Acession number: PRJNA608242).

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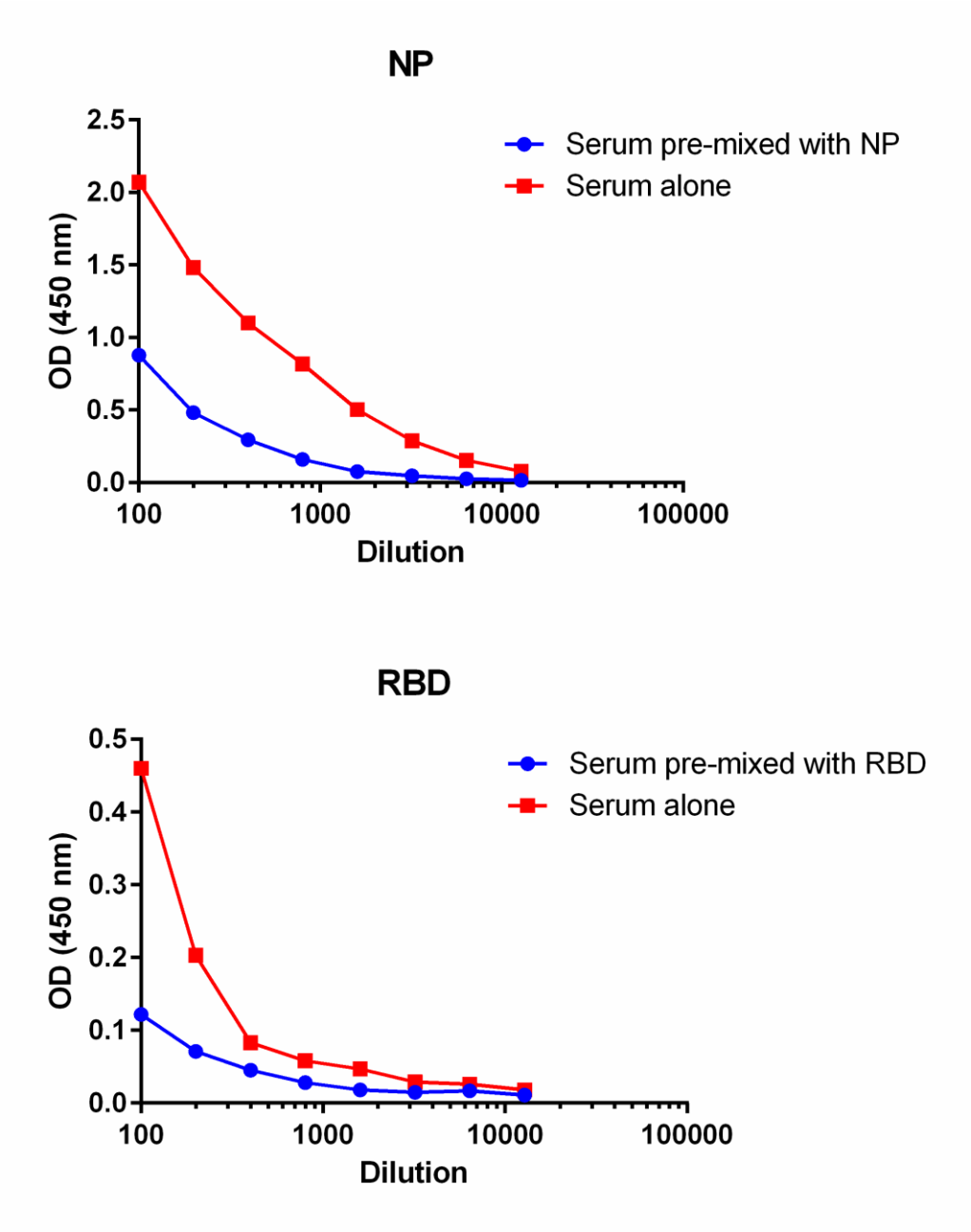
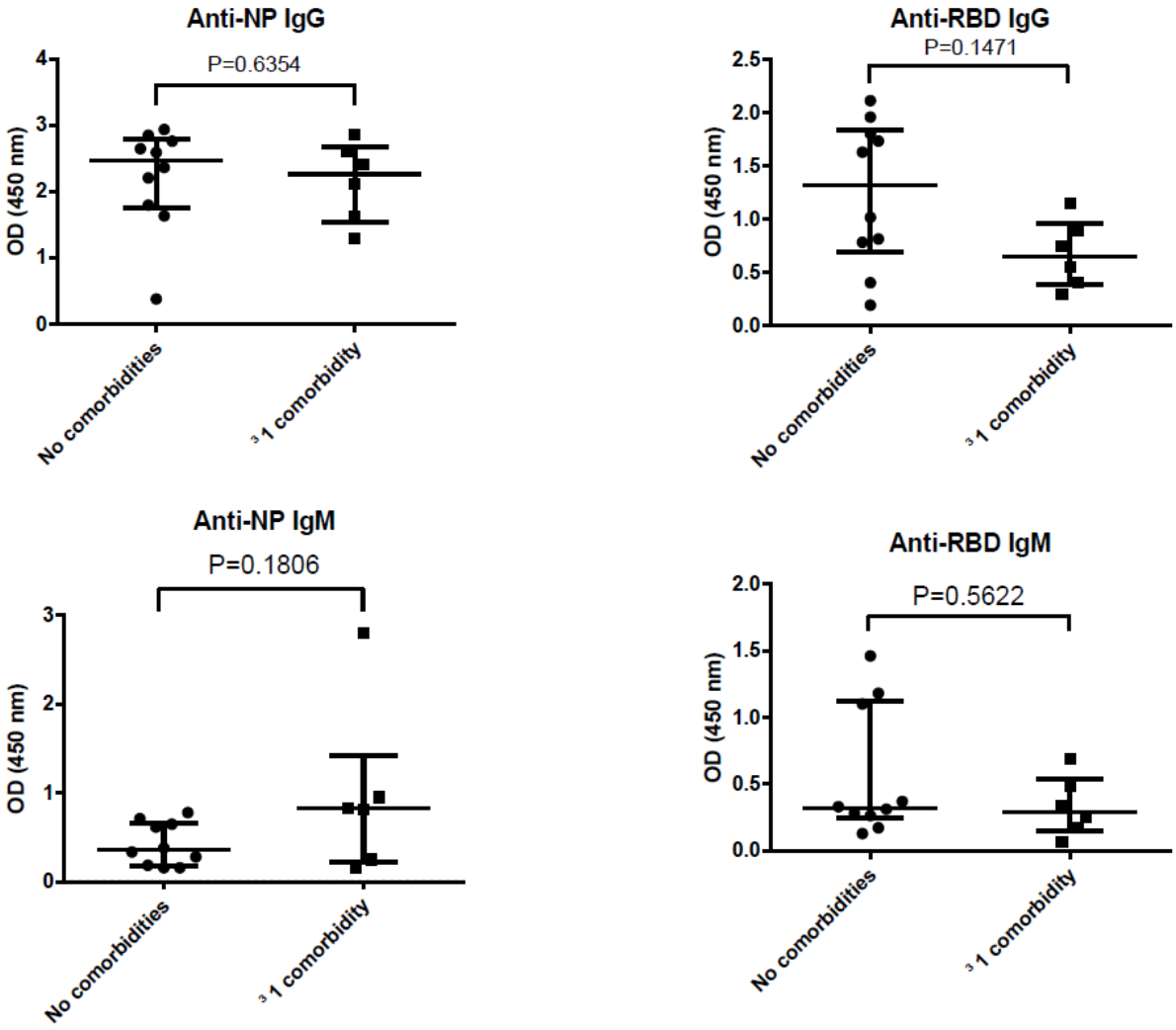
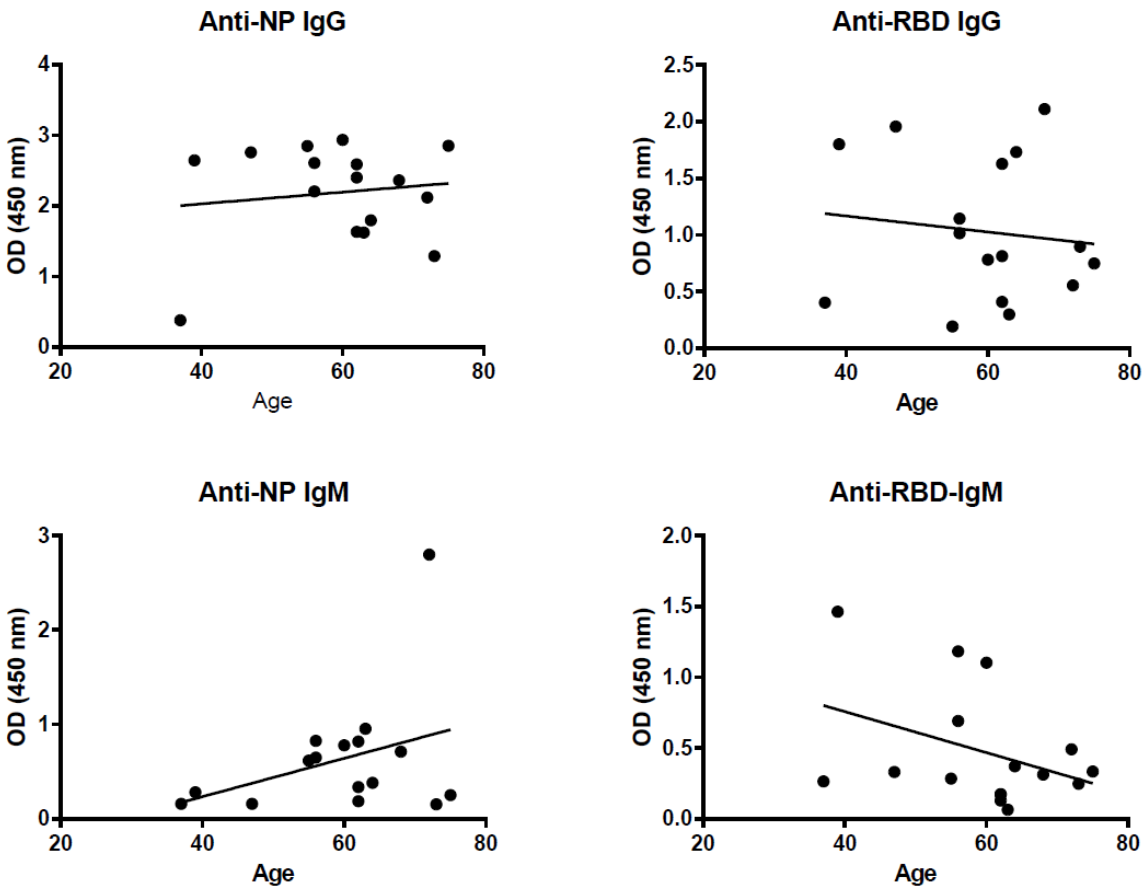
Supplementary Figure S1. Competitive enzyme immunoassay for SARS-CoV-2 NP and RBD IgG



Supplementary Figure S2. Comparison of antibody titers between patients with and without comorbidities



Supplementary Figure S3. Correlation between age and antibody titers.



Supplementary Figure S4. Original Western blot films of Figure 1D.