Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letters to the Editor

Immune responses to COVID-19 vaccine BNT162b2 in workers at a research institute in Japan: 6-month follow-up survey

Dear editor,

We read with interest the article by Tré-Hardy et al. on the kinetics of anti-spike IgG (S-IgG) levels after vaccination among healthcare workers who received the mRNA-1273 (Moderna) vaccine.[1] The authors reported that the antibody levels markedly reduced between 3 and 6 months after the second dose. We also investigated the kinetics of S-IgG levels for 6 months after receiving the BNT162b2 (Pfizer-BioNTech) vaccine and the *T*-cell response among low responders in a cohort of workers at the National Center for Geriatrics and Gerontology, including a hospital and a research institute, in Japan.

We have previously reported that seroprevalence against the nucleocapsid of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among our staff was equivalent to that observed among the local community and that 99.4% of the participants had S-IgG.[2,3] However, the kinetics of the antibody titer against S-IgG after vaccination remain unclear. Additionally, T-cell-mediated cellular immunity may affect COVID-19 recovery, even with a low antibody response.[4] The relationship between humoral and cellular immune responses to vaccination has been rarely investigated.

Of the 878 employees, 800 agreed to participate in the survey (participation rate: 91.1%). They received the first vaccination between February and June 2021 and the second dose 3 weeks later. Blood samples were obtained between June 14 and 18, 2021. This study was approved by the Institutional Review Board of the Ethics and Conflicts of Interest Committee (approval no 1481). All participants provided written informed consent.

We performed all laboratory tests in-house using two S-IgG chemiluminescence enzyme immunoassays: the SARS-CoV-2 S-IgG from Sysmex and ARCHITECT SARS-CoV-2 IgG assay from Abbott. The positive cutoff value was 20 BAU/mL for Sysmex's and 50 AU/mL for Abbot's assays, respectively.

The participants' characteristics are summarized in Table S1 ($N = 800$). The mean age ± SD was 41.0 ± 11.6 years, and 66.4% were women. Clinical staff (doctors, nurses, and allied healthcare professionals) accounted for 63.8%, whereas the others were engaged in basic research and investigation, general office duties, and other nonclinical work. Most participants ($n = 642$, 80.3%) received two doses of BNT162b2 vaccination at least 1 week before their annual health checkups in June. Seven participants received the second dose in March, seven in April, 515 in May, and 113 in June.

Of the 527 eligible participants who had received both vaccine doses by May 2021, all were positive for S-IgG except one participant. An age-dependent decline in antibody response was observed with Spearman correlation coefficient of –0.305 for the Sysmex tests ($p < 0.001$; Fig. 1A). Women tended to develop a higher S-IgG titer in each age group. The difference was significant in the age group ≥51 years (Mann–Whitney U test with Bonferroni correction, $p < 0.001$), and the age difference was statistically significant regardless of sex (Kruskal–Wallis test, $p < 0.001$) (Fig. 1B).

The antibody titer tended to decline post-vaccination in a time-dependent manner, with a Spearman correlation coefficient of −0.325 ($p < 0.001$; Fig. 1C). The median titer of samples collected 6–20 days after the second vaccination ($n = 118$) was 2636 BAU/mL for the Sysmex test. Among the individuals who received the second dose 21–50 days before the survey ($n = 512$), the median antibody titers decreased to 1599 BAU/mL. The antibody titer of participants who received the second dose >50 days before sample collection ($n = 12$) was reduced to 28.5%. Similar results were also obtained in the Abbott test (Fig. S1).

Seven participants with low antibody titers were invited to the additional investigation of T-cell-dependent cellular immunity in October 2021. We collected peripheral blood mononuclear cells and detected T cells secreting interferon-γ (IFN-γ) in response to SARS-CoV-2 peptides using the T-SPOT Discovery SARS-CoV-2 kit (Oxford Immunotec). Two participants had a count that was comparable with those showing higher antibody responses. One participant was receiving an immune suppressant for kidney transplantation; he showed negative responses in the cellular immunity and seroprevalence tests. Two participants— one was immunocompromised and the other had a history of Klippel–Trénanay–Weber syndrome and protein-losing gastroenteropathy—with a titer of <50 also showed negative responses. A 20-week pregnant woman showed a negative result in the cellular immunity test, although her antibody titers were relatively high compared with that of the other participants.

Further, 297 participants received additional medical checkups on December 16 or 17, 2021, who are required for employees engaged in specific work activities, such as night shift, donated a blood sample. The participant characteristics in the follow-up survey are presented in Table S2. A sharp decline in S-IgG titer was observed in the 6 months after receiving both vaccine doses (Fig. 2A). Median titers were reduced by 88.4% (from 2821 to 327 BAU/mL) for those who participated in the June survey 6–20 days at post-vaccination and by 88.9% (1537 to 172 BAU/mL) for those who participated at 21–50 days. However, most participants ($n = 293$, 98.7%) maintained a positive humoral immune response. Sex-dependent differences were not observed, whereas the titer significantly declined with age, with a Spearman correlation coefficient of −0.248 (Fig. 2B, C).

Consistent with the previous reports,[1,5,6] the anti-spike antibody titer resulting from mRNA vaccination was markedly reduced within a few months and continued to decline thereafter, sug-

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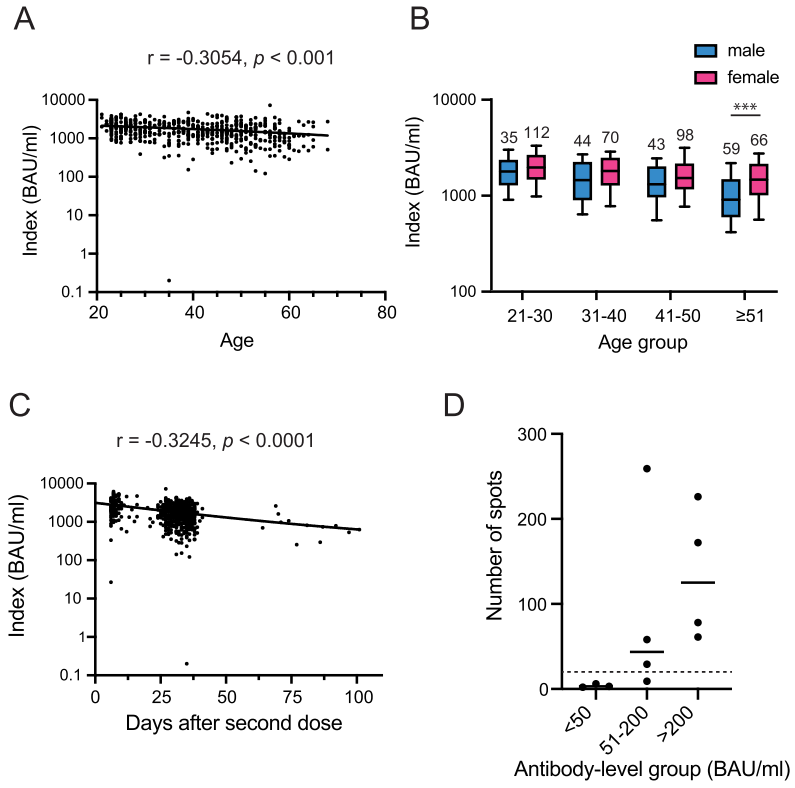

Fig. 1. Antibody titer and *T*-cell response following the second dose of the BNT162b2 vaccine. (A) Correlation between age and S-IgG levels 1–4 months after the second vaccination dose was measured using Sysmex test. Spearman r, as well as *p*-values, are presented in each graph. (B) Comparison of antibody levels among different age groups and between the sexes. Data are expressed as box plots: middle line, median; box edges, 25th–75th centile. $^{***}p < 0.001$. (C) Kinetics of the antibody titers following the second dose of the vaccine. Correlation between S-IgG levels and the number of days after the second dose. (D) Cellular immunity of participants who failed to develop antibodies against SARS-CoV-2 after the second dose of the vaccine. The number of spot-forming cells per 250,000 in the panel 1 of T-SPOT test was plotted based on the antibody-level groups. The horizontal solid lines indicate the mean of spot-forming cell numbers. The dashed line indicates the threshold of T-SPOT values.

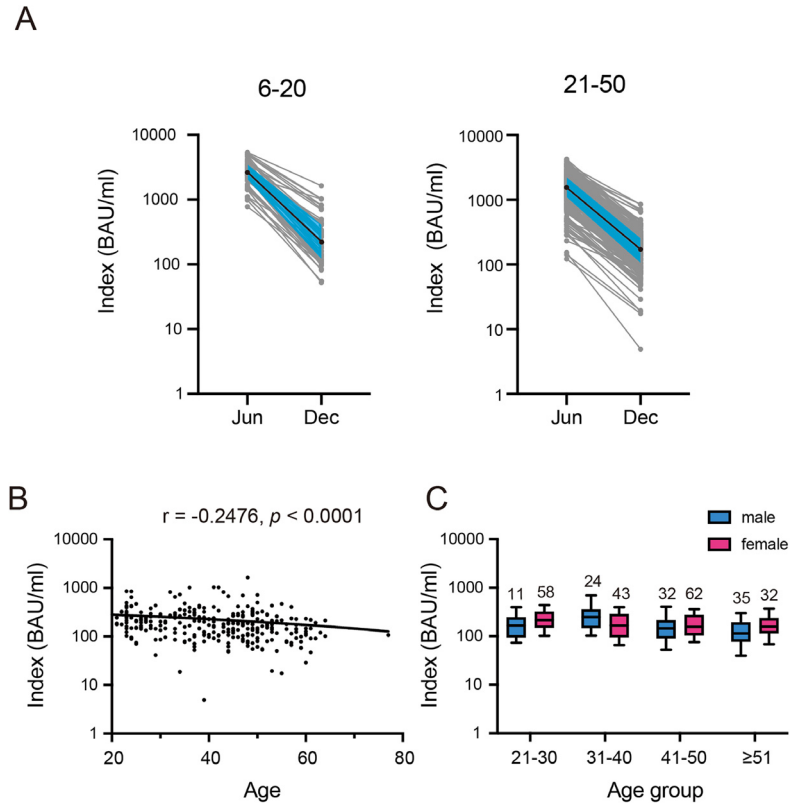

Fig. 2. Anti-spike IgG levels at 5–6 months following the second dose of the BNT162b2 vaccine. (A) Kinetics of anti-spike IgG levels of each participant as measured by the Sysmex test. The median values of each data point are displayed as solid black circles connected with solid black lines. Shaded areas in blue indicate the 25%–75% percentile. (B) Correlation between age and S-IgG levels in the December survey as measured with the Sysmex test. (C) Comparison of antibody levels among the different age groups and between the sexes. Data are expressed as box plots: middle line, median; box edges, 25th–75th centile.

gesting the requirement of additional doses. Conversely, cellular responses, which remain broad to wild type and variants,[7] were maintained even at 5–6 months after the second dose in those who developed a sufficient antibody titer. A survey of larger cohorts in the future is warranted to confirm our observation.

Funding

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Data availability statement

Not applicable.

Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.016.

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Bioavailable testosterone level is associated with COVID-19 severity in female: A sex-stratified Mendelian randomization study

Dear editor,

Recently in this Journal, Sergey Moiseev et al. reported that the mortality rate in intensive care unit patients with severe COVID-19 was higher in males older than 50 years old in contrast to females of similar age based on a nationwide cohort study.[1] The sex differences between mortality rates cannot be explained by comorbidities, given the increased occurrence of chronic illnesses in females that may worsen survival in COVID-19 patients. The results initially supported that sex steroid hormones underlie sex-related

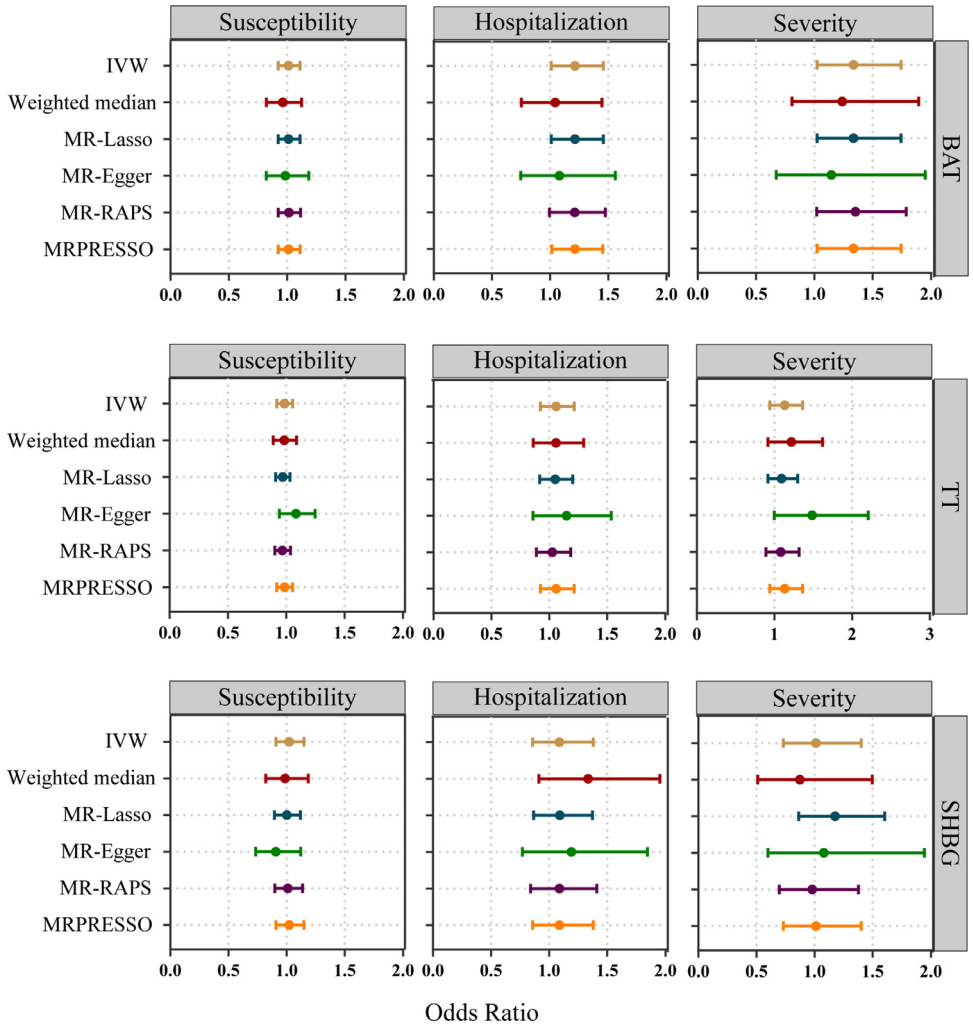

Fig. 1. The Mendelian randomization analysis of casual effect between sex hormones and COVID-19 outcomes in females. IVW: inverse variance weighted; BAT: bioavailable testosterone; TT: total testosterone; SHBG: sex hormone binding globulin.

differences in COVID-19 mortality. However, the alternations of sex hormones were accompanied by changes of many risk factors for severity and mortality of COVID-19, such as obesity, diabetes, and other comorbidities. It was unconvincing to judge the causality between sex hormones and COVID-19 outcomes based on epidemiological data due to above-confounding factors. Mendelian randomization (MR) analysis is an analytic method to estimate the causal effect, which overcome the limitations of measurement errors and confounding frequently encountered in observational studies. In this study, we performed a sex-stratified two-sample MR analysis to explore the causal relationship of serum sex hormone levels including bioavailable testosterone (BAT), total testosterone (TT), and sex hormone binding globulin (SHBG) on COVID-19 outcomes.

We obtained summary statistics for sex hormones including BAT, TT, and SHBG from previous genome-wide association analyses study based on genetic data in UK Biobank.[2] The datasets of sex hormones could be downloaded from https://www.ebi.ac.uk/gwas/. The GWAS summary statistics for COVID-19 susceptibility (C2: covid vs population), hospitalization (B2: hospitalized covid vs population) and severe disease (A2: critically ill covid vs population) outcomes were obtained from the COVID-19 Host Genetics Initiative (https://www.covid19hg.org/, Release 5, European ancestry cohorts but excluded UKBB). The selection of instrumental variables was followed by previous procedure (1) selected SNPs which effect on sex hormones was significant ($P<5 \times 10–8$); (2) matched these SNPs with the outcome dataset by rsid; (3) obtained independent SNPs through clumping procedure based on linkage disequilibrium with $r^2 < 0.001$ or the physical distance more than 10 000 kb via PLINK; (4) removed SNPs that were significantly associated with the outcome.[3] We calculated the proportion of variance explained for all remaining instruments to evaluate the strength of instrument variables. To investigate the causal effect, we performed the MR analysis via inverse variance weighted (IVW), weighted median, MR-Egger, MR-RAPS, MR-Lasso, and MRPRESSO method within males and females separately. All computations were performed using the Mendelian Randomization package.

In the study, we obtained 208, 141, and 216 instruments separately of serum TT, BAT, and SHBG levels when studied causal effect of sex hormones and COVID-19 susceptibility in females, which could explain 7.42%, 5.44% and 10.95% of the variance of TT, BAT and SHBG respectively. The number of instruments and the PVE for instruments used in analysis of causal effect of sex hormone on COVID-19 hospitalization and severe disease was approximately same as analysis in COVID-19 susceptibility. The results showed that each one standard deviation (SD) increase in serum BAT levels was associated with a higher risk of COVID-19 hospitalization (OR:1.214, 95% CI:1.011–1.458, $p = 0.038$) and COVID-

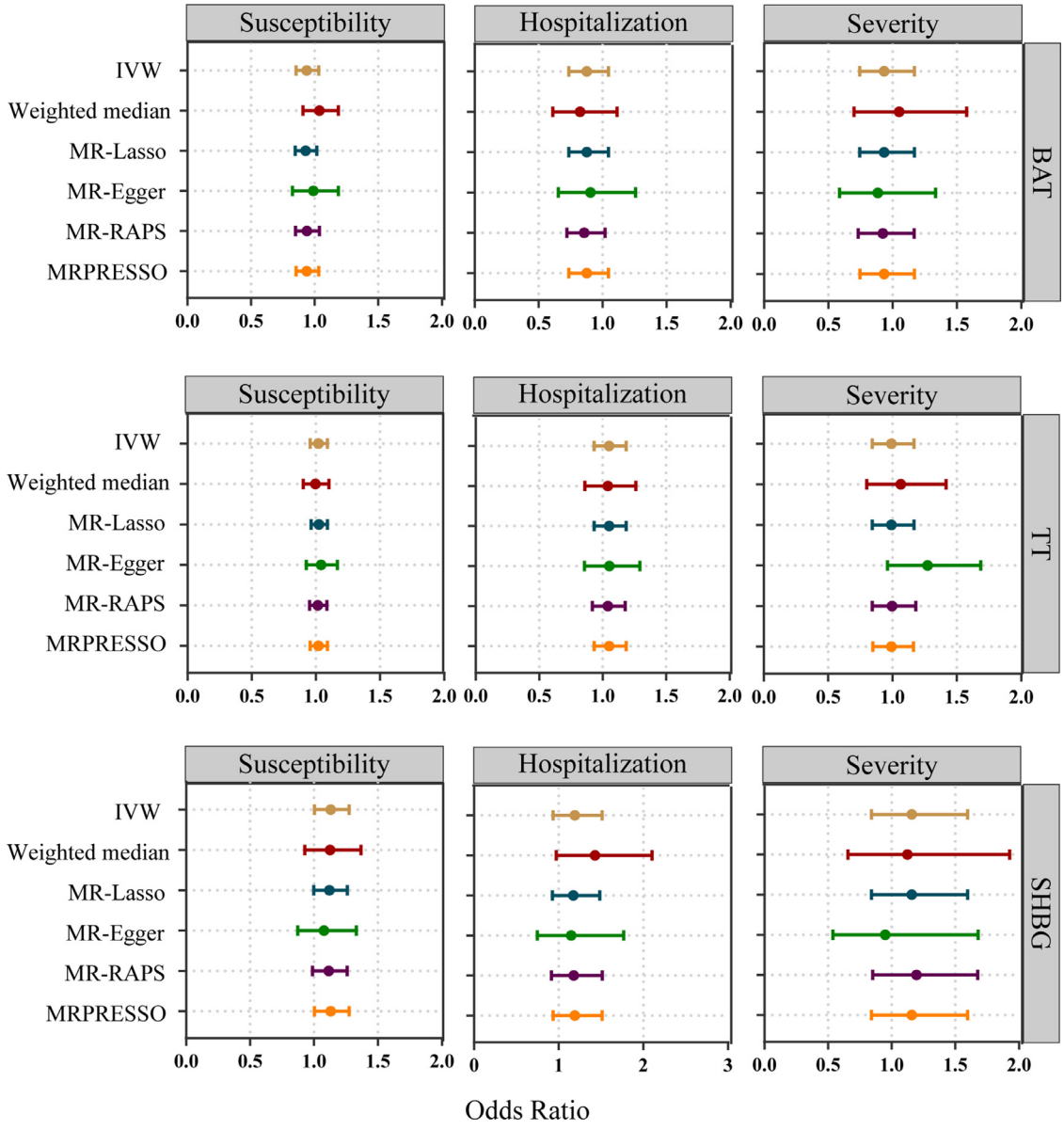

Fig. 2. The Mendelian randomization analysis of casual effect between sex hormones and COVID-19 outcomes in males. IVW: inverse variance weighted; BAT: bioavailable testosterone; TT: total testosterone; SHBG: sex hormone binding globulin.

19 severe disease (OR:1.336, 95% CI:1.024–1.744, $p = 0.033$) based on IVW method, and the results were verified by MR-Lasso, MR-PRESSO method (Fig. 1). For a SD increase in serum BAT level, we observed no statistically significant effect upon odds of COVID-19 susceptibility ($p = 0.800$) (Fig. 1). Similarly, we observed no significant difference in risk of COVID-19 outcomes associated with an SD increase in serum TT or SHBG levels based on all MR analysis methods (Fig. 1). The results suggested a causal effect of increased serum BAT levels on the higher risk of COVID-19 hospitalization and severe disease in females. No statistically significant difference in risk of COVID-19 outcomes associated with a SD increase in serum BAT, TT or SHBG levels was found in males (Fig. 2). The results demonstrated no causal relationship between sex hormones and COVID-19 outcomes in males.

In summary, we performed a sex-stratified two-sample Mendelian randomization analysis and demonstrated a causal relationship of increased BAT levels and higher risk of COVID-19 hospitalization and severe disease in females, not in males. A null causal relationship was observed for TT or SHBG levels with COVID-19 outcomes in females and males. The relationships between gender difference, sex hormone difference and COVID-19 outcomes have been reported based on several observational studies[1,4] and various hypotheses have been postulated to explain the relationships, including a sex-dependent difference in immune responses, sex-related expression difference of angiotensin-converting enzyme 2 and transmembrane protease serine 2.[5–7] Our study demonstrated a direct causal effect of BAT levels and COVID-19 hospitalization and severe disease in females based on MR analysis. The MR analysis exploited the natural random allocation of genetic variants and limited the potential confounding and reverse causal effect, thus providing powerful evidence for the causal effect of testosterone on COVID-19 outcomes.

In addition, the BAT referred to testosterone loosely bound to albumin and free form testosterone, which participated in the biological process in vivo and therefore may become more relevant to proposed causal relationship. This is consistent with our results that only BAT levels have a causal effect on COVID-19 outcomes, but not serum TT and SHBG levels. Another innovative

point in our present study was that the causal relationship of BAT and COVID-19 outcomes was only observed in females, but not in males. We inferred that the causal relationship between BAT and COVID-19 outcomes may be non-linear, and both high BAT and low BAT levels were risk factor for the poor prognosis of COVID-19. Further studies based on male population excluded patients with testosterone deficiency are needed to verify the relationship. In the study, we were unable to obtain gender-specific GWAS data of COVID-19 outcomes from update datasets, further study should be performed. Then, the findings that BAT increased the risk of COVID-19 hospitalization and severe disease in females helped better understand the role of sex hormones in COVID-19 occurrence and progression, and provided evidence for hormone therapy in the treatment of COVID-19 in females.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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A child with acute respiratory distress syndrome caused by avian influenza H3N8 virus

Dear editor,

Recently in this Journal, Li and colleagues showed that wild bird-origin H3N8 avian influenza virus can potentially adapt well to a mammalian host [1]. This suggests that H3N8 virus may pose a potential threat to human health. Several previous studies have also shown that H3N8 virus are associated with persistent infection outbreaks in dogs and horses, and have been isolated from pigs, donkeys, and most recently seals [2–4]. A case of acute respiratory distress syndrome in children caused by H3N8 influenza virus has been identified (Fig. 1).

A 4-year-old boy with no significant medical history was transferred to the pediatric intensive care unit of Henan Provincial People's Hospital on April 10, 2022. His chief complaints were fever, drowsiness for 5 days, cough for 2 days, dyspnea for 1 day and 5 h after extracorporeal membrane oxygenation (ECMO). Two days before admission, the child came to the local hospital, where he was considered to have community-acquired pneumonia and received antibiotics and aerosol therapy. One day before admission, the child developed dyspnea and hypoxemia, and was transferred to Zhumadian Central Hospital for endotracheal intubation and ventilator assisted breathing. On April 10, alveolar lavage fluid and peripheral blood samples were taken for metagenomics next-generation sequencing(mNGS). Chest CT showed pneumonia in the upper and lower lobe of the right lung and left lung (Fig. 2A). After 1 day of treatment with antibiotics and "oseltamivir", the fluctuation of blood oxygen saturation under high ventilator parameters was 60–80%, and the oxygenation index was 50. ECMO was recommended. With the consent of the children's parents, the ECMO team of our hospital rushed to the local hospital for femoral vein-internal jugular vein ECMO catheterization 5 h before admission to our hospital. The mNGS results of the bronchoalveolar lavage fluid and peripheral blood samples collected on April 10 showed that 7,888,701 reads suspected H3N8 viruses were detected in the bronchoalveolar lavage fluid and 192 reads in the blood. After admission, patients were given ECMO combined with ventilator for assisted respiration,"oseltamivir" for antiviral, "meropenem" for anti-infection, "methylprednisolone sodium succinate" to reduce inflammation, "plasma and immunoglobulin" infused and other treatments. On April 12, bronchoalveolar lavage

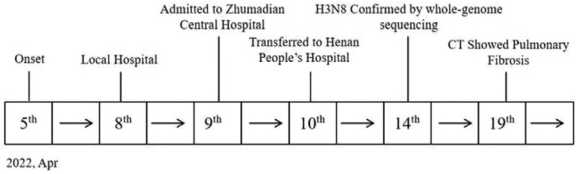

Fig. 1. Timeline of the avian-origin H3N8 patient's illness from onset of disease to the time when CT show pulmonary fibrosis.

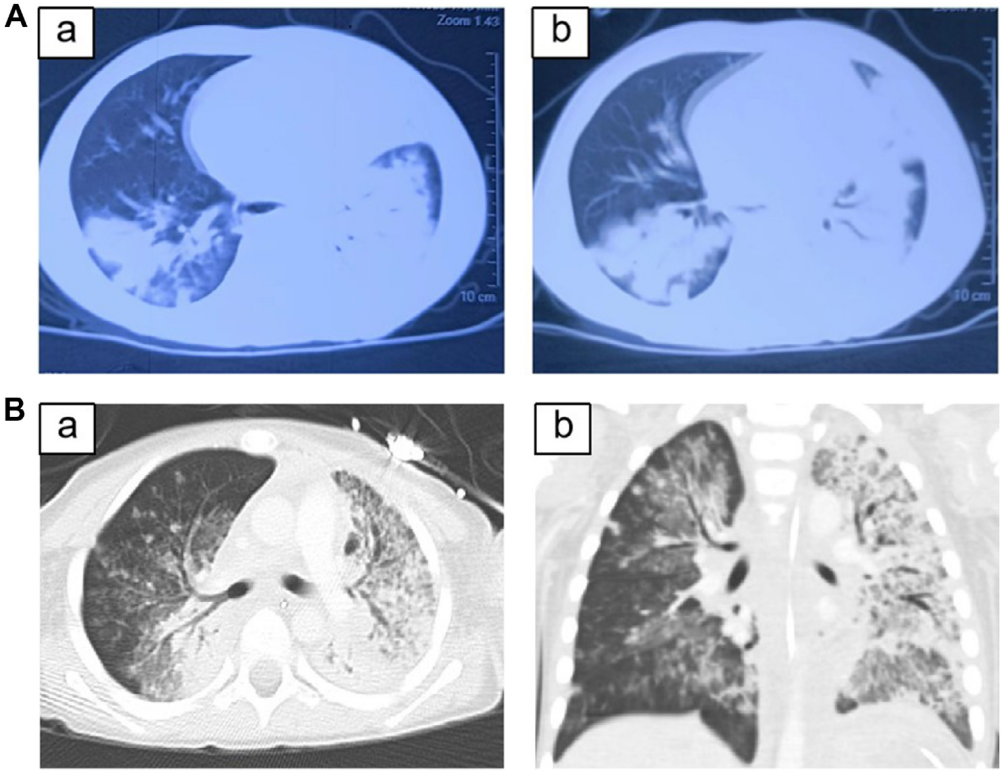

Fig. 2. Chest CT scan on April 10, 2022. (a) Large patchy high-density shadows in the right lower lobe. (b) Consolidation of the left lower lobe with air bronchus sign. Fig 2B. Chest CT scan on April 19, 2022. (a) Interstitial changes and consolidation in the lungs with obvious small airway involvement. (b) Coronal image of chest CT.

was performed again. The mNGS re-examination showed 183 reads virus sequences in the bronchoalveolar lavage fluid, and no virus sequences was detected in the blood. Part of bronchoalveolar lavage fluid sample was sent to the Center for Disease Control and Prevention. Whole genome sequencing confirmed the positive for avian H3N8 influenza virus, and H3N8 influenza virus was successfully isolated from the bronchoalveolar lavage fluid. On April 19, chest CT showed extensive interstitial changes and consolidation in the lungs, with obvious small airway involvement(Fig. 2B). Multiple deep lavage was performed with bronchoscope with an outer diameter of 2.8 mm. On April 20, the number of virus sequences in the bronchoalveolar lavage fluid decreased to 10 reads, and no virus was detected in peripheral blood. The child is in critical condition and is still receiving ECMO combined with ventilator support so far.

In 2014, Karlsson et al. found that the H3N8 avian influenza virus isolated from seals showed high affinity for mammalian receptors, could be transmitted via respiratory droplets, and could replicate efficiently in human lung cells in vitro [3]. Later studies by Hussein et al. also confirmed that seal H3N8 virus and bird H3N8 virus can bind to human lung tissue and replicate in human lung cancer cells [5]. These studies suggest the possibility of H3N8 virus transmission to humans. This case is the first human case of H3N8 infection. Fortunately, so far, no other cases of infection have been found in close contact with the child.

This patient started with mental symptoms of fever and lethargy, and then developed severe acute respiratory distress syndrome in a short period of time. The H3N8 virus can still be detected in the lavage fluid on the 11th day of admission, which is similar to other severe avian influenza such as H7N9, and the clearance rate in the body is slower. Pan et al. found that the time from the onset of infection to the virus turning negative in 13 cases of severe human infection with H7N9 avian influenza was (15.9 ± 6.4) days, and the time from antiviral treatment to the virus turning negative was (9.8 ± 7.4) days [6]. In this case, chest CT review on the 9th day of admission revealed partial pulmonary fibrosis, which may be related to the pulmonary interstitial and diffuse alveolar damage caused by virus. When healthy female mice were infected with H3N8 virus, the lung tissue of mice showed progressively aggravated interstitial inflammatory hyperemia at 3 and 6 days after infection [7]. There are no reports on lung imaging of H3N8-related cases, but some studies have shown that the absorption time of severe H7N9 avian influenza lesions is more than 1 month, and residual fibrotic lesions such as grid-like or cord-like are still gradually absorbed in patients with 1-year follow-up [6]. This suggests that even if the patient in this case can be cured and discharged, it is still necessary to follow up the child's imaging prognosis for a long time.

Declaration of Competing Interest

The authors have no competing interests to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.007.

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A novel clinical therapy to combat infections caused by Hypervirulent Carbapenem-Resistant *Klebsiella pneumoniae*

Dear editor,

We read with great interest on a recent study by Tao Lou and colleagues entitled "Risk factors for infection and mortality caused by carbapenem-resistant *Klebsiella pneumoniae*: A large multicentre case-control and cohort study" in this journal.[1] Carbapenem-resistant *K. pneumoniae* (CRKP) has been increasingly reported in China reaching 21.9% of *K. pneumoniae* infections in hospitals according to CHINET (China Antimicrobial Surveillance Network) 2021 report (http://www.chinets.com).[2] Data from this study showed a very high mortality rate caused by CRKP infections, reaching 24.2% for the 28-day crude mortality and over 45% for blood-stream infections. It is urgent to develop novel therapies to combat the infections caused by CRKP in China. Here, we report a novel and effective therapy that we have developed to treat infections caused by CRKP and Carbapenem-resistant and hypervirulent *K. pneumoniae* (CR-HvKP) that exhibited a much higher mortality rate than CRKP through oral administration.[3]

We screened over 1500 FDA-approved pharmaceutical compounds from a commercial drug library (Selleckchem, Drug library L1300)[4] and found a compound, zidovudine (a HIV drug), which exhibited antibacterial effect on various species of Gram-negative bacteria including *E. coli, P. aeruginosa, Salmonella* spp., and *K. pneumoniae*. The MIC of zidovudine on 30 clinical strains of CR-HvKP was determined and shown to be ranged from 1.25 to 5 μg/mL. Further tests showed that rifampicin exhibited very strong synergistic effect with zidovudine. The MICs of rifampicin alone on these clinical CR-HvKP strains were between 16 μg/mL to >64 μg/mL. However, when rifampicin was used in combination with zidovudine in a ratio of 8:5, all these 30 strains exhibited much lower MIC, ranging from 0.25 / 0.15625 μg/mL to 4 / 2.5 μg/mL (zidovudine / rifampicin).

A clinical CR-HvKP 1 strain was selected to perform further tests. Time-killing assay showed that at 20 μg/mL of zidovudine, the population size of CR-HvKP 1 was reduced at the first 4 h and then re-grew to reach the same size as no treatment control at 24 h; at 128 μg/mL of rifampicin, the bacterial population reduced at 6 h, but regrew after 24 h, suggesting that CR-HvKP 1 could develop resistance to zidovudine or rifampicin after long term treatment (Fig. 1a, b). When zidovudine and rifampicin were simultaneously present at 10 μg/mL and 16 μg/mL respectively, CR-HvKP 1 could be eradicated within 24 h, confirming the synergistic antimicrobial effect of these two drugs (Fig. 1c). The synergistic effect was further tested in the murine sepsis infection model, where the ICR mice were injected with 3×10^7 CFU of CR-HvKP 1 intraperitoneally. Treatment of different combinations of zidovudine and rifampicin were applied to the mice through oral gavage at 1-hour post-infection and every 12-hour intervals. The mortality rate of mice without any treatment was 100% in 24 h; the mortality rate of mice was 100% in 36 h when treated with zidovudine (10 mg/kg); the mortality rate of mice was 66% in 72 h when treated with rifampicin (8 mg/kg); the mortality rate was 0% in 72 h when treated with a combination of zidovudine (10 mg/kg) and rifampicin (8 mg/kg). This finding demonstrated that the treatment efficacy of simultaneous usage of zidovudine and rifampicin was much higher than single use of each drug (Fig. 1d).

A resistance development test was performed to assess the rate of drug-induced resistance in *K. pneumoniae* by rifampicin, zidovudine, or a combination of both. Incubation of rifampicin or zidovudine with CR-HvKP 1 led to the increased of MIC to 4096 μg/mL and 1280 μg/mL by the first or second generation, respectively, while combinational use of these two drugs led to increase the MIC to 32 μg/mL + 20 μg/mL in 6 days, a much slower resistance development rate when compared to single use of drugs (Fig. 1e). Both *in vitro* and *in vivo* toxicity tests were performed with results showing that single and combinational uses of rifampicin and zidovudine led to low level of hemolysis and *HepG2* cell lysis and no significant damage occurred in the liver and kidney of mice was observed after 7 days, 10-fold therapeutic dose treatment (Fig. 1f, g, h, i).

Zidovudine is an HIV drug that inhibits the propagation of HIV virus through interfering with the activity of viral reverse transcriptase.[5] We hypothesized that the antibacterial effect of zidovudine is probably due to the inhibition of bacterial RNA Polymerase complex (RNAp), and hence bacterial RNA transcription. We successfully co-expressed subunits of *K. pneumoniae* RNAp to prepare the core enzyme by a dual-plasmids expression system, pACYC-Duet and pET-Duet.[6] The interaction between RNAp and zidovudine was determined using isothermal titration calorimetry (ITC) tests with results showing that K_d of zidovudine was 7.8 μM, with the reaction stoichiometry (N sites) and enthalpy (ΔH) being 0.239 ± 0.273 and -335 ± 520 kJ/mol, respectively (Fig. 2a, b). It implied that zidovudine had a high affinity to RNAp from the thermodynamic aspect. Molecular docking of zidovudine to *E. coli* RNA polymerase (4YG2)[7] was performed using AutoDock Vina.[8] Zidovudine was shown to interact with the *β'* subunit of RNAp (RpoC), which formed a tight contact (2.3 Å) with the residues Asn^{495} in chain *J* of the *β'* subunit, while rifampicin binds with the *β* subunit of RNAp (RpoB)(Fig. 2c).[9] Binding pockets of zidovudine and rifampicin were located in the primary channel of RNAp near the

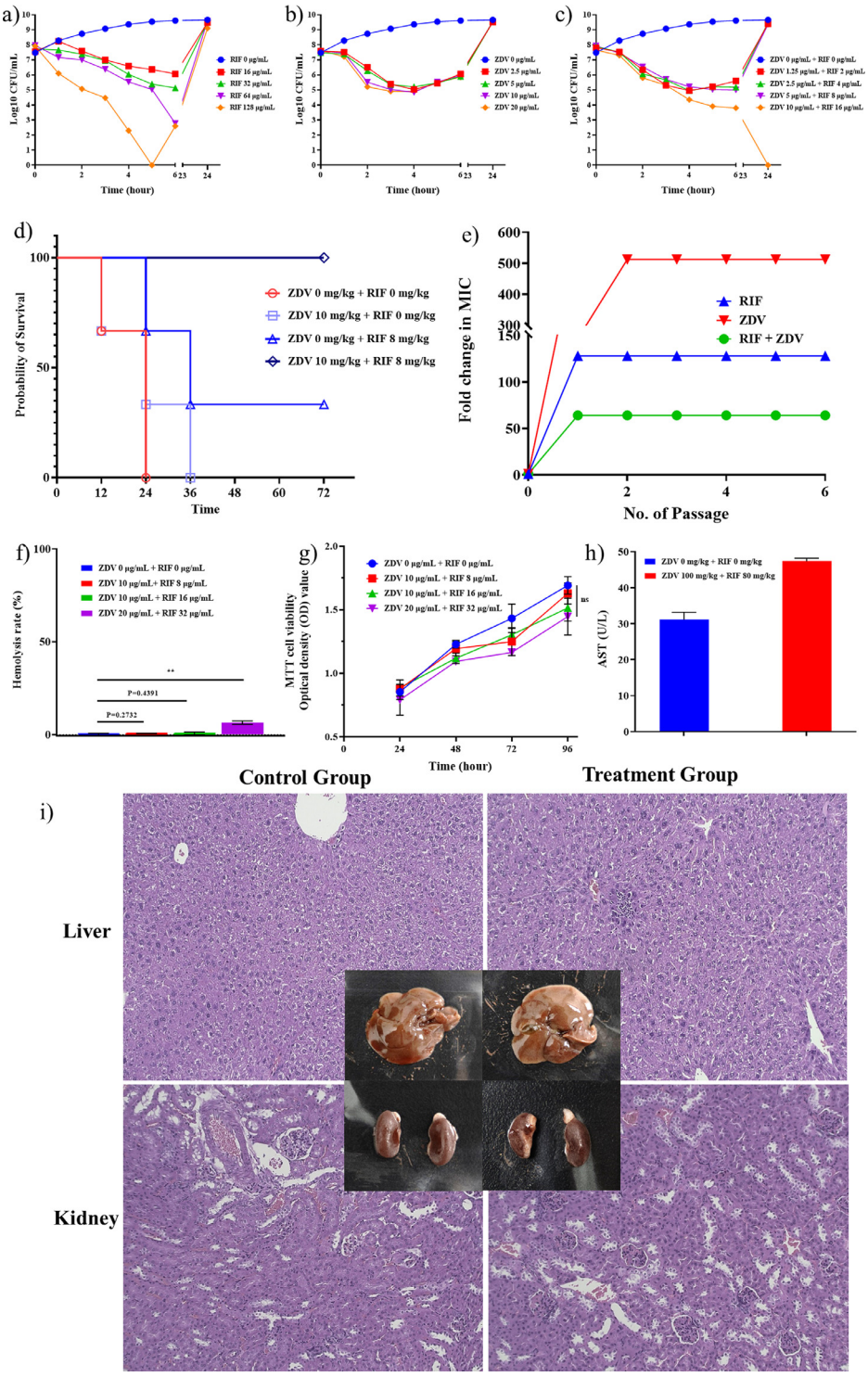

Fig. 1. *In vitro* and *in vivo* antibacterial activity, resistant development potential and toxicity of zidovudine and rifampicin combination therapy. In time killing assays, CR-HvKP 1 strains was treated with different concentrations of rifampicin (a), zidovudine (b), and combination of rifampicin and zidovudine (c). The CFUs of CR-HvKP 1 were measured over time and plotted. (d) Survival curve of mouse sepsis model where CR-HvkP 1 ($\approx 3 \times 10^7$ CFU bacteria) were used to infect mice followed by treatment with different drugs. The mortality of mice was recorded over 72 h. Log-rank (Mantel-Cox) test was performed for indicated curves ($p = 0.0031$). (e) *In vitro* resistance development study against CR-HvKP 1 strain: Changes in MIC upon 6 serial passages with incremental concentrations of zidovudine, rifampicin, and the combination were monitored. The fold change in MIC represented the ratio of the MIC after each passage to the initial MIC before the first passage. (f) Hemolysis ratio of RBCs under treatment with different concentrations of drug combinations, (g) *HepG2* cell viabilities determined by MTT Cell Proliferation Assay Kit (CyQUANTtm; Invitrogen, Carlsbad, CA) upon treatment with different concentrations of drug combinations. The absorbance (A) value of each well was measured at 570 nm. (h) Aspartate amino transferase (AST) concentration in serum of mice gavaged with saline or 10-fold therapeutic dose of zidovudine and rifampicin combination treatment after 7 days was determined using the assay kit (MAK055, Sigma Aldrich). The 450 nm absorbance (A) value of each sample was measured by Microplate Reader (SpectraMax 190, Molecular Devices). (i) Histopathology staining of kidney and liver from ICR gavaged with saline or 10-fold therapeutic dose of zidovudine and rifampicin combination treatment after 7 days was stained with Haematoxylin-Eosin (H&E), and evaluated for histological features. Data represents the mean ± SD ($n = 3$); statistical analysis was operated by one-way ANOVA for multiple groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

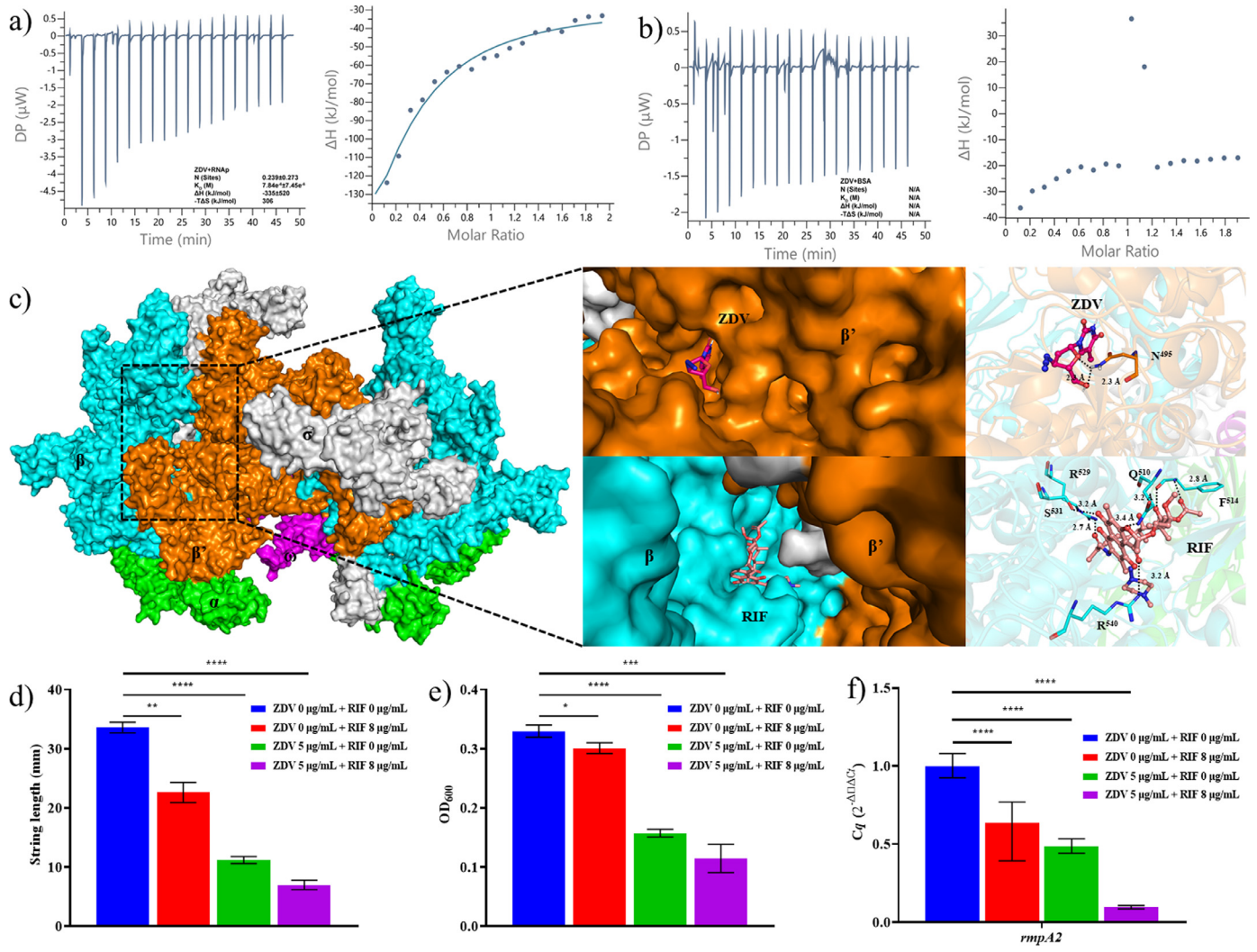

Fig. 2. Analysis of the inhibition mechanism of ZDV and regulation of the virulence of CR-HvKP 1. (a) Representative isothermal titration calorimetry (ITC) traces and binding curve of titration between 149.7 μM zidovudine and 14.79 μM RNA polymerase protein (molar ratio≈**10:1**). The test was performed at 25 °C using a Malvern MicroCal PEAQ-ITC automatic ultra-sensitive isothermal titration calorimeter (MicroCal (Malvern Instruments), Malvern, UK). (b) Representative isothermal titration calorimetry (ITC) traces and binding curve of titration between 149.7 μM zidovudine and 15.05 μM BSA protein (negative control) (molar ratio≈**10:1**). (c) Predicted binding mode of zidovudine with the *β'* subunit of *E. coli* RNAp which was analyzed by AutoDock Vina algorithm software and a binding mode of rifampicin with the *β* subunit of RNAp (PDB: 5UAC). (d) String length (mm) for CR-HvKP 1 colonies at sheep blood agar containing different drugs. (e) measurement of mucoviscosity of CR-HvKP 1 different drugs treatment. The cultures were diluted to OD_{600} at 1.0 and centrifuged for 2 min at 1000 x g. The supernatant was then obtained for OD_{600} measurement to determine the mucoviscosity. (f) Virulence gene *rmpA2* expression under different drugs treatment was determined by qRT-PCR. Data represents the mean ± SD ($n = 3$); statistical analysis was operated by one-way ANOVA for multiple groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

active center, suggesting that zidovudine would inhibit bacterial growth through a mechanism similar to that of rifampicin but involves an alternative pathway.[10]

We last check if inhibition of RNAp could inhibit the virulence expression of CR-HvKP 1. Our data showed that rifampicin or zidovudine alone could reduce the string length and hypermucoviscosity (Fig. 2d, e) as well as the expression of transcription regulators *rmpA2*, which were hallmarks of virulence of *K. pneumoniae* (Fig. 2f). In conclusion, this study has developed an effective combinational therapy against clinical CR-HvKP infections with low toxicity through drug repurposing approach and depicted its mechanisms of action to pave the way for further clinical trial.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Immunosuppression impaired the immunogenicity of inactivated SARS-CoV-2 vaccine in non-dialysis kidney disease patients

Dear editor,

Patients with chronic kidney disease (CKD) are at higher risk for coronavirus disease 2019 (COVID-19)-related morbidity and mortality than general populations and, early vaccination should be prioritized for this vulnerable population. However, numerous uncertainties exist regarding the safety and efficacy of vaccination, especially in CKD patients on immunosuppressive drugs.[1] Concerning safety and efficacy, a significant portion of CKD patients (1720/2509, 68.6%) showed hesitation toward vaccination in telephone survey of our center (Fig. S1). Previous studies focused on exploring immune responses to COVID-19 vaccine in patients on dialysis or receiving kidney transplant.[2,3] Three recent studies reported the humoral response to mRNA severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines in patients with CKD,[4–6] yet no serial data available for inactivated SARS-CoV-2 vaccines.

We conducted a pilot, prospective study to survey the safety and humoral response to inactivated SARS-CoV-2 vaccine in CKD patients receiving a 2-dose immunization of inactivated SARS-CoV-2 vaccine (Fig. S2). We recruited 45 CKD patients, and 100 healthy controls, 100 hypertension patients and 100 diabetes patients with matched sampling time after vaccination at Peking University First Hospital, Yunnan University Affiliated Hospital and Center for Disease Control and Prevention in Hainan. Baseline characteristics of the participants are described in Table 1. All participants received a 2-dose immunization (3–5 weeks between two doses) of inactivated SARS-CoV-2 vaccine (SinoVac or Sinopharm). We collected serum samples 20–33 days after the second dose of vaccination. To assess the humoral response to inactivated SARS-CoV-2 vaccines, we measured neutralizing antibody using competitive inhibition method, and anti-SARS-CoV-2 receptor-binding domain (RBD)-specific IgG and IgM using chemiluminescence immunoassay (MCLIA, Bioscience Co., Tianjin, China). We also recruited 31 CKD patients and 67 healthy controls receiving a third dose of inactivated SARS-CoV-2 vaccine (**Table S1**) to evaluate the immunological enhancement in this population. This study was approved by the Peking University First Hospital (2021[441]) and Yunnan University (CHSRE2021021).

Briefly, the average age of CKD patients was 42.4 years with 20 (44%) females. 21 of them received SinoVac, 20 received Sinopharm and 4 received both sequentially. The most common form of CKD was chronic glomerulonephritis (27/45, 60.0%) followed by podocytopathy (5/45, 11.1%) and metabolic kidney disease (5/45, 11.1%). There were 18 patients taking immunosuppressants during the vaccination period with 17 (94.4%) receiving monotherapy. We tightly controlled age, female ratio, types of administered vaccines and sampling time across groups, except that the average

Table 1
Demographic and clinical characteristics of study participants.

Characteristics	Chronic kidney disease patients (n = 45)	Healthy controls (n = 100)	Hypertension disease controls (n = 100)	Diabetes disease controls (n = 100)
Mean age (SD), y	42.4 (16.2)	38.4 (13.3)	39.7 (15.4)	56.3 (8.2)
Gender, n (%)				
Female	20 (44.4)	45 (45)	47 (47)	50 (50)
Median interval between the second dose and sampling (IQR), days	27 (20, 33)	30 (17, 30)	25 (16, 38)	28 (22, 31)
Applied vaccines, n (%)				
SinoVac	21 (46.7)	48 (48)	59 (59)	90 (90)
Sinopharm	20 (44.4)	43 (43)	25 (25)	5 (5)
Both	4 (8.9)	9 (9)	16 (16)	5 (5)
Kidney disease diagnosis, n (%)				
Chronic glomerulonephritis				
IgA nephropathy	21 (46.7)	–	–	–
IgA vasculitis	1 (2.2)	–	–	–
Chronic glomerulonephritis without kidney biopsy	5 (11.1)	–	–	–
Podocytopathy				
Membranous nephropathy	4 (8.9)	–	–	–
Focal segmental glomerular sclerosis	1 (2.2)	–	–	–
Metabolic kidney disease				
Diabetic nephropathy	4 (8.9)	–	–	–
Hypertensive nephropathy	1 (2.2)	–	–	–
Others				
Uninephrectomy	2 (4.4)	–	–	–
Fanconi syndrome	1 (2.2)	–	–	–
Alport syndrome	1 (2.2)	–	–	–
Acute interstitial nephritis	1 (2.2)	–	–	–
Kidney amyloidosis	1 (2.2)	–	–	–
Monoclonal gammopathy of renal significance	1 (2.2)	–	–	–
Kidney stone	1 (2.2)	–	–	–
Additional diagnosis, n (%)				
Hypertension	12 (26.7)	–	100 (100)	10 (10)
Diabetes	7 (15.6)	–	4 (4)	100 (100)
Obesity	1 (2.2)	–	–	1[1]
Gout	3 (6.7)	–	–	–
Viral B hepatitis	2 (4.4)	–	–	–
Fatty liver	1 (2.2)	–	–	–
Coronary heart disease	1 (2.2)	–	2 (2)	1 (1)
Asthma	1 (2.2)	–	–	–
Endometrial adenomyosis	1 (2.2)	–	–	–
Chronic lymphocytic leukemia	1 (2.2)	–	–	–
Hypothyroidism	1 (2.2)	–	–	1 (1)
Hyperthyroidism	–	–	–	1 (1)
Cerebral infarction	–	–	–	1 (1)
Endometrial carcinoma of uterus	–	–	–	1 (1)
Medication exposure, n (%)				
Prednisone	4 (8.9)	–	–	–
Bortezomib+dexamethasone	1 (2.2)	–	–	–
Hydroxychloroquine	7 (15.6)	–	–	–
Cyclosporin A	3 (6.7)	–	–	–
Mycophenolate mofetil	1 (2.2)	–	–	–
Tripterygium wilfordii	2 (4.4)	–	–	–
No immunosuppression[†]	27 (60)	–	100 (100)	96 (96)

[†] indicates angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, insulin, metformin, acarbose, sodium-dependent glucose transporter inhibitor, calcium channel blockers, β-blocker, propylthiouracil, or levothyroxin sodium tablets.

age of diabetes patients is significantly older than CKD patients. None of these participants reported severe adverse effects after vaccination.

Using neutralizing antibody titer of 2 as seroconversion cutoff, we found 84% (38 of 45) of CKD patients seropositive, which was lower than that in healthy controls (98%), hypertension patients (98%) and diabetes patients (95%). The median neutralizing antibody titers in CKD patients was 6.29 (IQR, 2.78–14.62), which was significantly lower than that in healthy controls [8.91 (IQR, 6.14–16.01), $P = 5.15 \times 10^{-3}$], hypertension patients [8.66 (IQR, 5.24–15.68), $P = 0.02$], and diabetes patients [9.14 (IQR, 4.62–16.22), $P = 0.04$] (Fig. 1**A**). Conversely, we did not observe anti-RBD IgG or IgM differences between CKD patients and controls (Fig. 1**B, C**), despite of strong association between neutralizing antibody and anti-RBD IgG levels (Fig. S3). To better understand the immunogenicity of inactivated SARS-CoV-2 vaccine in CKD patients, we further stratified CKD patients into subgroups according to their disease diagnosis, estimated glomerular filtration rate (eGFR) levels and medication status (receiving immunosuppressants or not). Notably, immunosuppressive medication (Fig. 1**D**) rather than eGFR levels (Fig. 1**E**) or disease types (Fig. S4) showed effect on the reduction of immunogenicity. There were only 72.2% (13/18) of CKD patients receiving immunosuppressants tested seropositive after 2-dose vaccination. Moreover, we observed an immunosuppressant–dependent association between neutralizing antibody level and eGFR after adjusting for age and gender ($r = 0.627$, $P = 0.02$), suggesting that immunosuppressive agents could sensitize neutralizing antibody response to kidney function in non-dialysis kidney patients. Interestingly, a third dose significantly boosted neutralizing antibody in CKD patients while immunosuppressants impeded the boosting effects (Fig. S5).

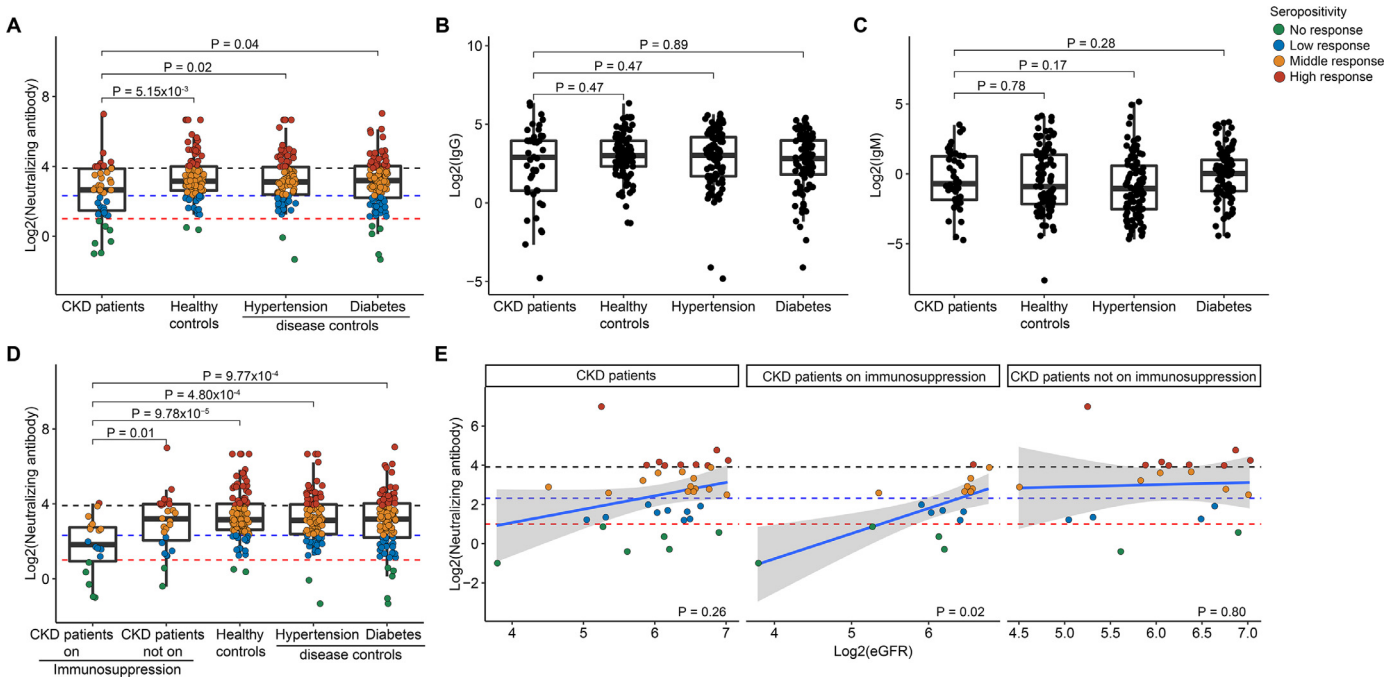

Fig. 1. *Immune responses after 2-dose inactivated SARS-CoV-2 vaccination in patients with chronic kidney disease.* (**A**) Neutralization antibodies response. (**B**) anti-SARS-CoV-2 receptor-binding domain (RBD)-specific IgG response. (**C**) anti-RBD IgM response. (**D**) Neutralization antibody responses in patients on immunosuppression. (**E**) The correlations between neutralizing antibody response and eGFR. Antibody titers were presented as median (IQR: interquartile range). The thresholds for neutralization antibodies is represented by the dashed lines, with <2.0 classified as no response, <5.0 as low response, <15.0 as middle response, and >=15.0 as high response. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

Our initial analysis showed that majority (84%) of CKD patients acquired detectable neutralizing antibody against SARS-CoV-2 without severe adverse effects, while the antibody titers were lower than controls. In contrast, we did not observe such difference in anti-RBD IgG or IgM. The deviance between neutralizing antibody and anti-RBD IgG responses in CKD patients indicates that neutralizing antibody rather than IgG might be the most important marker reflecting humoral immune response in CKD patients. Subgroup analyses showed that immunosuppressive therapies rather than eGFR levels or disease diagnosis impair SARS-CoV-2 vaccine-induced immunity. Our analysis in non-dialysis kidney disease patients receiving inactivated SARS-CoV-2 vaccine greatly supplemented previous studies on immunosuppressive therapies,[7,8] dialysis,[9] and kidney transplantation[10] impaired mRNA vaccine responses. We found that taking immunosuppressants hampers neutralizing antibody response in CKD patients and sensitizes neutralizing antibody response to kidney function. Additionally, our data demonstrates that CKD patients, even for those on immunosuppression treatment, can benefit from a third vaccination boost by improving their humoral immunity.

Disclosure statement

None declared.

Declaration of Competing Interest

Z.Z. served as a PI in a phase 4 clinical study sponsored by Sinovac Biotech Ltd. The funder has no role in study design, implementation and manuscript writing in this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.003.

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Declines in invasive pneumococcal disease (IPD) during the COVID-19 pandemic in Los Angeles county

Dear editor,

We read with great interest an article by Ji-Young Min and colleagues documenting the simultaneous administration of adjuvanted recombinant zoster vaccine (RZV) and the 13-valent pneumococcal conjugate vaccine (PCV) in adults aged ≥50 years.[1] Their results suggest that adults may benefit from receiving RZV and a PCV at the same healthcare visit. We would like to provide additional context for the implications of those conclusions by sharing results of a study we conducted to describe trends in invasive pneumococcal disease (IPD) incidence among LA County residents for six respiratory seasons from 2015 to 16 to 2020–21.

Los Angeles (LA) County recorded its first case of COVID-19 in January 2020 and implemented non-pharmaceutical interventions (NPIs), such as wearing face masks and social distancing, to mitigate community transmission beginning in March 2020.[2] Similar to the overall U.S. population, LA County experienced a reduction in circulating influenza and other viral respiratory pathogens after widespread implementation of NPIs.[3,4] Since *Streptococcus pneumoniae* is a respiratory pathogen that is also transmitted via contact with respiratory droplets, we hypothesized that implementation of COVID-19 related control measures would be associated with similar reductions in IPD incidence.

The LA County Department of Public Health (DPH) conducts surveillance for 10 million residents across 86 cities (excluding the cities of Long Beach and Pasadena). Healthcare providers and clinical laboratories are mandated to report IPD cases to DPH.[5] A confirmed IPD case is defined as the isolation of *S. pneumoniae* from a normally sterile body site (e.g., blood or cerebrospinal fluid) in a resident of LA County. All reported cases are investigated by DPH to determine if they meet the case definition. The COVID-19 pandemic required DPH to redirect staff to support the local response so no additional follow-up was conducted on IPD reports with incomplete information. Therefore, we established a probable IPD case definition for reports that occurred after January 1, 2020 and lacked sufficient information to establish if the confirmed case definition was met.

All reported cases are entered into a local surveillance data management system. We analyzed data by respiratory seasons, defined as July to June of the following year. The date of IPD cases' occurrence was calculated from the earliest of symptom onset date, specimen collection date, specimen result date, date of diagnosis, date report is received by public health department, or the date the case is added to the surveillance database. We described cases by age, sex, and race/ethnicity based on information included in the case reports to DPH. We were able to directly compare groups without statistical testing since we were analyzing a complete data set of all reported IPD cases in LA County.

During the 2015–16 through 2018–19 seasons (Fig. 1), 2089 confirmed IPD cases were reported to DPH (median cases/year: 482; minimum: 463, maximum: 667). During the 2019–20 and 2020–21 respiratory seasons, 456 and 174 confirmed/probable IPD cases were reported, respectively. Among 2719 LA County residents with confirmed/probable IPD across all seasons (Table 1), the median age was 60 years (1st quartile: 46, 3rd quartile: 74), 1549 (57%) were male, 694 (26%) were Hispanic, 752 (28%) were White, 460 (17%) were Black, and 411 (15%) were unknown. The characteristics of IPD cases by age, sex, and race/ethnicity were similar across all seasons.

We described trends in IPD among LA County residents during six respiratory seasons from 2015 to 16 to 2019–21. Corresponding with the introduction and widespread transmission of COVID-

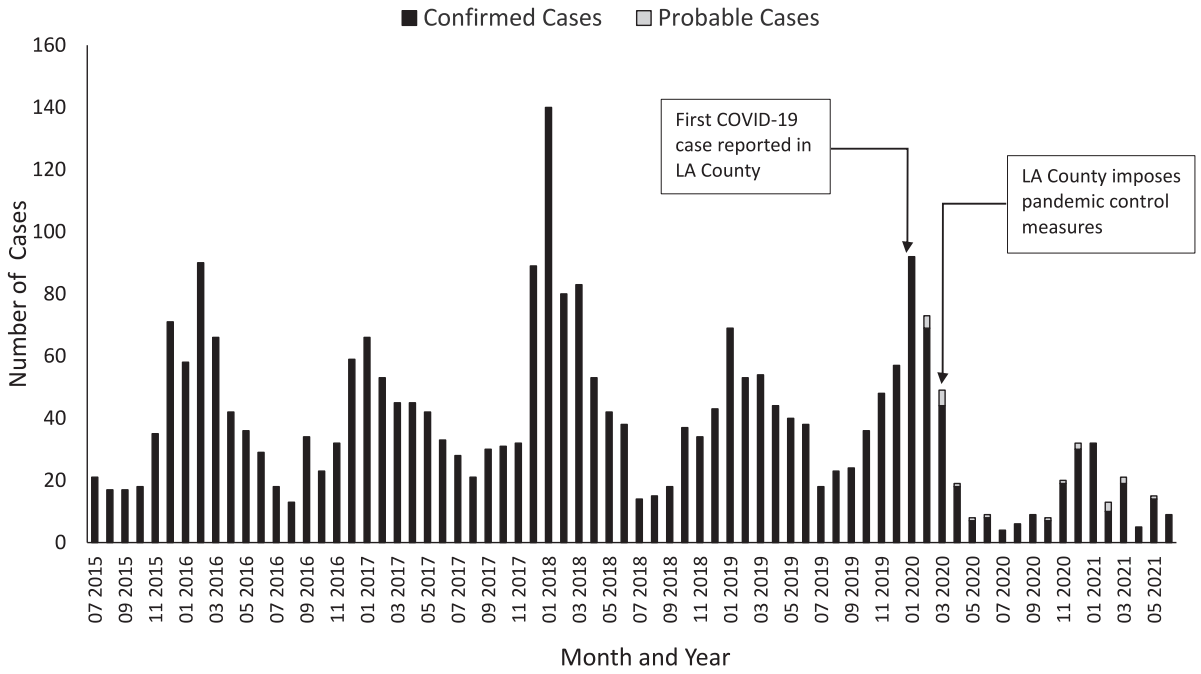

Fig. 1. Confirmed and probable invasive pneumococcal disease cases reported in Los Angeles (LA) County from July 2015 to June 2021.[1]

Table 1
Demographics of Persons with Invasive Pneumococcal Disease Reported in Los Angeles (LA) County from July 2015 to June 2021.

	Overall		July 2015 – Jun-16		July 2016 – Jun-17		July 2017 – Jun-18		July 2018 – Jun-19		July 2019 – June 2020[1]		July 2020 – June 2021[1]	
Total, N	2719		500		463		667		459		456		174	
Age (years), median (Q1,Q3)	60	(46,74)	61	(48,75)	60	(45,74)	61	(48,75)	61	(45,74)	61	(45,72)	58	(45,69)
Age group (years), n (%)														
<5	121	4%	21	4%	28	6%	29	4%	18	4%	22	5%	3	2%
5–17	90	3%	19	4%	17	4%	18	3%	15	3%	19	4%	2	1%
18–44	416	15%	72	14%	67	14%	89	13%	81	18%	72	16%	35	20%
45–64	959	35%	176	35%	154	33%	254	38%	142	31%	157	34%	76	44%
≥65 years	1129	42%	211	42%	196	42%	277	42%	202	44%	185	41%	58	33%
Missing	4	0%	1	0%	1	0%	0	0%	1	0%	1	0%	0	0%
Sex, n (%)														
Female	1154	42%	209	42%	194	42%	283	42%	220	48%	186	41%	62	36%
Male	1549	57%	290	58%	269	58%	384	58%	238	52%	260	57%	108	62%
Missing	16	1%	1	0%	0	0%	0	0%	1	0%	10	2%	4	2%
Race/Ethnicity, n (%)														
Asian	136	5%	14	3%	40	9%	30	4%	25	5%	21	5%	6	3%
Non-Hispanic Black	460	17%	79	16%	69	15%	112	17%	92	20%	80	18%	28	16%
Hispanic	694	26%	107	21%	132	29%	158	24%	127	28%	115	25%	55	32%
Non-Hispanic White	752	28%	130	26%	117	25%	204	31%	138	30%	124	27%	39	22%
Other	266	10%	12	2%	39	8%	73	11%	50	11%	67	15%	25	14%
Missing	411	15%	158	32%	66	14%	90	13%	27	6%	49	11%	21	12%

Abbreviations: Q1, first quartile; Q3, third quartile.
[1] A confirmed case is defined as the isolation of *Streptococcus pneumoniae* from a normally sterile body site (e.g., blood or cerebrospinal fluid) in a resident of LA County. All reported cases are investigated by DPH to determine if they meet the case definition. A probable case lacked sufficient information to establish if the confirmed case definition was met. There were 11 probable cases in the 2019–2020 season and 10 probable cases in the 2020–2021 respiratory season.

19 in 2020, the number of IPD cases declined from the 2019–20 season to the 2020–21 season. The reduction in IPD incidence during the COVID-19 pandemic has been observed in other regions of the world. Compared with the 2018–19 season, IPD incidence during the 2019–20 respiratory season in the United Kingdom was 30% lower.[6] In Hong Kong, where strict social control measures were put in place with near universal masking, IPD cases decreased by 74.7% during 2020 compared with the comparable pre-pandemic period of 2015–2019.[7] Conversely, IPD incidence increased after relaxing social control measures. In Switzerland, IPD incidence decreased by 73% from February to April 2020, remained low from April 2020 to February 2021, and then increased by 23% from March to May 2021 when social control measures were lifted.[8]

The decline in IPD cases associated with the COVID-19 pandemic is likely multifactorial. Public health recommendations/mandates for wearing facemasks, social distancing, and increased hand hygiene likely contributed to reduced transmission by decreasing the risk of coming into contact with respiratory droplets from someone with pneumococcal colonization. Moreover, school age children have a higher prevalence of pneumococcal colonization compared with adults, and they are an important source of transmission to adults in their household and community. Therefore, closure of LA County schools and childcare centers

during the early part of 2021 likely resulted in decreased community transmission of *S. pneumoniae*.[9] Finally, influenza activity declined substantially after the implementation of pandemic control measures; influenza activity was non-existent during the 2020–21 season.[10] As a result, it is possible that there were fewer IPD cases as a secondary complication of influenza infection.

Our analysis is limited by the fact we relied on a passive surveillance system. As a result, cases will not be reported if people were less likely to seek care or get tested for IPD during the pandemic. Under ascertainment of cases during the pandemic is unlikely, however, because IPD tends to be a serious illness and most people will present for medical attention. Therefore, the magnitude of the reduction in IPD incidence during the pandemic cannot be fully explained by differential health seeking behavior alone.

Additional analysis is necessary to determine the precise contribution of the various pandemic control measures to the overall reduction in incidence of IPD and other respiratory pathogens. At a minimum, persons at high risk for IPD should consider wearing a facemask when in public spaces even after pandemic control measures are lifted given the evidence for the protection they confer from respiratory infections.

Declaration of Competing Interest

None of the authors have any relevant conflicts of interest to disclose.

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Remdesivir use in COVID-19 patients might predispose bacteremia, matched case-control analysis

Dear editor,

We have read with great interest the paper by Yoon et al. (1) reporting infectious SARS-CoV-2 samples were not detected after the third dose of remdesivir despite concomitant dexamethasone treatment. Remdesivir is a nucleoside prodrug of an adenosine analogue that was approved for the treatment of COVID-19 based on reduced hospitalization duration and trend of reduced mortality in a randomized setting (2). Besides renal and liver toxicities, remdesivir has been shown to be associated with bradycardia (3), a favorable side-effect (4) of unknown mechanism that is potentially related to similarity of its metabolites to adenosine (5). Adenosine levels have an important role in control of inflammation and might dampen the hosts anti-microbial response (6) and consequently promote bacterial superinfections. Despite this potential association, there are no published data on occurrence of bacterial infections during remdesivir treatment in COVID-19 patients. In this paper we aim to investigate occurrence of bacteremia in a large real-life cohort of remdesivir-treated in comparison to matched control COVID-19 patients from our institution.

Among a total of 5959 consecutively hospitalized COVID-19 patients treated at our institution from 3/2020 to 6/2021, we retrospectively evaluated 876 consecutive remdesivir-treated patients. They were compared to a matched case-control cohort of 876 patients. Matching was based on age, sex, Charlson comorbidity index, WHO severity of COVID-19 at presentation and maximal level of oxygen requirement at the time of remdesivir application (to account for the fact that remdesivir was given to respiratory deteriorating patients). All patients were tested positive by either PCR or antigen test and had presence of COVID-19 symptoms. All patients were adults and Caucasian. Patients were treated according to the contemporary guidelines with majority receiving LMWH thromboprophylaxis and corticosteroids. Analyzed blood cultures were sampled during the whole hospitalization, and at least 48 h after initiation of remdesivir in remdesivir-treated patients. Clinically significant bacteremia was considered in the case of positive blood cultures which were sampled based on clinical reasoning of treating physicians, with exclusion of single blood cultures with isolates of contaminants such as coagulase-negative Staphylococcus (CoNS) or Corynebacterium spp, or more than one positive blood culture with same isolates but without clinical course consistent with blood-stream infection. MedCalc statistical program ver 20.104 (MedCalc Software Ltd, Ostend, Belgium) was used for all statistical procedures. The Mann-Whitney-U test, the X^2-test, the log-rank test and the Cox-regression were used. P values <0.05 were considered statistically significant.

A total of 1752 COVID-19 patients were evaluated (876 remdesivir-treated and 876 matched controls). In comparison of remdesivir vs control patients there were no significant differences in neither age (65 vs 66 years, $P = 0.109$), Charlson comorbidity index (3 vs 3 points, $P = 0.115$), male sex (61.8% vs 61.8%, $P = 1.000$),

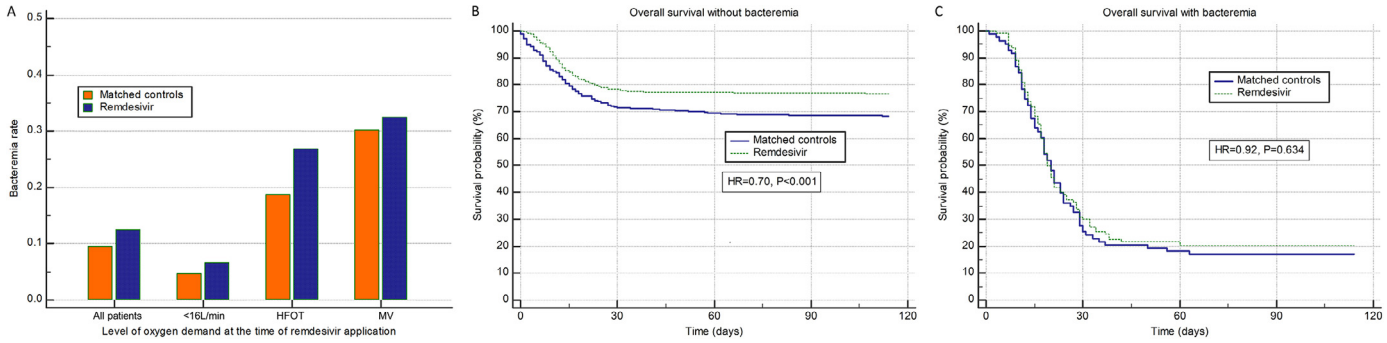

Fig. 1. A) Frequency of positive bacterial blood cultures in remdesivir-treated and matched control COVID-19 patients depending on the level of oxygen demand at the time of remdesivir application. **B)** Overall survival from hospital admission stratified by remdesivir use in patients without and **C)** with bacterial sepsis during hospitalization.

Table 1
Frequency of bacterial sepsis and specific pathogens isolated from blood cultures in remdesivir treated and matched control group of COVID-19 patients.

	Remdesivir treated (N = 876)	**Matched controls** (N = 876)	**P value**
Positive blood cultures	110 (12.6%)	83 (9.5%)	$P = 0.039$ *
Gram negative bacteria	81 (9.2%)	64 (7.3%)	$P = 0.141$
Gram positive bacteria	68 (7.8%)	44 (5%)	$P = 0.019$ *
Both Gram positive and negative bacteria (polymicrobial)	39 (4.5%)	25 (2.9%)	$P = 0.075$
Acinetobacter baumannii	63 (7.2%)	46 (5.3%)	$P = 0.093$
Staphylococcus aureus	21 (2.4%)	17 (1.9%)	$P = 0.512$
Enterococcus faecalis	22 (2.5%)	9 (1%)	$P = 0.019$ *
Enterococcus faecium	10 (1.1%)	8 (0.9%)	$P = 0.636$
Coagulase negative Staphylococcus	26 (3.0%)	18 (2.1%)	$P = 0.222$
Klebsiella pneumoniae	15 (1.7%)	14 (1.6%)	$P = 0.852$
Pseudomonas aeruginosa	5 (0.6%)	7 (0.8%)	$P = 0.562$
Corynebacterium species	6 (0.7%)	2 (0.2%)	$P = 0.157$
Escherichia coli	4 (0.5%)	8 (0.9%)	$P = 0.247$
Klebsiella aerogenes	0 (0%)	2 (0.2%)	$P = 0.157$
Proteus mirabilis	1 (0.1%)	4 (0.5%)	$P = 0.179$
Stenotrophomonas maltophilia	1 (0.1%)	0 (0%)	$P = 0.317$
Enterobacter cloacae	2 (0.2%)	1 (0.1%)	$P = 0.563$
Staphylococcus haemolyticus	1 (0.1%)	1 (0.1%)	$P = 1.000$
Serratia marcescens	1 (0.1%)	1 (0.1%)	$P = 1.000$
Providencia stuartii	1 (0.1%)	1 (0.1%)	$P = 1.000$
Staphylococcus epidermidis	1 (0.1%)	0%	$P = 0.317$
Beta hemolytic streptococcus	0 (0%)	1 (0.1%)	$P = 0.317$
Streptococcus pneumoniae	2 (0.2%)	0 (0%)	$P = 0.157$
Bactroides species	1 (0.1%)	0 (0%)	$P = 0.317$
Haemophilus parainfluenzae	0 (0%)	1 (0.1%)	$P = 0.317$
Providencia rettgeri	1 (0.1%)	0 (0%)	$P = 0.317$

*Statistically significant at level $P<0.05$.

severe or critical COVID-19 at the time of admission (97.9% vs 97.9%, $P = 1.000$) nor in requirement for high-flow oxygen therapy (HFOT, 36.5% vs 37.2%, $P = 0.766$) or mechanical ventilation (MV, 28.4% vs 27.9%, $P = 0.791$), as per matching procedure. A total of 552 (31.5%) patients died during hospitalization with remdesivir-treated patients experiencing lower mortality rate (29.2% vs 33.8%, $P = 0.039$).

Bacteremia was detected in 193 (11%) patients, more frequently in remdesivir-treated patients (12.6% vs 9.5%, $P = 0.039$). As shown in Fig. 1A, a trend of bacteremia was more pronounced with remdesivir treatment irrespectively of the level of oxygen demand at the time of remdesivir application (regardless whether remdesivir was given prior to or during requirement for HFOT and MV), although no significant difference could be shown for particular subgroups. There was a statistically significant interaction between remdesivir use, bacteremia and death ($P<0.001$), with patients without bacteremia during hospitalization experiencing significantly improved survival (HR=0.70, 95% CI (0.58–0.85), $P<0.001$, Fig. 1B) and no evident benefit of remdesivir use present in patients experiencing bacteremia (HR=0.92, 95% CI (0.66–1.27), $P = 0.634$, Fig. 1C). Frequencies of specific pathogens isolated from blood cultures in remdesivir-treated and matched control patients are shown in Table 1. Remdesivir use was significantly associated with a higher occurrence of bacteremia due to Gram-positive bacteria ($P = 0.019$), especially Enterococcus faecalis ($P = 0.019$). In addition, a nearly significant result was observable for bacteremia due to Acinetobacter baumannii ($P = 0.093$).

To the best of our knowledge, this is the first report on higher frequency of bacteremia in COVID-19 patients treated with remdesivir. Bacteremia occurs in a substantial proportion of severe and critical COVID-19 patients who are candidates for remdesivir and the drug might especially predispose Gram-positive bacteremia, particularly with Enterococcus faecalis. Mechanisms behind these observations remain uncertain. It can be speculated that remdesivir metabolites that are adenosine analogues alter innate and specific immunity in the same fashion as adenosine does (7). Thus, remdesivir use might attenuate inflammation and promote bacterial virulency resulting in higher frequency of sepsis. Since remdesivir is given intravenously, this might also facilitate bacteremia, especially in the context of personal protective equipment that might affect dexterity of health-care workers and impose difficulties in delivering health-care (8). Importantly, no increase in bacteremia due to CoNS or Corynebacterium spp. was observed. Thus, higher frequency of bacteremia is not driven by pathogens that could be as-

sociated with contamination of blood cultures. Also, since patients were matched based on the level of oxygen demand at the time of remdesivir application, two groups were balanced regarding HFOT and MV requirement and higher frequency of bacteremia is not likely to be driven by differences in intensive level of care. Our data show that occurrence of bacteremia significantly moderates the relationship of remdesivir use with survival and attenuates potential beneficial effects of remdesivir, implying that this phenomenon has important clinical consequences. Bacterial co-infections substantially affect in-hospital mortality of COVID-19 patients (9) and special considerations should be given to patients with bacterial co-infections who are candidates for remdesivir, carefully weighting risks and benefits on an individual basis, implementing measures of increased surveillance or completely avoiding the drug.

Limitations of our work are retrospective study design and single center experience. Our results are representative of a tertiary-level institution and treatment of severe or critical COVID-19 patients and might not be generalized to other clinical contexts. Nevertheless, our data based on a large real-life cohort of remdesivir-treated and matched control patients imply that remdesivir use might be associated with higher frequency of bacteremia and this might affect prognosis of remdesivir treated patients. Future studies on this very important subject are needed.

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Ethical approval

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Declaration of Competing Interest

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Outcome predictors in SARS-CoV-2 disease (COVID-19): The prominent role of IL-6 levels and an IL-6 gene polymorphism in a western Sicilian population

Dear editor,

Recently in this *Journal*, Grifoni and colleagues[1] reported that IL-6 levels at hospital admission seem to be the best prognosticator for negative outcomes in patients with coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Considering the preponderant role of host response in influencing the clinical evolution of SARS-CoV-2 infection, we studied polymorphisms of the *IL-6* gene -174G/C (rs1800795), as well as its receptor *IL-6R* (rs2228145), *TNF-α* (rs1800629), *ANGP2* (rs55633437), and *MX1* (rs2071430) in patients hospitalized with COVID-19 to identify any genetic predisposition to a worse outcome. Other clinical features, such as IL-6 serum level, and risk factors for severity and mortality were also analyzed.[2] We analyzed 316 Sicilian (Italy) patients, divided into two groups according to the type of oxygen and ventilation therapy they required during hospitalization: (1) non-critical ($n = 271$), patients admitted for reasons other than SARS-CoV-2 pneumonia with a positive preadmission screening, and patients hospitalized for SARS-CoV-2 infection without respiratory insufficiency; (2) critical ($n = 45$), subjects who underwent non-invasive ventilation (Suppl. Materials).

Table 1 shows some demographic features, the P/F ratio (pO/FiO) at admission, and the PSI as indicators of the severity of respiratory involvement in the two groups. Men were more prevalent in the two groups, but without statistical significance. Average age significantly increased with increasing disease severity, in line with the literature data.[1, 2, 3] The P/F ratio, indicative of the degree of respiratory failure at admission, was confirmed to be related to disease outcome ($p < 0.0001$). The PSI, split into two severity groups, was found to reflect the degree of disease impairment, being significantly less severe at baseline (grade 2) in non-critical than in critical subjects ($p < 0.0001$). Instead, other indicators of the severity of pulmonary involvement, such as absence of pneumonia, pulmonary thickening, pulmonary consolidation, ground glass, and mixed CT features, did not show statistically significant differences between the groups (Table 1).

The presence of comorbidities such as COPD, diabetes, and hypertension did not reach statistically significant percentages in the two groups (Table 1). Only the presence of ischemic heart disease was found to be significantly correlated with disease severity($p < 0.001$) (Table 1), a finding already described in the literature, with cardiovascular disease being one of the most important underlying diseases affecting the prognosis of patients with COVID-19.[4]

In addition, we found significantly lower platelet counts in subjects with more severe disease ($p < 0.03$). Furthermore, other parameters known to be related to the worst outcome of the disease (i.e., D-dimer and NLR)[2, 5] were confirmed in our series to be significantly related ($p < 0.005$ and $p < 0.05$, respectively) (Table 1). Finally, in accordance with Grifoni et al.,[1] we found maximum serum IL-6 levels, which were significantly higher in critically ill than non-critical subjects ($p < 0.0001$) (Table 1), to be strongly correlated with the worst prognosis among the markers of inflammatory status.

Successively, according to the literature data cut-off value of serum IL-6 > 55 pg/mL for identifying patients at high risk of severe COVID 19 forms,[6] we observed higher IL-6 values in 17/105 (16%) non-critical and 18/33 (53%) critical patients (OR 5.8 CI 95% 2.4-13.6).

For the genetic study, IL-6 rs1800795, IL-6R rs2228145, TNF-α rs1800629, ANGP2 rs55633437, and MX1 rs2071430 genotype distribution in critical and non-critical subjects is shown in

Abbreviations: COVID-19, Coronavirus 19 disease; GRS, genetic risk score; IL-6, Interleukin-6; MX1, MX Dynamin Like GTPase 1; NLR, Neutrophil-Lymphocyte Ratio; PSI, Pneumonia Severity Index; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 1
Demographic characteristics, blood gas analysis parameters, presence of comorbidities, laboratory parameters and characteristics of chest CT scan images of the study population, divided by degree of respiratory involvement.

	Non-critical $N = 271$ (%)	Critical $N = 45$ (%)	$p <$
Sex (M;%)	158 (58.3)	23 (51.1)	n.s.
Age (years; mean ± SD)	64.06 ± 14.1	71.1 ± 15.6	0.003
P/F	331.4 ± 90.1	261.9 + 106.6	0.0001
PSI			
1 (96)	187 (73.6)	16 (41)	
2 (%)	67 (26.4)	23 (59)	0.0001
COPD	15 (5.5)	1(2.2)	n.s.
Diabetes	58 (21.4)	7(15.6)	n.s.
Hypertension	154(65.8)	28 (62.2)	n.s.
Ischemic heart disease	41 (15)	17 (37)	0.001
IL-6 (pg/ml)	84.78 (1.5–1314)	1146(7.1–24526)	0.0001
D-dimer (ng/ml)	740 (82–64169)	867 (185–50386)	0.005
CRP (mg/l)	17.6 (0.5–307.26)	30.235 (0.88–201.52)	n.s.
NLR	4.529 (0.48–39.9)	6.33 (0.0008–22.5)	0.05
PCT (ng/l)	0.084 (0.02–40)	0.108 (0.03–55.26)	n.s.
Platelets (mm^3)	224000 (31000–575000)	195000(44000–337000)	0.03
NT-pro-BNP (ng/l)	186 (10–19514)	337 (28.8–33519)	n.s.
WBC (mm^3)	7590 (3040–46510)	7565 (1400–19610)	n.s.
Absence of pneumonia	29 (10.9)	3 (7.5)	n.s.
Pulmonary thickening	3(1)	1 (2.5)	n.s.
Pulmonary consolidation	1 (0.4)	2(5)	n.s.
Ground glass	5 (1.9)	3 (7.5)	n.s.
Mixed CT features	229 (85.8)	31 (77.5)	n.s.

P/F = pO2/FiO2; PSI = Pneumonia Severity Index; COPD = Chronic obstructive pulmonary disease; IL-6 = nterleukin 6; CRP = C-reactive protein; NLR = Neutrophil-Lymphocyte Ratio; PCT = Procalcitonin; NT-pro-BNP = N-terminal pro-hormone of brain natriuretic peptide; WBC = white blood cells

Table 2
Genotype distribution of IL-6 variants in the non-critical and critical groups.

	IL-6 - rsl800795	Non-critical $N = 103$ (%)	Critical $N = 33$ (%)	p
	GG	69 (67.0)	20 (60.6)	n.s.
Codominant model	CG	33 (32.0)	10 (39.3)	n.s.
	CC	1 (1.0)	3(9.1)	0.044
Dominant model	GG CG+CC	69 (67.0) 34 (33.0)	20 (60.6) 13 (39.4)	n.s.
Recessive model	GG+CG CC	102 (99.0) 1 (1.0)	30 (90.9) 3(9.1)	0.044

Table 2 and Suppl. Table 1, and all followed the Hardy-Weinberg equilibrium. The CC genotype frequency for IL-6 rs1800795 was significantly more frequent in critically ill than non-critical patients ($p = 0.044$).A significantly greater association was also observed between the rs1800795 polymorphism in critical subjects in the recessive model (CC vs. GG+CG) than in non-critical patients ($p = 0.044$) (Table 2). No significant associations were found in genotype frequencies for the other 4 SNPs analyzed (Suppl. Table 1).

Since the rs1800795 variant of *IL-6* affects gene transcription and serum IL-6 levels, we analyzed the association between this variant's presence and IL-6 serum levels ($N = 138$). In accordance with Smieszek et al.,[7] no significant correlation between IL-6 levels and the IL-6 rs1800795 genotype was found (Suppl. Fig. 1A). In addition, since some studies have reported that IL-6R variants may correlate with circulating IL-6 levels,[8] we correlated the rs2228145 genotype with IL-6 serum levels, finding no significant correlation between IL-6 levels and the IL-6R rs2228145 genotype (Suppl. Fig. 1B).

Subsequently, we examined the cumulative effects of the selected SNPs, developing a genetic risk score (GRS) by summing the number of risk alleles. For each SNP, a score of 0 was defined for homozygous non-risk alleles, 1 for heterozygous risk and non-risk alleles, and 2 for two homozygous risk alleles. A higher mean risk score was significantly associated with critical patients when the sum of the five scores for the rs1800629, rs1800795, rs2228145, rs2071430, and rs55633437 variants was considered for each patient ($p = 0.026$) (Suppl. Table 2). The mean of the gene count score was 3.67 ± 1.26 in the non-critical group and 4.26 ± 1.13 in the critical group. The cumulative effects of 4, 3, and 2 variants were also analyzed (Suppl. Table 2). After removing genetic variants, the contribution of IL-6 rs1800795 appeared to be crucial for risk prediction; in all cases where critical patients showed significant differences in GRS compared to non-critical patients, the *IL-6* gene variant was present. Moreover, in all cases of significant differences between critical and non-critical patients, the *MX1* variant was always present together with the IL-6 variant, except in one case (i.e., for the 4 variants IL-6, MX1, ANGP2, and TNF-α) in which no significant difference was observed ($p = 0.0516$), possibly due to the small number of patients. This suggests that the copresence of the MX1 and IL-6 variants could confer greater risk of disease severity.

Our study confirmed the prominent role of IL-6 levels and the genetic predisposition of an IL-6 gene variant in response to SARS-CoV-2 infection as predictors of COVID-19 disease severity and unfavorable outcomes, as well as the role of age and ischemic heart disease as important negative prognostic factors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.043.

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Reduced incidence of acute pharyngitis and increased incidence of chornic pharyngitis under COVID-19 control strategy in Beijing

Dear editor,

In this Journal, we described the impact of non-pharmaceutical interventions during COVID-19 pandemic on pertussis, scarlet fever and hand-foot-mouth disease in China.[1] As SARS-CoV-2 has swept the globe, the incidence spectrum of various diseases has been greatly affected in different pandemic prevention policies and people's coping strategies in various countries. In China, to achieve the strict COVID-19 control policy, nucleic acid testing and wearing mask are two of major control methods.[2,3] The typical nucleic acid testing largely relied on taking throat swabs. Both wearing mask and taking throat swabs could potentially affect the incidence of pharyngitis. However, the incidence trend of pharyngitis during COVID-19 pandemic in China remains inconclusive.

Pharyngitis is a common specific or nonspecific inflammatory reaction of pharyngeal mucosa, submucosal tissue and lymphoid tissue.[4] It can be divided into acute pharyngitis and chronic pharyngitis. Acute pharyngitis is often accompanied by upper respiratory infection diseases, which can be divided into bacterial pharyngitis, viral pharyngitis and fungal pharyngitis.[4] Chronic pharyngitis is a part of chronic inflammation of the upper respiratory tract. The course of disease is often and easy to aggravate repeatedly. Tissue lesions in adjacent parts, physical and chemical stimuli such as high temperature, dryness and dust can induce the onset or aggravation of chronic pharyngitis.

We compared the reported cases of acute pharyngitis and chronic pharyngitis before and after the outbreak of COVID-19 to explore the impact of COVID-19 control policies during the pandemic on pharyngitis. The monthly numbers of acute pharyngitis, chronic pharyngitis, and throat swabs were collected from 2019–2022 in Beijing Jiangong Hospital, most of whose patients are from Baizhifang Subdistrict and Taoranting Subdistrict of Xicheng District, Beijing, China. Data analysis and visualization were performed in statistical software (GraphPad Prism 8.0). A paired samples *t*-test was used to compare morbidity across different groups.

As shown in Fig. 1A, the monthly reported number of acute pharyngitis is gradually reduced after the outbreak of SARS-CoV-2. Based on the annual reported cases, the annual increased rate of acute pharyngitis is −32.9% and −48.7% for 2020 and 2021, respectively (Fig. 1B and Table 1). Compared with that in the first three months of 2021, the increment rate of acute pharyngitis in the first three months of 2022 is −81.4% (Fig. 1B and Table 1). We reason that more and more people wear mask and keep social distance during COVID-19 pandemic, which could reduce incidence of acute pharyngitis.

The monthly number of chronic pharyngitis in 2020 is decreased compared with that in 2019 (Fig. 1C). Interestingly, the monthly number of chronic pharyngitis is increased from 2020 to 2022 (Fig. 1C). Based on the annual reported cases, the annual increased rate of chronic pharyngitis is −18% and 110.1% for 2020 and 2021 respectively (Fig. 1B and Table 1). The increased rate of chronic pharyngitis in the first three months of 2022 is 57.5% compared with that in the first three months of 2021 (Fig. 1B and Table 1). In beijing, the strict regulation of COVID-19 required constant nucleic acid testing for SARS-CoV-2. In most cases, throat swabs were taken for nucleic acid testing. As shown in Fig. 1D, the number of throat swabs in Beijing Jiangong Hospital is greatly increased after the outbreak of SARS-CoV-2. The number of chronic pharyngitis and the number of throat swabs are positively linearly correlated (R^2=0.6525, p<0.0001), suggesting that taking throat swabs could increase the incidence of chronic pharyngitis (Fig. 1E).

During COVID-19 pandemic, wearing mask and social distance are required, which could reduce upper infections.[5,6] After the outbreak of COVID-19, China took a national SARS-CoV-2 nucleic acid testing strategy, which involved inpatient screening, rapid screening in fever clinics, travel screening, and large scale population screening.[7] Taking throat swabs become consistent, which could be a major physical stimulus for increasing chronic pharyngitis. Since August 2021, China has been sticking to "Dynamic COVID-Zero" strategy.[8] As a potential secondary disaster caused by COVID-19 control policy, chronic pharyngitis should be paid more attention during Dynamic COVID-Zero time.

Pharyngitis is more common in autumn, winter and spring, so there is a low incidence in summer (Fig. 1A and 1C). Under the influence of Beijing's medical insurance policy, the medical insurance balance returns to zero on January 1 every year. Combined with the upcoming Spring Festival from January to February, local residents in Beijing have more visits and follow-up visits before January 1, forming low visit to hospital from January to February (Fig. 1A and C).

In this study, we investigated the impact of the COVID-19 control regulation on the incidence of pharyngitis based on the data from Beijing Jiangong Hospital. From 2019 to 2022, cases of acute pharyngitis are decreased, while cases of chronic pharyngitis are increased. During COVID-19 pandemic, a strict control regulation is performed in Beijing. Wearing mask and social distance would reduce acute pharyngitis incidence. However, constantly taking throat swabs would increase the incidence of chronic pharyngitis. This study will advance our understanding of the potential benefit and secondary disaster caused by COVID-19 strict control policy.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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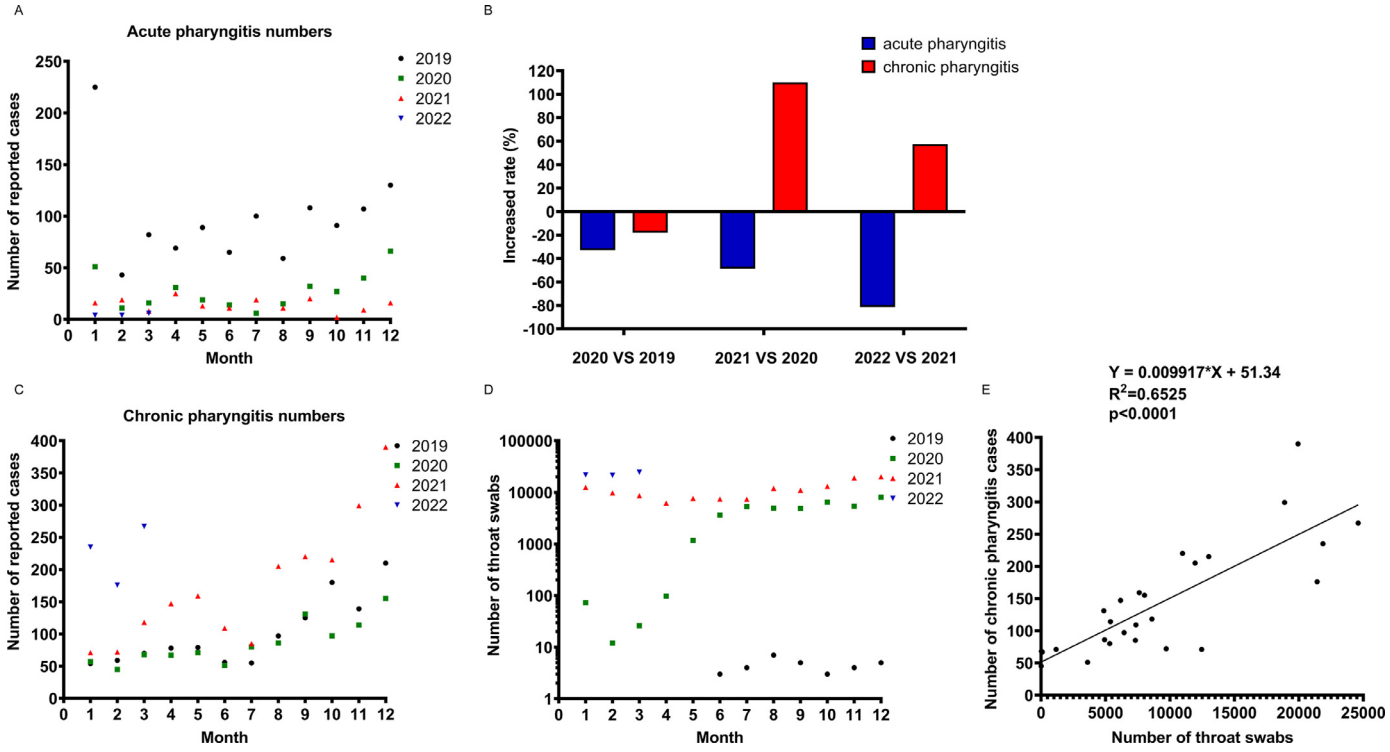

Fig. 1. Trends of pharyngitis numbers from January 2019 to March 2022. (A) Monthly trends of acute pharyngitis numbers. (B) Increase rate of acute and chronic pharyngitis. (C) Monthly trends of chronic pharyngitis numbers. (D) Monthly trends of throat swabs numbers. (E) Correlation of chronic pharyngitis numbers and throat swabs numbers.

Table 1
Epidemiological data of pharyngitis in Beijing Jiangong Hospital from 2019–2022.

Category	Number of reported cases in the first 3 months of 2021	Number of reported cases in the first 3 months of 2022	Increased cases (2022 VS 2021)	Increased rate	P value	Number of reported cases in 2020	Number of reported cases in 2021	Increased cases (2021 VS 2020)	Increased rate	P value	Number of reported cases in 2019	Number of reported cases in 2020	Increased cases (2020 VS 2019)	Increased rate	P value
Acute pharyngitis	43	8	−35	−81.4%	0.133	314	161	−153	−48.7%	0.030	468	314	−154	−32.9%	<0.0001
Chronic pharyngitis	261	411	150	57.5%	0.016	961	2019	1058	110.1%	0.016	1172	961	−211	−18%	<0.0001

A paired samples *t*-test was used to compare the increased rate across different groups.

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SARS-CoV-2 Omicron sublineage BA.2 replaces BA.1.1: Genomic surveillance in Japan from September 2021 to March 2022

Dear editor,

We read with interest the letter by Dimeglio et al. reporting the impact of vaccination and pre-immunity on the proliferation of Omicron BA.1 and BA.2 sublineages in France[1]. The new emerging Omicron strain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently spreading worldwide. The Omicron strain has multiple spike protein mutations compared with other variants of concern, such as the Alpha and Delta strains[2]. Consequently, there is concern that serum antibody activity against the Omicron strain in vaccinated or convalescent persons will be weaker than that against previous SARS-CoV-2 strains[3, 4]. Because the characteristics of infectivity and treatment response differ among Omicron sublineages[5, 6], it is important to understand the evolutionary process in real time.

To determine the viral lineage of SARS-CoV-2, we performed whole genome sequencing analyses or TaqMan assays using SARS-CoV-2-positive samples (n = 1298) collected consecutively in Yamanashi, Japan from September 2021 to March 2022 (Supplemental materials)[7, 8, 9]. During this period, we identified Delta strain (n = 159) and Omicron strain (n = 1139). After the first case of Omicron was identified in January 2022, Omicron rapidly replaced Delta as the prevalent strain of SARS-CoV-2 (Fig. 1A).

The whole genome sequencing data were analyzed using PANGOLIN (version 3.1.20), and BA.1 (n = 5), BA.1.1 (n = 992), and BA.2 (n = 142) were identified as sublineages of Omicron (Fig. 1B). Sublineage BA.1.1 was the dominant sublineage of Omicron from January to mid-February 2022; however, the incidence of sublineage BA.2 increased from mid-February 2022 onward, with this sublineage becoming dominant by the end of March (Fig. 1B and 1C). The average frequency for the seven-day period from March 8 to March 14 was 62.2% (51/82) for sublineage BA.1.1 and 37.8% (31/82) for sublineage BA.2, whereas from March 15 to March 21 it was 29.3% (27/92) for sublineage BA.1.1 and 70.7% (65/92) for sublineage BA.2. These results indicate an extremely rapid replacement of sublineage BA.1.1 by sublineage BA.2 and a higher transmissibility of sublineage BA.2 compared with sublineage BA.1.1.

To investigate the underlying factors for the high transmissibility of Omicron sublineage BA.2, we performed an RT-qPCR analysis of the viral load in the nasopharyngeal swabs collected from patients infected with sublineage BA.1.1 (n = 748) or sublineage BA.2 (n = 118). The median viral load ($\log_{10}$ copies/mL) was 5.7 (range: 0.2–7.9) for sublineage BA.1.1 versus 6.4 (range: 0.3–8.2) for sublineage BA.2 (Fig. 2A). The median Ct value for sublineage BA.1.1 was 19 (range: 11–38) versus 17 (range: 10–38) for sublineage BA.2 (Fig. 2B). There are significant differences in the viral load between cases of sublineage BA.1.1 and sublineage BA.2 (Fig. 2A, $p = 4.8 \times 10^{-4}$, Student's t-test) and Ct value (Fig. 2B, $p = 1.6 \times 10^{-3}$, Student's t-test). However, the median age of infected patients was not significantly different between these sublineages (35 years [range: 0–101 years] for BA.1.1 vs. 34.5 years [range: 0–90 years] for BA.2; p = 0.1, Student's t-test) (Fig. 2C). These results indicate that the viral load in nasopharyngeal swabs is higher for sublineage BA.2 than for sublineage BA.1.1 and that sublineage BA.2 is more contagious.

We next examined whether the viral load varied with patient age. There was no apparent correlation between patient age and

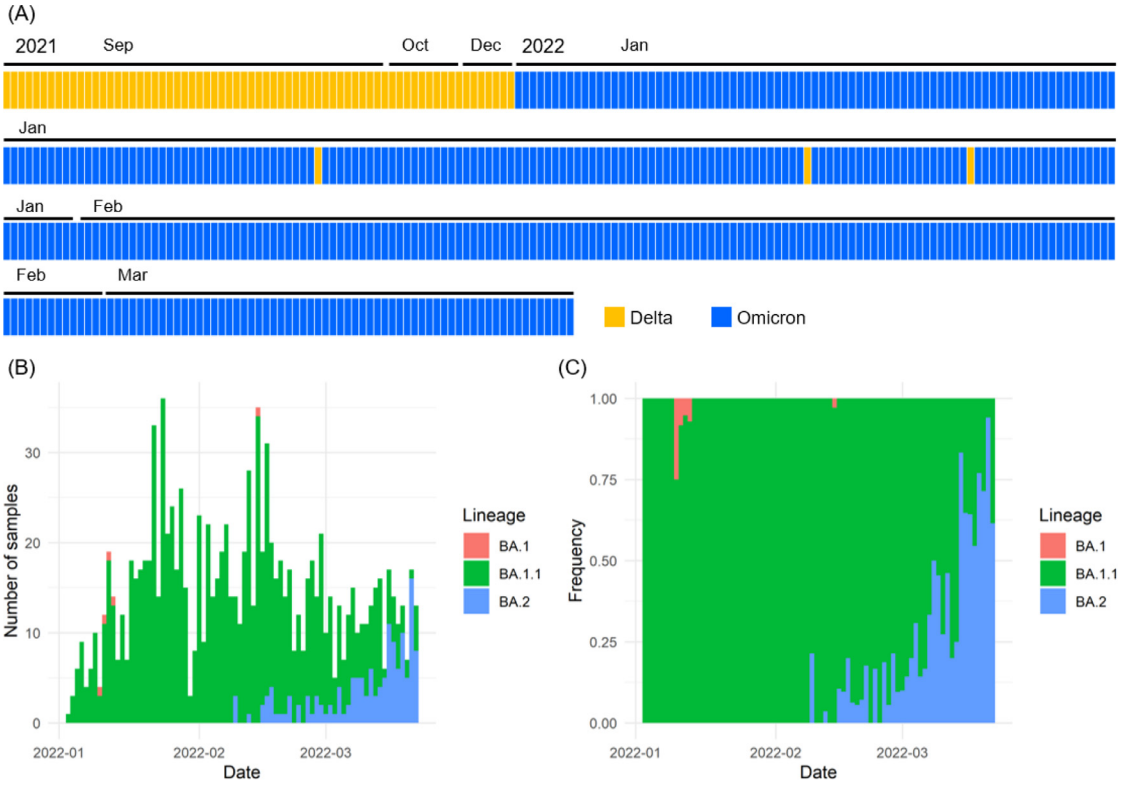

Fig. 1. Changes in Omicron strain prevalence
SARS-CoV-2 strains identified from September 2021 to March 2022. Orange boxes indicate Delta strains, and blue boxes indicate Omicron strains. (B, C) Sublineage of Omicron strains detected from January 2022 to March 2022, indicated by BA.1 (pink), BA.1.1 (green), and BA.2 (blue). The number of samples detected per day (B) and the frequency of detection (C) are shown.

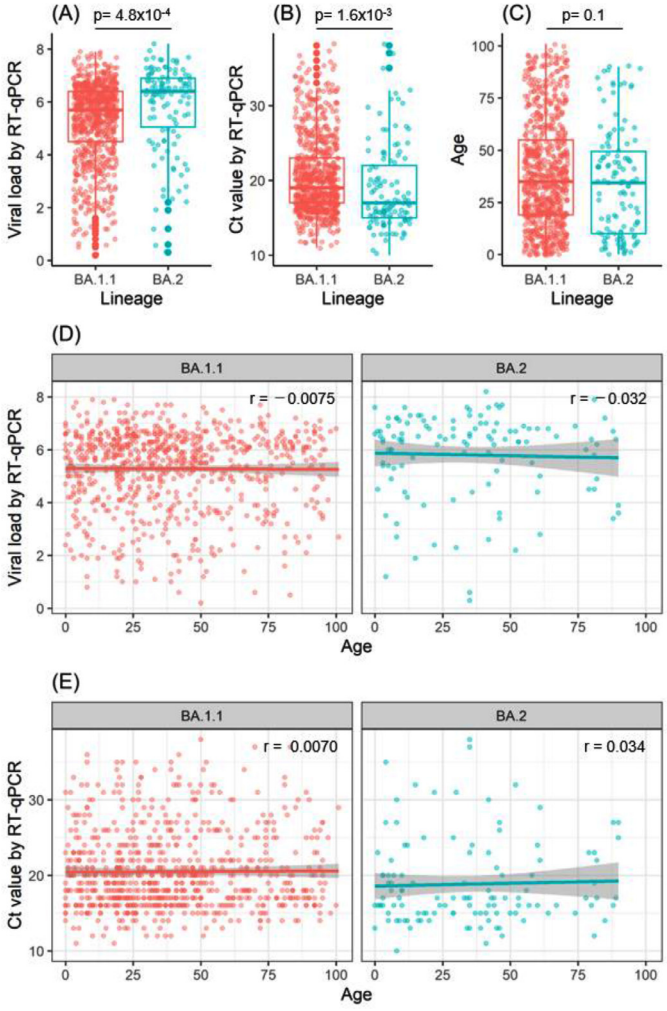

Fig. 2. Viral load and age of infected patients for sublineages BA.1. and BA.2. (A, B) The viral load and Ct values in Omicron sublineages BA.1.1 (n = 748) and BA.2 (n = 118) were analyzed by RT-qPCR. Box plots show the viral load (A) and Ct values (B) in BA.1.1 and BA.2. (C) Box plot shows the age of patients infected with sublineage BA.1.1 or BA.2. (D, E) Relationship between patient age and viral load (D) or Ct value (E). Pearson's correlation coefficient (r) is noted in the figures. The gray background of the regression line indicates the 95% confidence interval.

viral load or Ct value for either sublineage BA.1.1 or BA.2 (Figure 3D and 3E). The Pearson's correlation coefficients for sublineage BA.1.1 were $r = -0.0075$ ($p = 0.84$) for patient age and viral load and $r = 0.0070$ ($p = 0.85$) for patient age and Ct value, and those for sublineage BA.2 were $r = -0.032$ ($p = 0.73$) for patient age and viral load and $r = 0.034$ ($p = 0.71$) for patient age and Ct value (Figs. 2D and 2E). These results indicate that the viral load remained fairly high in Omicron-infected patients regardless of their age.

In summary, this study indicates that after the expansion of the SARS-CoV-2 Delta strain, a rapid spread of the Omicron strain occurred. Sublineage BA.1 was very minor in Japan when Omicron was first discovered. First, sublineage BA.1.1 expanded dominantly and was then gradually replaced by sublineage BA.2. A transition from sublineage BA.1.1 to sublineage BA.2 was clearly observed over approximately one month. The results of the present study show that the amount of viral load in the nasopharyngeal swab was higher for sublineage BA.2 than for sublineage BA.1.1. These epidemiological and viral characteristic results indicate that Omicron sublineage BA.2 is more transmissible than sublineage BA.1.1. Although a high incidence of household COVID-19 infections stemming from young children has been reported[10], our results indicate that the Omicron strain retains a fairly high viral load across age groups, which may contribute to the high infectivity of the Omicron strain and its accelerated spread. These data provide insights for determining appropriate COVID-19 prevention and control measures for homes, schools, workplaces, and facilities for the elderly during the spread of Omicron strain viruses.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.040.

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The negative impact of the COVID-19 Pandemic on immunization and the positive impact on Polio eradication in Pakistan and Afghanistan

Dear editor,

In a recent letter by Usman Ayub Awan et al. [1], the authors described the outbreak of the wild poliovirus in Afghanistan. We agree with the concerns raised by the authors and here we would like to discuss the current polio situation and the impact of the COVID-19 pandemic on polio vaccination in Pakistan and Afghanistan.

The global polio eradication initiative was launched in 1988, when the wild poliovirus was endemic in 125 countries, paralyzing 350,000 individuals per year. Enormous progress has been made towards the eradication of polio by reducing 99% of global incidence due to immunization efforts [2].

Vaccines and the importance of immunization have taken center stage in public discourse like never before. As the world focus on COVID-19 Vaccination campaigns, what does this mean for other vaccine-preventable diseases such as polio? The covid-19 pandemic has compromised public health systems around the world and disrupted the delivery of essential health services including efforts to achieve polio eradication. The three-decades-long derive to eradicate polio was going back even before COVID-19 hit, and the pandemic has made a bad situation worse. In 2019 and 2020, cases of wild poliovirus rose in Pakistan and Afghanistan, the last two countries where it is endemic.

The ongoing COVID-19 Pandemic is disrupting life-saving immunization services globally, but with a particular impact in low-income countries including Pakistan and Afghanistan. The highest number of unimmunized children live in south Asia, with 97% of them living in Pakistan, Afghanistan, and India [3]. The evidence from previous Ebola epidemics has demonstrated that even temporary interruptions of routine immunization services can lead to secondary public health crises such as outbreaks of vaccine-preventable diseases including polio and measles.

Pakistan and Afghanistan are the fifth and thirty-seventh most populous countries in the world. Both countries spend less than 1% of their gross domestic product on health services. Owing to the low investment in the health sector many vaccines preventable diseases (VPDs) are still endemic in Pakistan and Afghanistan including polio and measles. The polio eradication program in Pakistan and Afghanistan successfully reduces the number of confirmed polio cases from 306 in 2014 to only 12 in 2018 in Pakistan and from 28 cases in 2014 to 21 cases in 2018 in Afghanistan. Unfortunately, there was a resurgence of polio cases to 147 in 2019 and 84 polio cases in 2020 were reported in Pakistan. Similarly, 29 polio cases in 2019 and 56 in 2020 were reported in Afghanistan. Surprisingly, in the presence of millions of unimmunized children, only one polio case was reported in Pakistan and 4 polio cases were reported in Afghanistan in 2021. Only one polio case is reported in Afghanistan and zero polio cases in Pakistan in 2022. From 2010-to 2022, a total of 1122 and 342 polio cases were reported from Pakistan and Afghanistan respectively. Detail of reported polio cases in Pakistan and Afghanistan is given in Fig. 1.

The coverage of routine immunization including polio and measles vaccination in Pakistan and Afghanistan is far lower than the 90% that is required for herd immunity and immunization against polio has further declined by 50% during the COVID-19 pandemic when 50 million children in Pakistan [3] and 23 million children in Afghanistan [4] did not receive a polio vaccination owing to the suspension of immunization activities. Before the COVID-19 pandemic, more than 600,000 Afghan children miss polio vaccination because of the refusal of their parents [5]. There is an 18,593 live birth average per day in Pakistan and a 3986 live birth average per day in Afghanistan, the suspension of door-to-door polio immunization campaigns has left a huge and growing pool of children who are susceptible to polio in both countries. When the virus finds them, it will tear through the unimmunized population and may lead to more polio outbreaks in near future. Recently polio outbreak has been declared in Malawi, the poliovirus strain found in the child in Lilongwe has been linked to one circulating in Pakistan [6].

Due to the disruption in immunization, the crystal-clear evidence of the unprecedented upsurge in vaccine-preventable diseases such as measles in Pakistan and Afghanistan resulted in 28,125 suspected measles cases, including 800 deaths reported

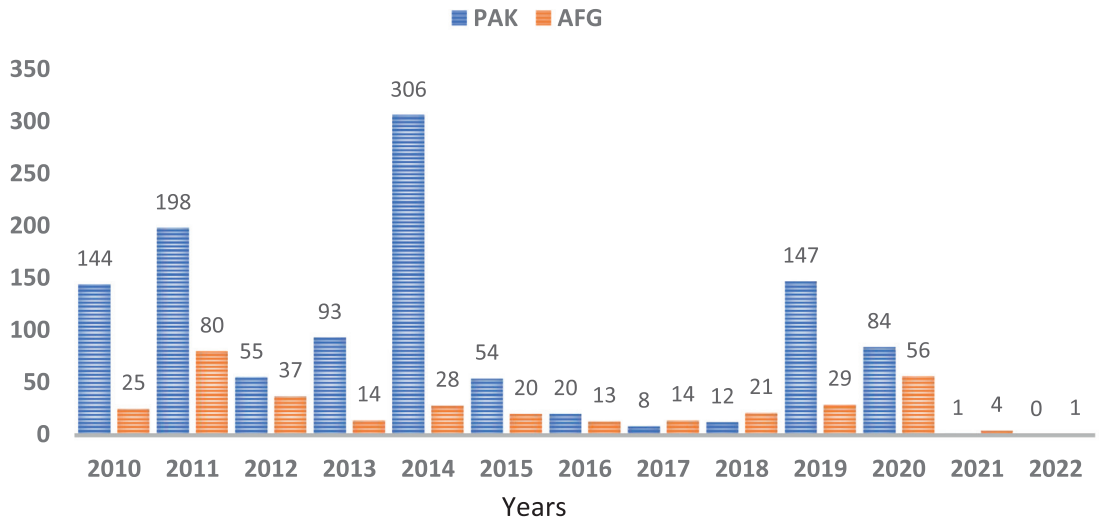

Fig. 1. Reported polio cases 2010–2022 in Pakistan and Afghanistan.

from Pakistan [7] and 35,300 suspected measles cases including 156 deaths were reported from Afghanistan in 2021. Most other VPDs such as Diphtheria, Pertussis, typhoid, TB, Hepatitis, Rota, and Rubella virus are on the rise due to the disruption in immunization owing to a pandemic, but poliovirus is unprecedently declined in both countries.

Multiple hurdles in polio eradication are still there in both countries such as poor immunization coverage, vaccine hesitancy, vaccine refusal, conspiracy theories, political instability, overcrowded population, killing of polio workers, population movement, remote locations, and conflict. In the presence of multiple hurdles fueled by the COVID-19 pandemic, the more than 99% decline in polio cases in Pakistan and Afghanistan is difficult to understand for the scientific community. The sudden absence of polio circulation is a daunting task that cannot be verified reliably because of the limitations of existing surveillance tools, which include acute flaccid paralysis surveillance supplemented by environmental surveillance. In a fully susceptible population, acute flaccid paralysis surveillance can detect one in 100 to 1000 infections. However, with increasing population immunity, surveillance for clinical signs of poliovirus infection become much less sensitive, allowing poliovirus to circulate undetected for many years. If the decline in polio cases in Pakistan and Afghanistan is due to the vaccination campaigns, now the key is to prevent a resurgence in the coming peak season for polio transmission in both countries.

World Health Organization and patterners in the global polio eradication initiative are committed to fully supporting the government of Pakistan and Afghanistan to tackle polio in its last strongholds and failure to eradicate polio now could result in a resurgence of the disease, with as many as 200,000–300,000 new cases worldwide every year.

Although Pakistan and Afghanistan face distinct polio eradication challenges, they are linked epidemiologically because of high rates of cross-border population movement. Transit-point vaccination must be maintained as emigration from Afghanistan potentially increases after the Taliban control.

The persistence of polio in Pakistan and Afghanistan, and the increase in circulating vaccine-derived poliovirus, has been a warning call for intensifying efforts for control despite the disruption imposed by the COVID-19 pandemic. Disruption in immunization in both countries coupled with Taliban rule in Afghanistan threatens to reverse significant achievements in polio eradication and the world must seek innovative approaches to work with the Taliban to get polio and other VPDs under control.

The COVID-19 pandemic is a magnifying glass that has highlighted the larger threat of existing public health challenges such as polio. The long-term trajectory of the pandemic is uncertain, and the risk of resurgence of epidemic-prone diseases including polio is very high. Additionally, at the same time, the health system's capacity to perform a meaningful epidemiological analysis of the situation may also be impacted. Pakistan and Afghanistan are already struggling to eradicate polio and disruption in immunization owing to the pandemic the risk of polio outbreak is on the brink and it may provide the virus a milieu to spread further and faster and could also result in the worldwide spread of polio infection. The world cannot afford to have another epidemic of polio after the devastating impact of the COVID-19 pandemic.

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Characteristics of genotypes and prevalence of high-risk human papillomavirus in different economic development regions in China

Dear editor,

A study with population-based published by He-Ling Bao and colleagues entitled "Prevalence of cervicovaginal human papillomavirus infection and genotypes in the pre-vaccine era in China: A nationwide population-based study" in this journal.[1] We read with great interest. Yet, some points remain to be discussed, including whether the prevalence of high-risk human papillomavirus (HR-HPV) was different in level of economic development. A lot of previous studies have showed that there were disparities significantly in HPV prevalence across varying economic development regions.[2–5]

There might be several reasons for this results. Firstly, the different women population was selected. As Bao et al.[1] study mentioned, main groups are mainly from urban or developed areas in their study that might affect the estimation. Secondly, the sensitivity and specificity of different HPV testing and reagents are still vary significantly,[4,6] which might cause within-group variations in HPV prevalence. In addition, the data lack of HPV infection in some provinces and derived from existing database also may be another reason of inconsistence.

We conducted a prospective study based on large population to investigate the epidemiological characteristics of HR-HPV infection and the 14 genotype distribution of HR-HPV in different regions of China using self-collected from vaginal samples. The included population was divided into the undeveloped, middle developed and highly developed region groups according to the reginal Gross Domestic Product (GDP), the relationship of HPV subtypes especially HPV16 /18 and GDP were analyzed.

A total of 20,103 women aged 30–59 years were recruited from 13 provinces in China from September 2018 to July 2020. 35.81% of them was from remote and rural areas. All participants registered, signed electronic informed consent, and filled in individual information voluntarily using an internet-based cervical cancer screening (CCS) platform. The exfoliated cells of the vagina and cervix with self-sampling was obtained using a designed the sampling kit including a sampling brush, a storage card or preservation solution, a printed graphic sampling instruction. The samples were tested for 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using the SeqHPV and BMRT HPV PCR assays. SPSS 22.0 statistical analysis software was used for data analysis in this study.

The overall prevalence of HR-HPV infection was 13.86% among 20,103 women. The vast majority of HR-HPV (77.18%) were still a single type. Inner Mongolia had the highest prevalence (21.55%), while the lowest prevalence was in Jilin (9.51%) in 13 regions. HPV prevalence in Qinghai (14.08%,195/1384) and Guangxi(10.26%,195/1900) were included. The infection rate of overall HPV in different regions are shown in Fig. 1.

According to the various GDP, namely highly developed, middle developed, and undeveloped regions, the significant differences were observed in the prevalence of HR-HPV in analyzed data of three stratifications ($p < 0.01$).It also shows that the prevalence of HPV16/18 decreases with the increases of the reginal GDP. In addition, the undeveloped regions had the highest prevalence of combined- and single-type prevalence (HPV16/18, HPV16/18/ 31/ 33/ 52/58, single-type infection). All HR-HPV infections except for multiple-type infections were significantly associated with the reginal economic status (Table 1).

The binary logistic regression have shown, the education level was positively correlated with HR-HPV prevalence (aOR = 0.64, 95%CI 0.48–0.85); and first sexual intercourse after 20 years and older was correlated with a decrease in the odds of HR-HPV infection (21–25 years: aOR = 0.86, 95%CI 0.76–0.97; ≥26 years: aOR = 0.72, 95%CI 0.62–0.85).

The present study was conducted based on population-based study involved 13 provinces in China. It is so far the first as well as the largest prospective study in China that used HR-HPV testing of self-collected vaginal samples, which 13.86% of women were tested positive of HR-HPV. The HR-HPV prevalence in this cervical screening program were found significantly different in geographical regions, with a range from 9.51% to 21.55%. Reginal variation on HR-HPV prevalence was also evidenced by a previous study based on physician-sampling.[3,4] In addition, our study included not only urban and suburban women, but also rural women, so the data is more comprehensive.

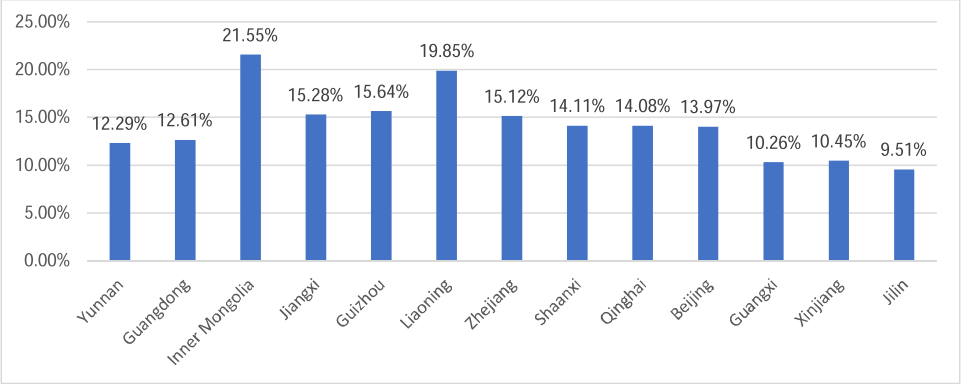

Fig. 1. Prevalence of HR-HPV infections in 20,103 women from 13 provinces.

Table 1
Distribution and association of HPV infections with regions stratified by GDP.

Regions stratified by GDP	Total	HPV16+18 [a] [n (%)]	HPV16+18+31 +33+52+58[b] [n (%)]	Overall HR-HPV [n (%)]	Single-type infection [n (%)]	Multiple-type infection [n (%)]
Highly developed regions [c]	7032	116 (1.65)	750 (10.67)	971 (13.81)	744 (10.58)	227 (3.23)
Middle developed region [d]	6331	180 (2.84)	586 (9.26)	814 (12.86)	630 (9.95)	184 (2.91)
Undeveloped regions [e]	6740	204 (3.03)	731 (10.85)	1002 (14.87)	777 (11.53)	225 (3.34)
χ^2		31.74	10.67	11.06	8.66	2.13
P-value		<0.001	0.005	0.004	0.013	0.340

[a] HPV16+18, women who were infected with HPV16 or HPV18.
[b] HPV16+18+31+33+52+58, women who were infected with HPV16, HPV18, HPV31, HPV 33, HPV 52, or HPV 58.
[c] Highly developed regions are regions with a per capita GDP greater than 100,000 RMB (Beijing, Guangdong, Zhejiang, and Qinghai).
[d] Middle developed regions are regions with a per capita GDP between 60,000 RMB and 100,000 RMB, (Xinjiang, Liaoning, Jilin, Shaanxi, and Yunnan).
[e] Undeveloped regions are regions with a per capita GDP less than 60,000 RMB (Guizhou, Inner Mongolia, Jiangxi, and Guangxi).Acronyms: GDP, Gross Domestic Product; NA, Not Applicable.

In this analysis of HR-HPV prevalence and subtypes in term of GDP, the positive rate of HR-HPV, combined- and single-type prevalence (HPV16/18, HPV16/18/ 31/ 33/ 52/ 58, single-type infection) in diverse GDP was statistically different, while development status in Bao et al.[1] study there was no statistical difference ($p = 0.7301$). In another study of large sample the level of GDP per capita(low, middle, and high), the prevalence of HPV infection were 16.72, 18.01 and 13.41%.[4] Meantime, the undeveloped regions in our study had the highest prevalence of specific combinations of HR-HPV subtypes, of which HPV16/18 infections decreased with increasing GDP.

In addition, the highest prevalence of HPV single-type in the undeveloped regions in this study, and the relationship between education level and HR-HPV infection also provide the evidence for varying HPV infection rates in diverse states of economic development. These data also provide a strong basis for HPV vaccination and accurate CCS in various regions, in particular, lower coverage of CCS, large population, imbalanced economic development, and culture variations in China.[7,8]

In summary, based on self-collected HPV testing, the undeveloped regions had the highest prevalence of specific combinations and single-type of HR-HPV subtypes, of which HPV16/18 infections decreased with increasing GDP. These demonstrate that it is crucial to develop specific plans for CCS according to the distribution of various subtypes. Finally, with WHO recommending self-collected HPV testing as one of the optional primary CCS method[9], using the internet-based self-sample screening can improve a much greater coverage of CCS in different level of GDP.

Ethics approval

Ethical approval of the study was granted by the Ethics Committee of the Peking University People's Hospital (2018PHB056-01), and registered on the Chinese Clinical Trial Website (https://www.chictr.org.cn, ChiCTR2000032331).

Declaration of Competing Interest

All authors declare no conflict of interest.

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Proctitis and prostatitis by *Neisseria meningitidis* among MSM: A case series

Dear editor,

In this Journal, Liu and colleagues have recently described the case of a men who have sex with women with urethritis by *Neisseria meningitidis* (Nm).[1] Nm infections of the urogenital and anorectal tracts have been described also among men who have sex with men (MSM) and in outbreaks, either without clinical manifestations or with gonorrhea-like symptoms. Transmission occurs through droplets or though sexual contact with a carrier.[2]

Here we present a case-series of Nm infections of proctitis and prostatitis in MSM.

Among 3354 rectal swabs performed for culture exam for Ng, Nm was isolated in 13 MSM. Nm was isolated through gonococcal-specific cultures on anal swab or semen, collected from a cohort of MSM, in care at the Infectious Diseases Unit of IRCCS San Raffaele, Milan, from 2014 to 2022. Samples (E-Swab, Copan, Brescia, Italy) were collected upon symptoms in people living with HIV (PLWH) or during the routine screening in pre-exposure prophylaxis (PrEP) clients, which encompasses rectal and pharyngeal swabs for *N. gonorrhoeae* (Ng), rectal swab for *C. trachomatis* (Ct) and urine samples for nucleic acid amplification tests (NAATs) for Ng and Ct. Diagnosis of Nm infection was based on gonococcal-specific culture-based detection method, performed on rectal and pharyngeal swabs and on semen sample of a patient with prostatitis. Antimicrobial susceptibility breakpoints were defined according to the EUCAST recommendations.[3]

All the four PLWH reported symptoms of proctitis, while no symptoms were documented among the nine PrEP users. At least one previous sexually transmitted infection (STI) was recorded in all subjects (Table 1). Concomitant oropharyngeal carriage was confirmed by oropharyngeal swab in most PrEP clients. All subjects received ceftriaxone-based therapy (1 gram, intramuscular injection, single dose), according to European recommendations,[4] as all isolates were susceptible. CDC-recommended therapy for NG with ceftriaxone plus azithromycin (1 gram, oral, single dose) or doxycycline (100 mg, oral, twice, for 7 days) was introduced in PLWH upon symptoms.[5] In asymptomatic PrEP users, ceftriaxone alone was administered, in addition to specific treatment for concomitant infections. We obtained symptoms resolution and infection clearance in all cases. Contacts were not identified, nor treated. Finally, no one was immunized against Nm serotypes A, C, W, Y or B prior to meningococcal isolation.

Among them, we present the case of a 30-years old MSM PrEP client (Case 13, Table 1) who had six positive cultures from anal swabs for Nm, performed during screening visits; the subject reported tenesmus once. Concurrent oropharyngeal Nm carriage was found in five episodes with pharyngodynia documented once. He reported engaging in multiple unprotected receptive sexual intercourses (Smith DK index >25),[6] including receiving anilingus from different partners. The isolates showed variable susceptibility to beta-lactams over time and treatment was always administered with either ceftriaxone or ciprofloxacin (500 mg) with clearance of infection at rectal site. Notably, a persistent carriage of Nm at oropharynx was recorded following treatment with ciprofloxacin or with doxycycline administered for concomitant Ct infection. Interestingly, Nm was isolated also after MenACWY vaccination, administered more than 2 years after first episode (Table 2). As recurrent Nm infections have been associated with complement deficiencies,[7] we also measured complement serum activity (CH50) by turbidimetry (Optilite-WKCMET189), being 33 U/mL (normal values >32 U/mL).

Here, we also report the case of a symptomatic acute prostatitis caused by Nm in a MSM living with HIV (Case 14, Table 1), confirmed by a positive semen culture, which was successfully treated with ceftriaxone for one month, with resolution of symptoms and subsequent clearance of infection. Notably, just one case of prostatitis by Nm has already been described.[8] On the other hand, as NAAT techniques currently available in clinical setting fail to identify Nm, urethritis might be underreported in our sample; selective whole genome amplification techniques should be implemented.

Currently, no guidelines are available for diagnosis, treatment and prevention of meningococcal anal infections.

As most of our cases were asymptomatic and diagnosis was made during a screening visit, it should be clarified whether the presence of Nm in the anal mucosa should be considered as carriage or a disease (i.e. proctitis). In a dated study, a mild chronic inflammatory cell infiltration of the lamina propria was found in a biopsy of rectal tissue retrieved from two men with meningococcal infection, being possibly related to anal intercourse.[9] Since meningococci are not adapted to colonize the urogenital and the anorectal tracts, we considered the microbiological isolate as a sign of infection, even in the absence of symptoms, and we treated all cases. Moreover, we cannot exclude a potential role of Nm infection in favoring sexual HIV transmission, as it has been demonstrated for the phylogenetically-related gonococci. In particular, in the case of recurrent proctitis, we believe that having at-risk sexual behaviors, engaging anal intercourses and the variable susceptibility to beta-lactams over time of Nm suggest re-infections rather than colonization or treatment failure.

Reassuringly, in contrast to Ng, antibiotic resistance is not worrisome; consistently, we found no resistance for ceftriaxone and low MIC levels for azithromycin, one ciprofloxacin-resistant and three rifampin-resistant strains. We advise treating all Nm anal infections, with or without symptoms, with ceftriaxone (1 g), as for Ng urethritis, according to CDC and European guidelines.[4,5] Moreover, we have shown that ceftriaxone-based regimens were always effective in clearing infections at both oropharynx and anal sites. Finally, treatment of contacts should be considered, particularly of engaging with the same sexual partner, while treatment of oropharyngeal carriage of Nm is discouraged with implications both for the clinical and public health management.

Table 1
Characteristics of 13 cases of proctitis and 1 case of prostatitis caused by *Neisseria meningitidis*.

	Age (y.o.)	HIV status	Sample	Dates of positive rectal swabs for Nm	Dates of positive oropharyngeal swabs for Nm	Symptoms	Concomitant infection	Treatment	Previous STIs
Case 1	68	Positive	Rectal swab	11.06.2014	Not assessed	Tenesmus	Syphilis	Ceftriaxone and azithromycin, penicillin G	Proctitis by Ct and *M. genitalium*, proctitis and urethritis by Ng
Case 2	26	Positive	Rectal swab	14.06.2016	Not assessed	Proctorrhagia Mucorrhea	Urethritis by *U. urealyticum*	Ceftriaxone and doxycycline	Syphilis, HPV
Case 3	48	Positive	Rectal swab	29.07.2016	Not assessed	Diarrhea	None	Ceftriaxone and azithromycin	Syphilis, HBV, HPV, proctitis by Ct, urethritis by *U. urealyticum*, pediculosis
Case 4	39	Negative	Rectal swab	03.12.2019	03.12.2019 11.08.2020	None	None	Ceftriaxone and azithromycin	Syphilis, proctitis by C. trachomatis and proctitis by Ng
Case 5	32	Negative	Rectal swab	18.05.2021	Always negative	Dysuria, Urethral discharge	None	Ceftriaxone and azithromycin	Syphilis, HAV, HPV, proctitis and urethritis by Ct and proctitis by Ng
Case 6	32	Negative	Rectal swab	01.07.2021	Always negative	None	None	Ceftriaxone	Syphilis, proctitis by Ct and by Ng
Case 7	32	Negative	Rectal swab	07.07.2021	07.07.2021	None	Proctitis by Ng	Ceftriaxone and azithromycin	Proctitis by Ct
Case 8	27	Negative	Rectal swab	16.08.2021	16.08.2021	None	Proctitis by Ng and Ct	Ceftriaxone and azithromycin	Syphilis, proctitis by *M. genitalium* and by Ng
Case 9	35	Negative	Rectal swab	14.09.2021	14.09.2021	None	None	Ceftriaxone	Unknown
Case 10	34	Negative	Rectal swab	15.09.2021	Not assessed	None	None	Ceftriaxone	Proctitis by Ng and Ct
Case 11	28	Negative	Rectal swab	11.01.2022	11.01.2022	Pharyngitis	None	Ceftriaxone	Syphilis
Case 12	45	Negative	Rectal swab	27.01.2022	27.01.2022	None	None	Ceftriaxone	Urethritis by Ng
Case 13^	30	Negative	Rectal swab	06.11.2017	04.10.2017 06.11.2017	None	Proctitis by Ct	Ceftriaxone and azithromycin	HPV, pharyngitis and proctitis by Ct and proctitis by Ng
			Rectal swab	01.03.2021	01.03.2021	None	None	Ceftriaxone	
Case 14	53	Positive	Semen	18.09.2015	Not assessed	Pelvic pain, dysuria	None	Ceftriaxone	Anal HPV carriage, prostatitis by Ng

^ **Only first and last positive rectal swabs reported; details for this subject in Table 2.** Abbreviations: y.o.: years old; STI: sexually transmitted infection; Ng: *Neisseria gonorrhea*; Ct: *Chlamydia trachomatis*; HBV: Hepatitis B virus; HPV: Human Papillomavirus; *M. genitalium: Mycoplasma genitalium; U. urealyticum*: *Ureaplasma urealyticum*.

Table 2
Summary of clinical history of Case 13.

Date	Rectal swab	Pharyngeal swab	Intervention
Month 0	Negative	Negative	–
Month 2	Not assessed	Negative	–
Month 4	Negative	Negative	–
Month 6	Negative	***N. meningitidis***	–
Month 8	***N. meningitidis + C. trachomatis***	***N. meningitidis***	Ceftriaxone + Doxycycline
Month 10	Negative	Negative	–
Month 12	***N. meningitidis***	Negative	Ceftriaxone
Month 14	Negative	Negative	–
Month 16	***N. meningitidis***	***N. meningitidis***	Ciprofloxacin
Month 18	Negative	***N. meningitidis***	–
Month 20	***N. gonorrhoeae***	***N. meningitidis***	Ceftriaxone + Azithromycin
Month 22	Negative	Negative	–
Month 24	Negative	***N. meningitidis***	Ciprofloxacin
Month 26	Negative	Negative	–
Month 28	Negative	Negative	–
Month 30	***N. meningitidis***	***N. meningitidis***	Ceftriaxone
Month 32	Not assessed	Negative	–
Month 34	***C. trachomatis***	***N. meningitidis + C. trachomatis***	Doxycycline
Month 36	Not assessed	***N. meningitidis***	–
Month 38	Negative	Negative	–
Month 40	***N. meningitidis***	***N. meningitidis***	Ceftriaxone
Month 42	Not assessed	Negative	–
Month 44	Negative	Negative	–
Month 46	Negative	Negative	MenACWY vaccination
Month 48	Negative	Negative	–
Month 50	***N. meningitidis***	***N. meningitidis***	Ceftriaxone
Month 52	***C. trachomatis***	Negative	Doxycycline
Month 54	Negative	Negative	–

As most reported cases of Nm proctitis were caused predominantly by serogroup C lineages,[2] we believe that MenACWY vaccination should be provided to MSM, particularly in those at high-risk of STIs. Moreover, vaccines targeting Nm might help in reducing the probability of reinfection, even though they failed to reduce oropharyngeal carriage at 12 months.[10]

In conclusion, proctitis and prostatitis by Nm were described in 14 MSM, with and without HIV infection, successfully treated with ceftriaxone.

Declaration of Competing Interest

Authors have no conflicts of interest to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.04.037.

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Cervical lymph node dissection on the treatment of cervical tuberculosis

Dear editor,

We have read with great interest a recent study of Dr. Ying Luo regarding GBM model may be of great benefit served as a tool for the accurate identification of ATB.[1] In their study, A total of 2619 participants (1025 ATB and 1594 LTBI) were enroled in discovery cohort. ATB patients had significantly higher levels of tuberculosis-specific antigen/phytohemagglutinin ratio and coefficient variation of red blood cell volume distribution width, and lower levels of albumin and lymphocyte count than those of LTBI individuals.[1] In a similar study, we have further found out that Functional cervical lymph node dissection can shorten the anti-tuberculosis medication treatment time in patients with cervical lymph node tuberculosis.

We gathered inpatient and post-discharge follow-up data on 360 patients clinically diagnosed with cervical lymph node TB at Wuhan Pulmonary Hospital between January 2017 and August 2021, including surgical and non-surgical patients. The number of cervical lymph nodes and the period of anti-tuberculosis medication treatment were compared after patients were home.

The data distribution was described in Table 1. The patients ranged in age from 2 to 83 years old, with an average age of 38 and a standard deviation of 20. The surgical treatment group's patients ranged in age from 2 to 75 years old, with an average age of 38 and a standard deviation of 16. The surgical treatment group's patients ranged in age from 6 to 83 years old, with an average age of 45 and a standard deviation of 20. The number of lymph nodes in the neck of the patients ranged from 8.15 to 1.61 according to the follow-up of the patients from one month to six months after discharge from the hospital, the number of lymph nodes in the surgical treatment group ranged from 1 to 0.69 with a standard deviation of less than 1, and the number of lymph nodes in

Table 1
Summary table of the patient characteristics.

	Total	Surgery	Non-surgery
Age, years			
Range	2–83	2–75	6–83
Mean(SD)	38(16)	36(14)	45(20)
Gender(%)			
Male	29.44%	31.01%	2.00%
Female	70.56%	68.99%	98.00%
The number of lymph nodes			
1 month	8.15(2.87)	1.00(0.91)	4.57(1.12)
2 months	1.84(1.77)	0.86(0.84)	4.46(1.07)
3 months	1.72(1.75)	0.77(0.83)	4.32(1.11)
6 months	1.61(1.73)	0.69(0.7)	4.17(1.16)
The time of anti-tuberculosis treatment after discharge			
Range	1–24	1.41–12	1–24
Mean (SD)	244.8(86.1)	205.34(42.39)	372.72(71.54)

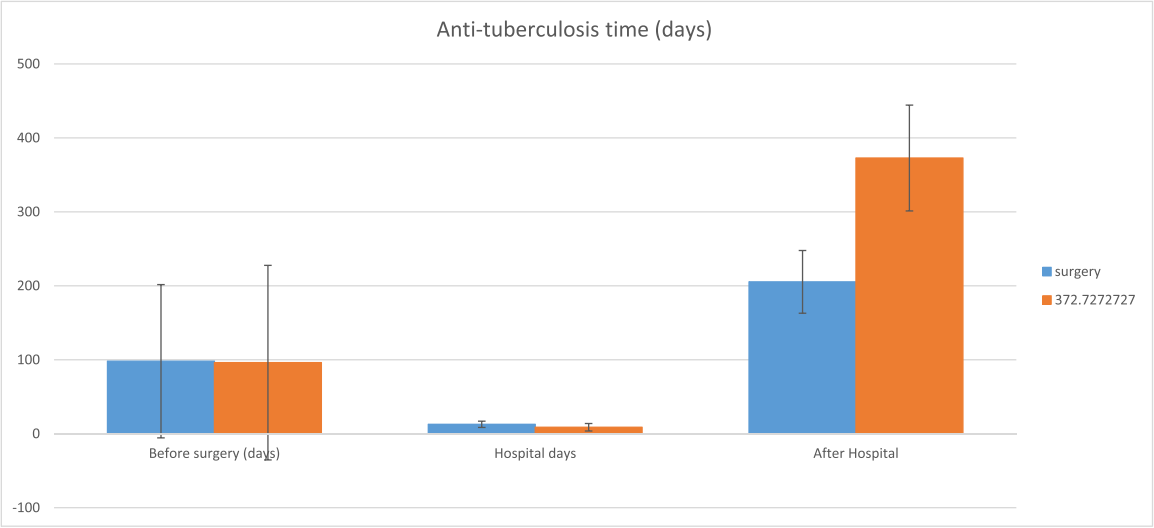

Fig 1. The anti-tuberculosis time between the surgery group and non-surgery group.

the non-surgical treatment group ranged from 4.57 to 4.17 with a standard deviation of around 1. This indicates that after surgery, patients with cervical lymph nodes have fewer remaining lymph nodes in the neck.

In this paper, we had collected 360 HNTB patients, 258 of them were with surgery treatment and 66 of them were without surgery treatment during their hospital days. The color doppler ultrasonography prompt was adopted to keep tracking the quantitative analysis of lymph nodes for one month, two months, three months, and six months for paper. Standard deviation (SD) analysis was shown in Fig. 1, the average anti-tuberculosis time of the patients with the surgical treatment was 98.02 days and for the patients without surgery treatment was 96.13 days before the hospital stage. The average anti-tuberculosis time in the hospital stage was 12.76 days for surgical treatment patients and 8.74 days for without surgical treatment patients. The average anti-tuberculosis time after the hospital was 205 days with a standard deviation of 42.39 for surgical treatment patients, and 372 days with a standard deviation of 71.54 for without surgery treatment patients.

The diagnosis of HNTB is usually challenging due to the nonspecific presentation of systemic symptoms and the paucibacillary nature, hindering the clinical effectiveness of conventional testing.[2]

In our study, out of a total of 360 patients, 269 patients (74.7%) required surgical treatment, while only 91 patients (25.3%) were cured with medical treatment alone, which is a high proportion compared to the study by Mohapatra and Janmeja,[3] who operated on half of the patients (50.8%, 33), and much higher than Monga S, who successfully treated 83% of patients in a prospective study of 140 cases with short-term chemotherapy for six months.[4]

In functional cervical lymph node dissection, a transverse streak incision in the corresponding area of the cervical lymph node is selected as the surgical approach. The broad neck muscle and sternocleidomastoid muscle are separated and the internal jugular vein is found, and the internal cervical lymph nodes are cleaned one by one along the outside of the internal jugular vein.

The anti-tuberculosis medicine therapy utilized in this hospital was based on the WHO's anti-tuberculosis treatment guideline. Patients with cervical lymph node tuberculosis got anti-tuberculosis medications whether they had surgery or not.[4]

After the patient was discharged from the hospital, he or she would return to the hospital for regular evaluation since the paient required to continue taking anti-tuberculosis medications. We also did follow-up and re-examination for patients with cervical lymph node TB in the outpatient department of Wuhan Pulmonary Hospital. We measured the size of the patient's cervical lymph nodes on a regular basis. The number of cervical lymph nodes was determined using color Doppler ultrasonography to better quantify the alterations in the lymph nodes.[5]

Tuberculosis treatment necessitates long-term treatment with a cocktail of medications.[6] These medications are linked to a number of side effects that can cause serious morbidity, such as deafness, and in some cases, death.[7]

Our research found that functional cervical lymph node dissection can shorten the anti-tuberculosis medication treatment time in patients with cervical lymph node tuberculosis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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High rate of reinfection with the SARS-CoV-2 Omicron variant

Dear Editor,

We read with interest that infection with Omicron variant can occur in patients who presented a high antibody titer, even though their concentration was at 2.4 higher than infection with Delta variant [1]. The SARS-CoV-2 pandemic has shown the succession or superposition of epidemics linked to numerous viral variants [2]. Until recently, the overall rate of reinfection with SARS-CoV-2 has been relatively low, below 2% according to several international studies [3, 4]. The Omicron (B.1.1.529) variant has been described for the first time on November 2021 in Gauteng province, South Africa and spread rapidly worldwide. One study conducted in South Africa demonstrated that it was associated with an increased hazard ratio of reinfection, suggesting its substantial ability to evade immunity from prior infection [5]. In addition, vaccine efficacy against this variant was reported to be reduced to around 56% for the Pfizer vaccine [6].

We report here the incidence and proportion of reinfections with the Omicron variant among patients diagnosed in our institute.

Our laboratory has massively screened SARS-CoV-2 infections by real-time reverse transcription-PCR (qPCR) under the same conditions since the emergence of this virus in France in February 2020. We thus have a cohort of patients screened and diagnosed as infected for the period February 27, 2020-March 6, 2022, making it possible to calculate the rate of reinfections over this entire period without the bias of variable screening strategy or capacity. An automatic reinfection detection system has been implemented through the laboratory information system of our institute's laboratory, on the basis of two qPCR-positive samples spaced at least 90 days apart with a negative qPCR between two episodes, according to the CDC definition of reinfection case [https://www.cdc.gov/coronavirus/2019-ncov/php/invest-criteria.html]. SARS-CoV-2 RNA genotyping was performed by using sequencing or variant-specific qPCR, as described elsewhere [3].

From February 7, 2020 to March 6, 2022, 1646 of 80,863 patients found SARS-CoV-2-positive experienced a reinfection. Their mean age ± standard deviation at time of the second infection was 38.3 ± 16.4 years, ranging from 9 months to 97 years, and 60.1% were female. In Marseille, we observed five major epidemics of SARS-CoV-2 infections due to different mutants or variants (Fig. 1)

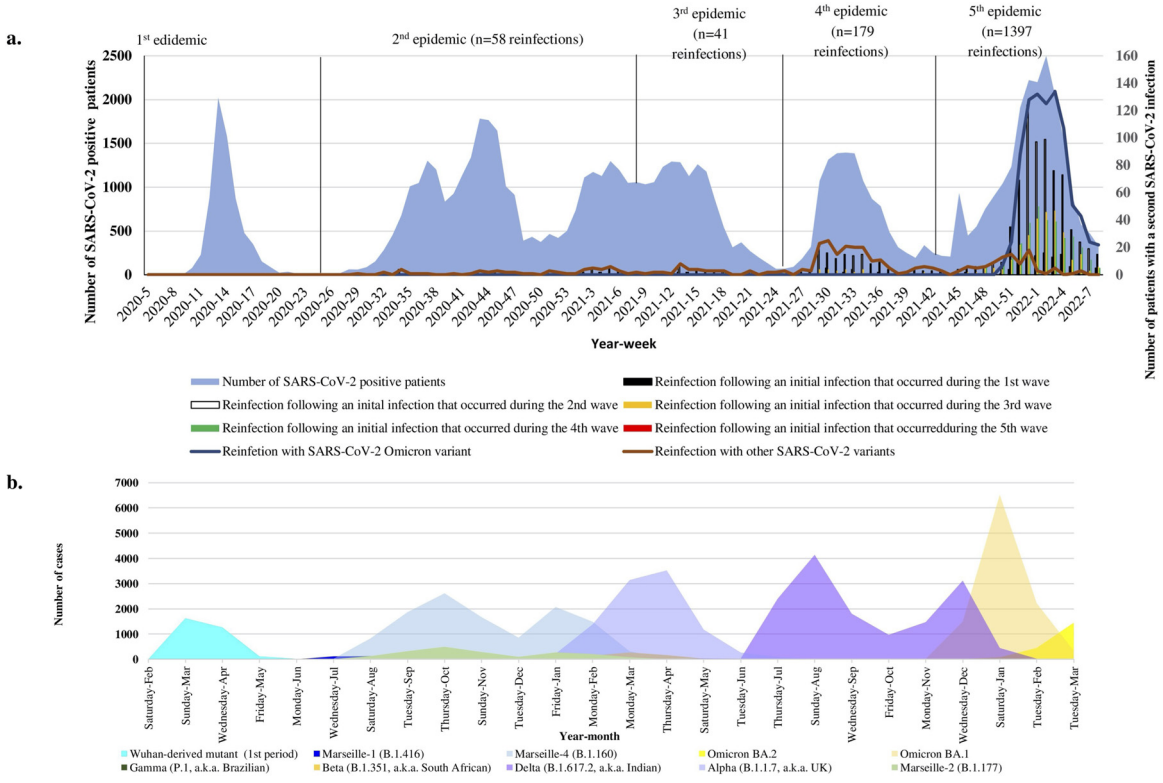

Fig. 1. Dynamics of SARS-CoV-2 infections (left axis) and reinfections (right axis) (a) and of major SARS-CoV-2 variants determined (b) in patients diagnosed at IHU Méditerranée Infection, 2020–2022.

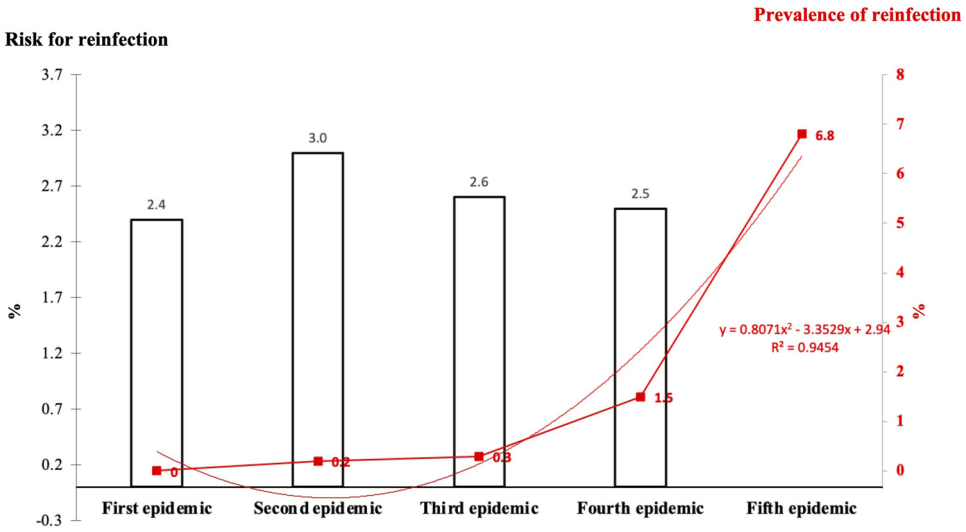

Fig. 2. Frequency of reinfection (proportion of infected patients during a given epidemic who had previous infection with SARS-CoV-2, red curve) and estimated risk for reinfection (proportion of patients first infected during a given epidemic who get reinfected at the time the study.

[7]. The first epidemic from February to early June 2020 was driven by three mutants derived from the Wuhan-Hu-1 isolate that were classified into Nextstrain clades (Pangolin lineages) 20A (B.1), 20B (B.1.1) and 20C (B.1). The second epidemic from mid-June 2020 to February 2021 was due to multiple variants among which the 20A (B.1.416, a.k.a. Marseille-1 [7] variant, the 20A.EU2 (B.1.160, a.k.a Marseille-4) variant, the majority one, and the 20E.EU1 (B.1.117, a.k.a. Marseille-2) variant. The third epidemic during March 2021-June 2021 was mainly due to the Alpha (20I, B.1.1.7) variant. The Delta (B.1.617.2) variant was the one mostly responsible for the fourth epidemic that lasted from July 2021 to early November 2021. Finally, the fifth epidemic, due to the Omicron (B.1.1.529) variant, started in November 2021 and is still going on as of March 6, 2022.

Reinfection cases were observed since the second epidemic among patients whose first infection occurred during one of the five epidemics (Fig. 1). The overall mean time span between first infection and reinfection was 334 ± 146 days and significantly increased overtime from one epidemic to another (Supplementary Figure). The first patient reinfected with the Omicron variant was detected mid-December. Then, this variant rapidly became predominant in reinfected patients until the study's endpoint, as of 6 March 2022, with 885 cases out of 1397 (Fig. 1). In earlier studies, we reported that the prevalence of reinfection among SARS-CoV-2 infections diagnosed in our institute was 0.2% (58/29,154 cases), 0.3% (41/12,283 cases) and 1.5% (110/7152 cases) during the second, third and fourth epidemic (until 24 August 2021), respectively [3 ,8]. In the present study, we confirm a 1.5% reinfection rate (179/12,135 cases) during the entire fourth epidemic (until November 2021), and observe a marked increase in the reinfection rate that reaches 6.8% (1397/20,542 cases) during the on-going fifth epidemic (Fig. 2). Among 13,060 cases of first infections with the Omicron variant, 10,590 were due to the Omicron BA.1 variant (81.1%) and 2470 (18.9%) to the BA.2 variant. Among 885 cases of reinfection with the Omicron variant 834 (94.2%) were due to the BA.1 variant and 51 (5.8%) to the BA.2 variant. There were no cases of first infection with the BA.1 variant that were reinfected with the BA.2 variant.

Fig. 2 represents the prevalence of reinfection and the estimated risk for reinfection in SARS-CoV-2-infected patients according to the period of first infection. Contrary to our previous assessment that this estimated risk decreased over time [3], we observed little variation, between 2.4 and 3.0%, in this updated study. This is likely because of cumulative numbers of reinfections overtime with occurrence of new cases of reinfection that were diagnosed after our previous assessment.

The increase in the proportion of reinfections with the Omicron variant is additional evidence that the genetic variability of SARS-CoV-2 has resulted in antigenic changes leading to reduced protection conferred by a previous infection. Our recent observations of a lower severity of infections with the Omicron variants as indicated by low rates of hospitalization, transfer to intensive care units, and death is good news in this context [9, 10]. The Omicron variant is likely distantly related to other SARS-CoV-2 variants which may account for a higher rate on reinfection and lower efficacy of vaccines. Indeed, despite the currently considerable proportions of people vaccinated and/or infected in France, present data and recently published data [5] suggest that resulting immunity could not prevent an endemicization of SARS-CoV-2.

Author contributions

Conceptualization: Didier Raoult and Philippe Gautret; Investigation: Philippe Colson, Linda Houhamdi, Didier Stoupan and Nhu Ngoc Nguyen; Formal analysis: Van Thuan Hoang and Nhu Ngoc Nguyen; Writing original draft: Philippe Gautret, Philippe Colson and Nhu Ngoc Nguyen; reviewing and editing: Philippe Gautret, Philippe Colson and Didier Raoult. All authors have contributed to this study and approved the final version of the manuscript and its revision.

Ethical approval

This retrospective study has been approved by the ethics committee of the University Hospital Institute Méditerranée Infection (No. 2022–016). Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019–73

Supplementary Material

Supplementary Figure. Mean time (days) between first infection and reinfection according to time when reinfection occured.

Declaration of interest

The authors have no conflicts of interest to declare relative to the present study. Didier Raoult was a consultant for the Hitachi High-Technologies Corporation, Tokyo, Japan from 2018 to 2020. He is a scientific board member of the Eurofins company and a founder of a microbial culture company (Culture Top). Funding sources had no role in the design and con-duct of the study, the collection, management, analysis, and interpretation of the data, and the preparation, review, or approval of the manuscript.

Supplementary materials

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Surface electrostatic shift on spike protein decreased antibody activities against SARS-CoV-2 Omicron variant

Dear editor,

In this Journal, Pascarella and colleagues recently described the value of electrostatic potentials of the spike receptor binding and N-terminal domains in addressing transmissibility and infectivity of SARS-CoV-2 variants of concern.[1] They interestingly found that the Omicron B.A1 variant has the highest net surface charge of SARS-CoV-2 receptor-binding domain (RBD), which may enhance affinity of Omicron RBD binding to the receptor angiotensin-converting enzyme 2 (ACE2).[1] However, whether the protein surface electrostatic shift affect antibody activities is still unknown.

A vaccine's efficacy or effectiveness against SARS-CoV-2 infection usually ranges from 19% to 99%.[2] However, some vaccines show notably lower efficacy, and the reason for this remains unknown. Marked reductions in neutralizing activity have been observed against Omicron relative to the ancestral pseudovirus in plasma from convalescent individuals and from individuals who had been vaccinated against SARS-CoV-2.[3-5] The SARS-CoV-2 Omicron variant encodes 37 amino acid substitutions in the spike protein, 15 of which are in RBD, thereby raising concerns about the effectiveness of available vaccines and antibody-based therapeutics.[3] In addition to these mutations, the other possible reasons for the considerable decline in the neutralizing activity against Omicron have yet to be documented.

In this study, we computed sequence-based antibody epitopes on spike proteins of SARS-CoV-2. Four epitopes with high surface accessible scores have been found and named #RBD, #CS (S1/S2 cleavage site), #S2–1 (S2 subunit-1) and #S2–2 (S2 subunit-2), respectively (**Fig. 1A** and **Supplementary Fig. 1**, and **Supplementary Table 1**). Then these epitopes were synthesized chemically. The ascites production of monoclonal antibodies against these epitopes was generated by inoculation of mice. However, unlike the other three epitopes, the epitope #CS failed to generate any ascites antibodies, which may be because the cleavage impairs its antigenicity.

Mouse monoclonal antibody against the epitope #RBD showed a relatively high endpoint (dilution) titer against the SARS-CoV-2 original strain (**Fig. 1B**). A human neutralizing monoclonal antibody, CV30,[6,7] also in complex with the RBD, showed a much higher endpoint titer to the original strain than mouse anti-#RBD antibody (**Fig. 1C**). This may be because CV30 binds more residues (32 residues) than the epitope #RBD (14 residues). However, endpoint titers of both antibodies against RBD were significantly lower when binding to the Omicron S protein, by a factor of 10 for CV30. This is consistent with a previous report stating that the neutralizing activity against Wuhan-Hu-1 and retained detectable neutralization against Omicron, with decreases about 21–

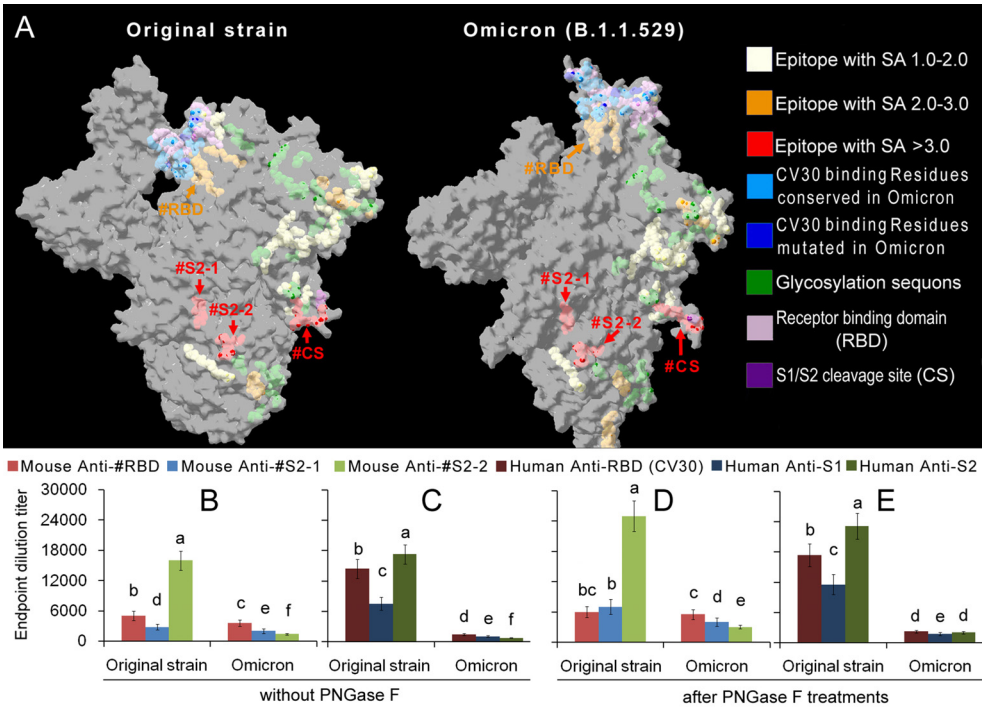

Fig. 1. Glycosylation affects antibody activities against SARS-CoV-2 original strain and Omicron variant. (A) Distribution of glycosylation sequons and antibody epitopes on SARS-CoV S. S1/S2 cleavage sites (CS) are marked with the dark purple color. The receptor-binding domain (RBD) is marked with the pale lavender color. Putative epitopes with different surface accessibilities (SA) are marked in yellow (SA 1.0–2.0), orange (SA 2.0–3.0), and red (SA > 3.0) respectively. CV30 binding residues conserved in all SARS-CoV-2 strains are marked with the sky-blue color. CV30 binding residues mutated in Omicron are marked with the dark blue color. Glycosylation sequons are marked with the green color. To present the sites more clearly, only one of the three monomers is labeled. (B) Endpoint (dilution) titers of mouse monoclonal antibodies against epitopes #RBD, #S2–1, and #S2–2 respectively. (C) Endpoint titers of human monoclonal antibodies against RBD, S1 subunit, and S2 subunit, respectively. (D) Endpoint titers of mouse anti-#RBD, anti-#S2–1, and anti-#S2–2 antibodies after PNGase F treatments. (E) Endpoint titers of human anti-RBD, anti-S1, and anti-S2 antibodies after PNGase F treatments. Bars represent standard deviations of three independent replicates. Values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

39-fold.[3] Five of 9 residues binding to CV30 at the C-terminal of RBD were found to be mutated in Omicron (**Supplementary Fig. 1**). This might be an important reason for its decreased binding activity.

The epitope #S2–1 with the highest SA score of 4.431 is located on the interface between subunits S1 and S2, which might be uncovered by transmembrane protease serine 2 (TMPRSS2) cleavage (**Supplementary Fig. 2**). However, neither TMPRSS2 nor its inhibitor Camostat[8] affected antibody activity (**Supplementary Fig. 2**).

Unexpectedly, mouse monoclonal antibody against the epitope #S2–2 showed the highest endpoint (dilution) titer to the original strain (3 times higher than the antibody against #RBD; **Fig. 1B**). Human monoclonal antibody against S2 subunit confirmed this finding (2 times higher than the antibody titer against S1 subunit; **Fig. 1C**). The unexpectedly high activity of non-neutralizing antibodies against S2 subunit were consistent with the fact that the vaccine efficacies against severe disease are usually higher than 90%, no matter how low the vaccine efficacies against SARS-CoV-2 infection are.[2] Nevertheless, activities of both anti-S2 antibodies declined 11–23-fold when binding with Omicron S protein (**Fig. 1BC**).

Possible reasons for the dramatically reduced antibody activity against the Omicron variant were further investigated. Previous studies have suggested that N-linked glycosylation on the S protein may compromise its antibody activities.[9,10] Most SARS-CoV-2 epitopes are shielded by glycans, and only areas of the protein surface at the apex of the S1 domain (RBD region) are not surrounded by glycosylation sequons (**Fig. 1A**). Consistent with the structure analysis, oligosaccharide-removing treatments involving PNGase F increased all antibody titers, especially for anti-S2 antibodies (**Fig. 1DE**). However, after PNGase F treatments, antibody titers against Omicron were still much lower than those against the original strain (**Fig. 1DE**).

Given that all the three epitopes #RBD, #S2–1, and #S2–2 are completely conserved in all SARS-CoV-2 strains (**Supplementary Fig. 1**), the large decline in the antibody activity against Omicron cannot be attributed to the mutations. We noticed that 11 residues had been substituted into the alkaline amino acid on Omicron S protein, which may change the electrostatic potential on the surface of the protein. We computed the electrostatic potential on the S protein. The surface of the Omicron S protein is uniformly positively charged. However, a large part of original SARS-CoV-2 S protein surface is electrically neutral or negatively charged, but its RBD is positively charged (**Fig. 2A**). For the original strain, epitopes #RBD and #S2–2 distribute on electrically neutral areas, which are positively charged in the Omicron strain. When the pH value of the binding system was adjusted to 5.0 (the positive charge could be neutralized), antibody titers against Omicron increased exponentially. This effect was doubled for anti-RBD antibodies, 7–11-fold increases for anti-S2 antibodies (**Fig. 2BC**). However, pH 9.0 decreased all antibody activity (**Fig. 2DE**).

The large decline in antibody activity against Omicron RBD (neutralization) may be attributed to both residue mutations and the shift in electrostatic potential. The large decline in antibody activity against the Omicron S2 subunit (non-neutralizing antibody activity) may be mainly attributable to a shift in electrostatic potential on the surface of the protein. Because the heterogeneity in antigen surface-charge distribution causes charge-related heterogeneity in monoclonal antibodies,[11] new vaccines against the full-

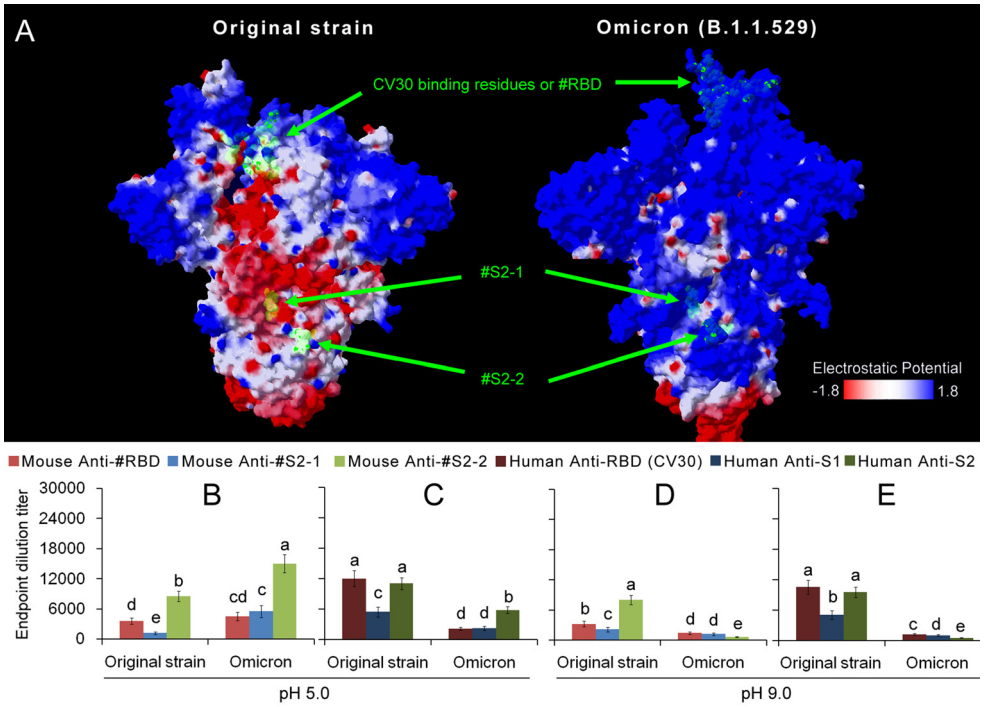

Fig. 2. Shift in electrostatic potential affect antibody activities against SARS-CoV-2 original strain and Omicron variant. (A) Electrostatic potential of SARS-CoV-2 S. The red-to-blue color on the molecular surface indicates the electrostatic potential (red: −1.8; blue: 1.8). Epitopes #RBD, #S2–1, and #S2–2 and CV30 binding residues are marked with the dark blue color. (B) Endpoint titers of mouse anti-#RBD, anti-#S2–1 and anti-#S2–2 antibodies at pH 5.0. (C) Endpoint titers of human anti-RBD, anti-S1, and anti-S2 antibodies at pH 5.0. (D) Endpoint titers of mouse anti-#RBD, anti-#S2–1, and anti-#S2–2 antibodies at pH 9.0. (E) Endpoint titers of human anti-RBD, anti-S1, and anti-S2 antibodies at pH 9.0. Bars represent standard deviations of three independent replicates. Values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

length Omicron S protein may be developed that have both negatively charged neutralizing antibodies and negatively charged non-neutralizing antibodies with high affinity to the Omicron variant.

Declaration of Competing Interest

The authors declare no competing interests.

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Supplementary materials

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