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COVID-19: From bedside to follow-up

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COVID-19: From bedside to follow-up

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Editorial: COVID-19: From bedside to follow-up

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COVID-19, SARS-CoV-2, bedside, follow-up, immunogenicity, post-acute COVID-19 syndrome, post-acute COVID-19 condition

Editorial on the Research Topic
COVID-19: From bedside to follow-up

Many Research Topics have been established regarding coronavirus disease 2019 (COVID-19) "*From Bench to Bedside*," i.e., the research process by which laboratory results generated at the bench are directly used at the bedside to treat the patients. In this Research Topic entitled "*COVID-19: Bedside to Follow-up*," we explore the effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on the clinical course from bedside to follow-up, as well as the effects of SARS-CoV-2 vaccination over time.

In comparison studies, perfect controls do not differ genetically from the cases, as seen with monozygotic twin pairs. In a case report, de Castro et al. present follow-up data from a monozygotic twin pair after severe COVID-19 before vaccination. These twin brothers were both admitted to the intensive care unit but one of them, who had a longer hospitalization, had persistent muscle weakness and fatigue during long-term follow-up. Consequently, this report highlights the role of non-genetic factors in the pathogenesis of severe COVID-19 and post-COVID-19 conditions. Data from large databases, before the introduction of COVID-19 vaccines, have shown that the most relevant non-genetic risk factor is age (1). The age-related risk of severe COVID-19 increases gradually and is more than doubled in the age interval 60–69 years compared with 50–59 years. Other relevant risk factors for disease progression among the unvaccinated include male sex, obesity (BMI $>$ 35), and co-morbidities, such as cancer, organ transplantation, and chronic kidney, heart, and lung disease. Today, in the Omicron era, advanced age ($\geq$65 years) is still one of the most relevant risk factors for developing severe COVID-19 and death, followed by vaccination status and immunocompromised conditions (2–4). Although the risk of severe COVID-19 or death for unvaccinated patients with Omicron is lower than with Delta variants, the risk is still relevant and similar to ancestral lineages (5).

For prediction models estimating the mortality risk in acute COVID-19 to be useful, they need to be based on easily accessible clinical parameters and routinely available laboratory tests. By using point-based scores of only four parameters, age, oxygen saturation, C-reactive protein, and creatinine levels, in a large cohort in Austria, Horvath et al. validated their mortality risk prediction tool (6), which can be assessed at (https://www.cbmed.at/covid-19-risk-calculator/). Robertson et al. present data from 19 moderate and 104 severe COVID-19 patients compared with 20 matched disease-free controls on another biomarker, the level of the circulating soluble form of angiotensin-converting enzyme 2 (sACE2) protein. The level of sACE2 decreased with disease severity in men but increased with disease severity in women, suggesting sex-specific differences in how the level of sACE2 correlates with

COVID-19 severity. A longitudinal study has shown that sACE2 levels are lower in children than in adults and increases only in males from the age of 12 years (7). Why opposite trends in sex-specific sACE2 levels are observed among severe COVID-19 patients needs to be further investigated.

In a substudy of the Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 vaccines (ENFORCE) cohort (8), Hvidt et al. present direct comparative analyses of four COVID-19 vaccines following primary and booster vaccination, focusing on the vaccine-induced humoral immune responses against diverse SARS-CoV-2 variants. The COVID-19 vaccine immunogenicity as measured by SARS-CoV-2 spike IgG levels and antibody neutralization titers reached similar levels among the four COVID-19 vaccines.

Early in the pandemic, there was insufficient knowledge about the risk of developing severe COVID-19 among immunocompromised patients. Today, we know that organ transplant recipients are poor vaccine responders and are one of the most important risk group for developing severe COVID-19, as presented in the umbrella review on kidney health by Yang et al. Interestingly, Benning et al., in a prospective observational cohort study, show that COVID-19 vaccine immunogenicity could be improved in kidney transplant recipients with a fourth vaccine dose after short-term withdrawal of the immunosuppressant mycophenolic acid. However, for safety reasons, short-term withdrawal of mycophenolic acid can only be considered in kidney transplant recipients without prior or current anti-HLA donor-specific antibodies.

To prevent severe COVID-19, Ouyang et al. theoretically reviewed the pros and cons of SARS-CoV-2 pre-exposure prophylaxis using antivirals, as well as other anti-SARS-CoV-2 agents, for high-risk groups, including healthcare workers, immunodeficient individuals, and poor vaccine responders. A personalized medicine approach consisting of risk stratification and decisions on early antiviral treatments based on measurements of an individual's vaccine response is an attractive option. However, this is often hindered by limitations in screening resources. An example on how this can be facilitated is shown in the study by Schmetzer et al., in which healthcare workers and patients with immune-mediated inflammatory diseases, who are likely to be poor vaccine responders, used self-collection of capillary blood and saliva to determine COVID-19 vaccine immunogenicity. Despite a limited study cohort size ($n = 60$), self-sampling was shown to be accurate and feasible. The study was conducted under controlled conditions but self-collection could potentially also be used "at home" to increase flexibility. Follow-up studies from larger cohorts are needed to conclude the effectiveness of self-collection to determine COVID-19 vaccine immunogenicity in clinical practice.

Health outcomes after 15 months of follow-up are described by Sun et al. in a cohort of 534 COVID-19 patients hospitalized in Wuhan during the first wave. The most prevalent self-reported symptoms were sleep disorders (19%) and fatigue (17%). Of note, there are generally good correlations between self-reported symptoms and validated health scores (9). In multivariate logistic regression analyses, the risk of sleep disorders was significantly associated with females compared with males, and glucocorticoid use during hospitalization was significantly associated with an increased risk of fatigue. Five percent of the COVID-19 patients suffered from post-traumatic stress disorder (PTSD), which was significantly associated with persistent symptoms during follow-up compared with no persistent symptoms in a multivariate logistic regression analysis. No significant associations were observed between COVID-19 severity and sleep disorders, fatigue, or PTSD. Of note, even subtle cognitive symptoms have previously been shown to worsen when returning to normal life following viral infection (10). Reports on the Omicron variant from East Asia are sparse, but Shen et al. present clinical characteristics and 1-month recovery of subjective hyposmia in a cohort of 349 hospitalized patients infected with Omicron. Among these non-severe COVID-19 patients, the prevalence of Omicron-related hyposmia was 6%. The patients with hyposmia had more clinical symptoms than patients without hyposmia, which might have contributed to longer hospitalization.

We look forward to the many reports on data from bedside to long-term follow-up after COVID-19, as well as the effects of SARS-CoV-2 booster vaccination over time in the coming years. Lastly, we would like to thank all the authors and reviewers for their contribution.

Author contributions

JDG drafted the manuscript. All authors critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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15-Month Health Outcomes and the Related Risk Factors of Hospitalized COVID-19 Patients From Onset: A Cohort Study

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Objective: The long-term impact of COVID-19 on patient health has been a recent focus. This study aims to determine the persistent symptoms and psychological conditions of patients hospitalized with COVID-19 15 months after onset, that patients first developed symptoms. The potential risk factors were also explored.

Methods: A cohort of COVID-19 patients discharged from February 20, 2020 to March 31, 2020 was recruited. Follow-ups were conducted using validated questionnaires and psychological screening scales at 15 months after onset to evaluate the patients' health status. The risk factors for long-term health impacts and their associations with disease severity was analyzed.

Findings: 534 COVID-19 patients were enrolled. The median age of the patients was 62.0 years old (IQR 52.0–70.0) and 295 were female (55.2%). The median time from onset to follow-up was 460.0 (451.0–467.0) days. Sleep disturbance (18.5%, 99/534) and fatigue (17.2%, 92/534) were the most common persistent symptoms. 6.4% (34/534) of the patients had depression, 9.2% (49/534) were anxious, 13.0% (70/534) had insomnia and 4.7% (25/534) suffered from post-traumatic stress disorder (PTSD). Multivariate adjusted logistic regression analysis showed that glucocorticoid use during hospitalization (OR 3.58, 95% CI 1.12–11.44) was significantly associated with an increased risk of fatigue. The OR values for anxiety and sleep disorders were 2.36 (95% CI 1.07–5.20) and 2.16 (95% CI 1.13–4.14) in females to males. The OR value of PTSD was 25.6 (95% CI 3.3–198.4) in patients with persistent symptoms to those without persistent symptoms. No significant associations were observed between fatigue syndrome or adverse mental outcomes and disease severity.

Conclusions: 15-month follow-up in this study demonstrated the need of extended rehabilitation intervention for complete recovery in COVID-19 patients.

Keywords: long-term health consequence, COVID-19, persistent symptom, mental health, PTSD

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen responsible for the coronavirus disease 2019 (COVID-19) pandemic, which has resulted in global healthcare crises and strained health resources (1). Globally, as of 12 March 2022, there have been 452,201,564 confirmed cases of COVID-19, including 6,029,852 deaths, reported to WHO (2).

COVID-19-related symptoms have been intensively studied in different systems since the pandemic outbreak. Most COVID-19 patients suffer from respiratory symptoms (such as fever, cough, and dyspnea) and are subjected to multiple organ injuries caused by SARS-CoV-2 infection together with the drugs used in the treatment of this disease (3, 4). Currently, researchers are aware of the persistent symptoms of COVID-19 after recovery, which are defined as "post-COVID condition," "long COVID" or "post-COVID syndrome," indicating a long-term course of various physical and neuropsychiatric symptoms lasting more than 12 weeks without other explanation (5, 6).

Long COVID is a rapidly evolving medical problem that requires action now. Several recent studies have reported specific persistent symptoms in discharged patients, such as fatigue and dyspnea (6). The severity of this disease in acute phase is likely to be related to the long-term adverse outcome of the disease, and gender may be an important risk factor affecting the adverse psychological outcome (7). However, to date, most studies have only examined adverse health effects up to 6 months after Covid-19 diagnosis, and little is known about the long-term mental health effects. It is still unclear how long COVID lasts, what the risk factors for long COVID are, and the relationship between long COVID and disease severity during the acute phase. Therefore, there is an urgent need to clearly define the long-term impact of COVID-19 on health in recovered patients and its potential risk factors.

Recently, we conducted a research to describe the detailed symptomatic features of COVID-19 at the onset and rehabilitation stages (8). The data showed that COVID-19 patients presented atypical but diverse symptoms. The most common remaining symptoms at the 3-month recovery stage were cough and fatigue. The proportion and severity of dyspnea as a remaining symptom after discharge in severe patients were higher than those in non-severe patients.

In this study, we aimed to explore the clinical characteristics of long COVID and especially to discuss the remaining long-term mental and psychological problems and their related risk factors. This study provide an important and critical update to our previously published data on the symptomatic characteristics and prognosis of COVID-19 (8).

METHODS

Study Design and Participants

All the patients enrolled in this study were from the same cohort in our other recently published study (8). The subjects included in our cohort were diagnosed with COVID-19 by reverse transcription-polymerase chain reaction (RT–PCR) and were discharged from the Optical Valley Branch of Hubei Maternal and Child Hospital, a designated hospital for COVID-19 patients in Wuhan, from February 20 to March 31, 2020.

The following patients were excluded: (1) patients who died after discharge; (2) patients who were difficult to follow up due to mental illness, dementia, or underlying diseases; (3) patients who refused to cooperate; (4) patients who could not be contacted; and (5) patients who lived in nursing homes or welfare homes. All the study participants provided informed consent. The Research Ethics Committee of Shanghai Changzheng Hospital approved this study (2020SL007).

A total of 1,524 patients with COVID-19 discharged from the Guanggu District of Hubei Maternal and Child Healthcare Hospital between February 20 and March 31, 2020 were screened. As shown in **Figure 1**, 990 patients were excluded, of which 454 refused to cooperate, 366 could not be contacted, 78 had dementia or psychotic disease who could not complete the interview, 77 lived in nursing or welfare home, and 15 died. Lastly, 534 patients were enrolled in this study, including 114 severe cases and 420 non-severe cases.

Procedures

The collected data of acute phase were extracted from electronic medical records of patients with COVID-19 hospitalized in Optical Valley Branch of Maternal and Child Hospital of Hubei Province, including demographic information and clinical characteristics, which were described in our previous study (8). We confirmed the data for demographic and self-reported comorbidity with participants at the 15-month follow-up visit.

All participants were interviewed by a group of certified doctors by telephone and were asked to complete a series of questionnaires, including a self-reported symptom questionnaire (shown in **Appendix**), the modified British Medical Research Council (mMRC) dyspnoea scale, psychological status questionnaire and Ischemic Stroke and Cardiovascular Events Registry. In the self-reported symptom questionnaire, participants were asked to report new and persistent symptoms or any more severe symptoms than before the onset of COVID-19(9). The mMRC dyspnea scale is a five-level scoring scale used to describe the degree of dyspnea caused by physical activity. A higher mMRC dyspnea scale score indicates more severe dyspnea (10).

Psychological conditions were measured using various scales: the GAD-7 anxiety scale (0–4 points for no anxiety disorder, 5–9 points for mild anxiety, 10–14 points for moderate anxiety, and 15–21 points for severe anxiety) (11), the PHQ-9 depression scale (0–4 points for no depression, 5–9 points for mild depression, 10–14 points for moderate depression, 15–19 points for moderate-severe depression, and 20–27 points for severe depression) (12) and the ASI scale for insomnia (0–7 points indicate no insomnia, 8–14 points indicate mild insomnia, 15–21 points indicate moderate insomnia and 22–28 points indicate severe insomnia) (13). We used the PC-PTSD (primary care PTSD screen) to identify PTSD symptoms, which was developed to quickly detect PTSD based on DSM-IV PTSD diagnostic criteria (14). The PC-PTSD included four items, and each item was designed to report whether the following symptoms were present or not, including reexperiencing, avoidance,

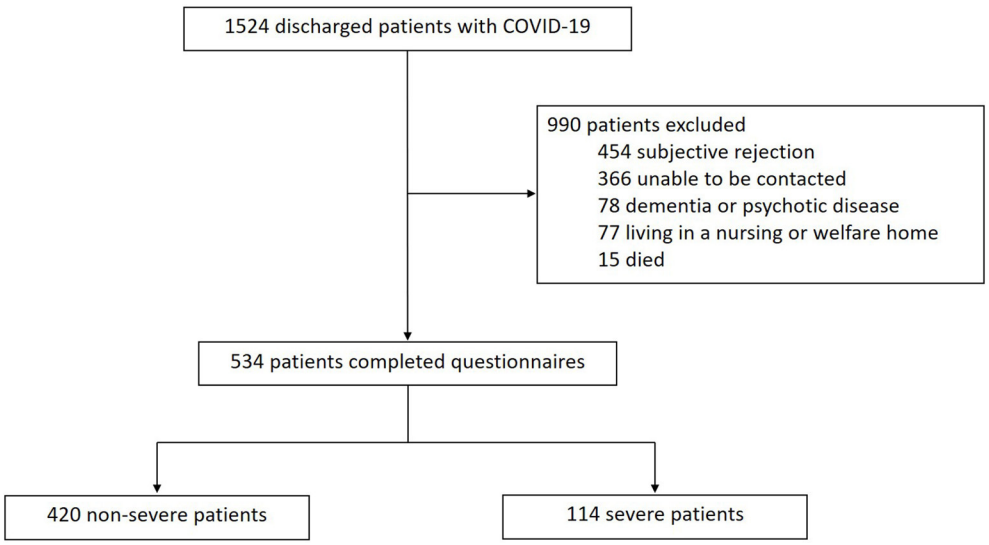

FIGURE 1 | Flow chart of hospital patients with COVID-19.

hyperarousal and numbing. Answering "yes" was scored as 1, answering "no" as 0, and the scores of four items were added to get a total score. Generally, a total score of 3 or above is considered a positive result, indicating a clinically significant PTSD.

The EuroQol five-dimension five-level (EQ-5D-5L) questionnaire was used to assess patient quality of life by evaluating the following five factors: mobility, self-care, daily activities, pain or discomfort, and anxiety or depression (15). The classification of each element is divided into five levels, ranging from no problem to extreme problems. The post-COVID-19 functional status (PCFS) scale is recommended for use during the current COVID-19 pandemic (9). It is proposed that it could be used to display the direct retrieval and functional sequelae of COVID-19.

The follow-up was conducted from April 30 to May 9, 2021. A group of certified medical staff completed the follow-ups through telephone interviews. REDCap electronic data collection tools were used to manage the data, which helped to minimize missing inputs and allow for real-time data verification and quality control.

Definition

Onset was defined as the date on which patients with confirmed COVID-19 first developed symptoms, excluding those with asymptomatic infection.

Severe cases are defined in accordance with the seventh edition of Chinese COVID-19 diagnosis and treatment guideline (16), which means that adults meet any of the following: ① Shortness of breath, RR > 30 times/min; ② In resting state, oxygen saturation when inhaling air degree of ≤93%; ③ arterial partial pressure of oxygen (PaO2)/inhaled oxygen concentration (FiO2) ≤300 mmHg; ④ progressive worsening of clinical symptoms, and lung imaging showed that the lesions progressed significantly within 24–48 h > 50%.

The new-onset diabetes mellitus in our study was based on glycated hemoglobin A1C (HbA1C) with a threshold of ≥6.5% or fasting plasma glucose of above 7.0 mmol/L. Deep venous thrombosis (DVT) was defined as forming a blood clot within a deep vein. The diagnosis of DVT of the lower limbs in our study was performed by duplex ultrasound imaging. Autoimmune thyroid disease (AITD) was defined as having thyroid antibodies that can be detected in the blood, including thyroglobulin antibodies, thyroid microsomal antibodies, and TSH receptor antibodies.

Patient Outcomes

Primary outcomes included persistent symptoms (fatigue, sleep disturbance, cough, dyspnea, loss of taste, loss of smell, loss of appetite, hair loss, or photophobia) and psychological consequences (anxiety, depression, insomnia and PTSD as assessed by a series of standard scales) at the 15-month follow-up.

Secondary outcomes included health-related quality of life (pain or discomfort, anxiety or depression, mobility, personal nursing, and daily activities), PCFS scales, and all-cause death and extrapulmonary organ function (including major adverse cardiovascular events, deep vein thrombosis of the lower extremities, new-onset autoimmune thyroid disease, new-onset diabetes, and newly diagnosed cancer) at the 15-month follow-up.

Statistical Analysis

Continuous variables are expressed as the median, and categorical variables are expressed as a percentage of the sum of absolute values. The participants were divided into two groups according to their symptom severity during hospitalization: severe and non-severe. We compared the

demographic characteristics and long-term health outcomes of the two groups of participants. We also compared the long-term health outcomes of males and females. To compare the symptoms, physical activity, and health-related quality of life between men and women, we used the Mann–Whitney *U* test, χ^2 test, or Fisher's exact test where appropriate.

The multivariate-adjusted logistic regression model was used to estimate the odds ratio (OR) and 95% confidence interval between disease severity and subtype outcome. For the relationship between disease severity and continuous outcome, a multivariate-adjusted linear regression model was used to estimate β estimates and 95% Cis. Confounding factors include age, sex, smoking (never smoker, current smoker, and former smoker), comorbidities (hypertension, diabetes, cardiovascular disease, cerebrovascular disease, malignant tumor, chronic obstructive pulmonary disease, and chronic kidney disease), corticosteroids, antiviral drugs (arbidol, chloroquine phosphate, and hydroxychloroquine), convalescent plasma therapy, and intravenous immunoglobulin.

Multivariate adjusted logistic regression analysis was used to explore the risk factors related to PTSD, ASI-sleep disorders, GAD-anxiety, and fatigue syndrome. Adverse mental outcomes occurred in ∼20% of enrolled subjects, we followed accepted statistical practice and considered 10 variables in our multiple regression model. Variables associated with outcome measures (age, sex, comorbidities, severity of illness, corticosteroids, special oxygen therapy, length of hospital stay, symptoms remaining after discharge, and COVID-19 recovery status scale) were all included in the model. For the association of comorbidity with outcome, the above-mentioned variables except for disease severity were all included.

All the tests were two-sided, and a *P*-value of <0.05 was considered statistically significant. We included all the follow-up participants in the final analysis without entering any missing data. All statistical analyses were performed using SAS version 9.4.

RESULTS

Baseline Characteristics of the Study Population

The demographic and clinical characteristics of the participants are shown in **Table 1**. The median age of the enrolled patients was 62.0 (52.0–70.0) years old, with 239 males (44.8%) and 295 females (55.2%). The most common comorbidities were hypertension (198 patients, 37.08%), followed by diabetes (85 patients, 15.92%) and atherosclerotic cardio-cerebrovascular disease (ASCVD) (71 patients, 13.30%). A total of 403 (75.47%) of 534 participants required supplemental oxygen therapy during hospitalization, 15 (2.81%) required high-flow nasal oxygen inhalation (HFNC), non-invasive mechanical ventilation (non-IMV), or both, and 3 (0.56%) required extracorporeal membrane oxygenation (ECMO), IMV, or both. The median duration of hospitalization was 29.0 (17.0–40.0) days. The median time from symptom onset to follow-up was 460.0 (451.0–467.0) days, and the median time from discharge to follow-up was 414.0 (408.0–420.0) days (**Table 1**).

Persistent Symptoms and Psychological Consequences at the 15-Month Follow-Up

There were still many patients who had persistent symptoms. As shown in **Table 2** and **Supplementary Table S2**, 44.57% of participants (238 of 534 patients) reported at least one symptom at follow-up, and a higher percentage was observed in women. The most common self-reported symptoms at 15 months after SARS-CoV-2 infection were sleep difficulties (99/534, 18.54%, **Table 2**) and fatigue (92/534, 17.23%), followed by memory loss (86/534, 16.10%). In addition, at 15 months after SARS-CoV-2 infection, 11.42% (61/534, **Table 2**) of patients still reported chest tightness, 9.93% (53/534) reported cough, and 8.05% (43/534) reported hair loss. A total of 5.43% (29/534) of patients reported dyspnea, 3.18% (17/534) reported smell disorder, 2.81% (15/534) reported taste disorder, and 2.62% (14/534) reported photophobia.

The long-term impact of COVID-19 on the psychological consequences of patients after discharge from the hospital should not be ignored. As measured by the PHQ-9 and GAD-7 scales, 6.4% (34/534, **Table 2**) of patients had varying degrees of depression, and 9.2% (49/534) had different degrees of anxiety. According to the ASI questionnaire test, 13.0% (70/534) had various degrees of insomnia. The results from the PTSD screening scale showed that 4.7% (25/534) of patients had PTSD at 15 months after acute infection. The incidence rates of these adverse psychological conditions were higher in women than in men (see **Supplementary Tables S2, S3**, $P < 0.05$).

Health-Related Quality of Life, PCFS Scales and All-Cause Death and Extrapulmonary Organ Function at the 15-Month Follow-Up

The results from the EQ-5D-5 L questionnaire showed that 19.10% (102/534) of the patients had trouble with mobility, 13.11% (70/534) had personal care problems, 15.92% (85/534) reported difficulties with performing their usual activities, 19.10% (102/534) had pain or discomfort and 20.79% (111/534) had anxiety or depression. The severe COVID-19 patients reported more problems in each sub-item of the EQ-5D-5L questionnaire and had worse quality of life than non-severe patients (all $P < 0.05$, **Table 3**).

The PCSF rating results showed that 65.17% (348/534) of patients recovered well in functional status, reaching the F0 grade. That means 65.17% of the patients were able to recover to their pre-sick condition, and their life and work were not affected by COVID-19. There was no significant difference in the proportion of F0 grade individuals between severe patients and non-severe patients ($P > 0.05$, **Table 3**).

Notably, 15 patients died after discharge. The primary reason was the deterioration of lung, heart, and kidney conditions. The detailed characteristics are shown in **Supplementary Table S1**. In addition, seven patients reported non-fatal myocardial infarctions or ischemic strokes after discharge. Five patients were readmitted for hospitalization again due to heart failure. Eleven patients underwent arterial revascularization or stent implantation. Nine patients suffered

TABLE 1 | Characteristics of 534 enrolled patients with COVID-19.

	Total (n = 534)	Non-severe (n = 420)	Severe (n = 114)	P-value
Age, years				
Median (IQR)	62.0 (52.0, 70.0)	60.0 (50.0, 68.0)	70.0 (61.0, 78.0)	<0.0001
Distribution-n (%)				<0.0001
14–49	106 (19.85)	99 (23.57)	7 (6.14)	
50–64	205 (38.39)	177 (42.14)	28 (24.56)	
>65	223 (41.76)	144 (34.29)	79 (69.30)	
Sex				<0.0001
Male	239 (44.76)	169 (40.24)	70 (61.40)	
Female	295 (55.24)	251 (59.76)	44 (38.60)	
Cigarette smoking				0.0463
Never-smoker	293 (83.24)	236 (84.29)	57 (79.17)	
Current smoker	23 (6.53)	22 (7.86)	1 (1.39)	
Former smoker	36 (10.23)	22 (7.86)	14 (19.44)	
Comorbidities				
Hypertension	198 (37.08)	145 (34.52)	53 (46.49)	0.0190
Diabetes	85 (15.92)	61 (14.52)	24 (21.05)	0.0911
ASCVD	71 (13.30)	38 (9.05)	33 (28.95)	<0.0001
Asthma	2 (0.37)	2 (0.48)	0 (0.00)	1.0000
COPD	22 (4.12)	10 (2.38)	12 (10.53)	0.0003
Chronic kidney disease	7 (1.31)	2 (0.48)	5 (4.39)	0.0053
Chronic liver disease	24 (4.49)	19 (4.52)	5 (4.39)	0.9498
Cancer	23 (4.31)	16 (3.81)	7 (6.14)	0.4082
Highest seven-category scale during hospital stay				
3: not requiring supplemental oxygen	113 (21.16)	108 (25.71)	5 (4.39)	<0.0001
4: requiring supplemental oxygen	403 (75.47)	312 (74.29)	91 (79.82)	0.2229
5: requiring HFNC or non-IMV, or both	15 (2.81)	0 (0.00)	15 (13.16)	<0.0001
6: requiring ECMO or IMV, or both	3 (0.56)	0 (0.00)	3 (2.63)	0.0095
Admission into ICU	3		3	
Length of ICU hospitalization	32.0 (20.0–42.0)		32.0 (20.0–42.0)	
Treatment received during hospital stay				
Antivirals	499 (93.45)	394 (93.81)	105 (92.11)	0.5144
Antibiotics	231 (43.26)	160 (38.10)	71 (62.28)	<0.0001
Corticosteroids	35 (6.55)	16 (3.81)	19 (16.67)	<0.0001
Tocilizumab	11 (2.06)	0 (0.00)	11 (9.65)	<0.0001
Convalescent plasma therapy	20 (3.75)	11 (2.62)	9 (7.89)	0.0186
Intravenous immunoglobulin	18 (3.37)	9 (2.14)	9 (7.89)	0.0064
CRRT	1 (0.22)	0 (0.00)	1 (1.09)	0.1991
Length of hospital stay, days	29.0 (17.0, 40.0)	28.0 (17.0, 40.0)	30.50 (20.0, 42.0)	0.0354
Time from symptom onset to admission, days	12.0 (4.0, 26.0)	13.0 (4.0, 27.0)	8.0 (2.0, 22.0)	0.0033
Time form discharge to follow-up, days	414.0 (408.0, 420.0)	415.0 (409.0, 420.0)	411.0 (404.0, 419.0)	0.0016
Time form symptom onset to follow-up, days	460.0 (451.0, 467.0)	461.0 (451.0, 468.0)	456.0 (444.0, 467.0)	0.0123

Data are n (%) or median (IQR). IQR, interquartile range; ASCVD, atherosclerotic cardio-cerebrovascular disease; COPD, chronic obstructive pulmonary disease; HFNC, high-flow nasal cannula for oxygen therapy; ECMO, extracorporeal membrane oxygenation; IMV, invasive mechanical ventilation; CRRT, continuous renal replacement therapy; COVID-19, corona virus disease 2019.

from acute pulmonary embolism due to deep lower limb venous thrombosis. One patient underwent dialysis treatment due to worsening renal failure. Nineteen patients were diagnosed with new-onset diabetes, ten reported new-onset autoimmune thyroid disease, and four were newly diagnosed with malignant tumors.

Risk Factors for Long-Term Health Impacts and Their Association With Disease Severity

After adjusting for confounding factors such as age, sex, smoking, comorbidities, length of stay, oxygen therapy, and medication, the risk of chest tightness, chest pain, and photophobia in severe

TABLE 2 | Persistent symptoms and psychological consequences at 15-month follow-up.

	Total ($n = 534$)	Non-severe ($n = 420$)	Severe ($n = 114$)	*P*	OR or β (95%CI)*	*P* for regression
Self-report symptoms—*n* (%)						
Any	238 (44.57)	175 (41.67)	63 (55.26)	0.0096	1.46 (0.92, 2.34)	0.1097
Sleep disorder	99 (18.54)	80 (19.05)	19 (16.67)	0.5618	0.81 (0.44, 1.48)	0.4902
Fatigue	92 (17.23)	68 (16.19)	24 (21.05)	0.2228	1.25 (0.69, 2.29)	0.4601
Memory loss	86 (16.10)	61 (14.52)	25 (21.93)	0.0564	1.34 (0.74, 2.43)	0.3399
Arthralgia	66 (12.36)	50 (11.90)	16 (14.04)	0.5399	1.30 (0.67, 2.49)	0.4354
Chest tightness	61 (11.42)	37 (8.81)	24 (21.05)	0.0003	2.55 (1.34, 4.87)	0.0046
Dizziness	55 (10.30)	37 (8.81)	18 (15.79)	0.0297	1.73 (0.87, 3.42)	0.1177
Cough	53 (9.93)	40 (9.52)	13 (11.40)	0.5517	0.93 (0.45, 1.95)	0.8574
Sore throat	52 (9.74)	35 (8.33)	17 (14.91)	0.0356	1.46 (0.71, 3.02)	0.3029
Headache	47 (8.80)	35 (8.33)	12 (10.53)	0.4636	1.37 (0.64, 2.93)	0.4251
Hair loss	43 (8.05)	34 (8.10)	9 (7.89)	0.9444	1.40 (0.59, 3.34)	0.4440
Myalgia	41 (7.68)	26 (6.19)	15 (13.16)	0.0132	2.14 (1.00, 4.60)	0.0506
Palpitation	37 (6.93)	24 (5.71)	13 (11.40)	0.0339	1.99 (0.88, 4.50)	0.0974
Chest pain	36 (6.74)	23 (5.48)	13 (11.40)	0.0252	2.63 (1.18, 5.86)	0.0180
Anorexia	31 (5.81)	20 (4.76)	11 (9.65)	0.0478	1.89 (0.79, 4.48)	0.1503
Dyspnea	29 (5.43)	18 (4.29)	11 (9.65)	0.0250	2.21 (0.92, 5.31)	0.0777
Diarrhea	25 (4.68)	23 (5.48)	2 (1.75)	0.0953	0.35 (0.08, 1.57)	0.1682
Rash	22 (4.12)	15 (3.57)	7 (6.14)	0.3379	1.87 (0.66, 5.29)	0.2410
Smell disorder	17 (3.18)	13 (3.10)	4 (3.51)	1.0000	1.54 (0.45, 5.31)	0.4923
Taste disorder	15 (2.81)	12 (2.86)	3 (2.63)	1.0000	0.72 (0.17, 2.97)	0.6480
photophobia	14 (2.62)	6 (1.43)	8 (7.02)	0.0029	6.93 (2.08, 23.11)	0.0016
Nausea or vomiting	10 (1.87)	7 (1.67)	3 (2.63)	0.7760	1.43 (0.31, 6.57)	0.6439
Intermittent fever	3 (0.56)	3 (0.71)	0 (0.00)	1.0000	0.00 (0.00, 454E94)	0.9361
mMRC				0.0124	0.45 (0.19, 1.07)	0.0702
mMRC4	3 (0.56)	0 (0.00)	3 (2.63)			
mMRC3	4 (0.75)	3 (0.71)	1 (0.88)			
mMRC2	6 (1.12)	4 (0.95)	2 (1.75)			
mMRC1	16 (3.00)	11 (2.62)	5 (4.39)			
mMRC0	505 (94.57)	402 (95.71)	103 (90.35)			
PHQ-9 scale of depression				0.0030	0.57 (0.30, 1.06)	0.0768
No depression	470 (88.01)	375 (89.29)	95 (83.33)			
Mild depression	37 (6.93)	30 (7.14)	7 (6.14)			
Moderate depression	12 (2.25)	9 (2.14)	3 (2.63)			
Severe depression	15 (2.81)	6 (1.43)	9 (7.89)			
GAD-7 scale of anxiety				0.0944	0.65 (0.31, 1.34)	0.2397
No anxiety	485 (90.82)	386 (91.90)	99 (86.84)			
Mild anxiety	36 (6.74)	27 (6.43)	9 (7.89)			
Moderate anxiety	6 (1.12)	4 (0.95)	2 (1.75)			
Severe anxiety	7 (1.31)	3 (0.71)	4 (3.51)			
ASI scale of insomnia				0.2412	1.25 (0.62, 2.53)	0.5321
No insomnia	464 (86.89)	364 (86.67)	100 (87.72)			
Mild insomnia	52 (9.74)	43 (10.24)	9 (7.89)			
Moderate insomnia	15 (2.81)	12 (2.86)	3 (2.63)			
Severe insomnia	3 (0.56)	1 (0.24)	2 (1.75)			
PTSD screen				0.0671	2.00 (0.77, 5.17)	0.1546
Negative	509 (95.32)	404 (96.19)	105 (92.11)			
Positive	25 (4.68)	16 (3.81)	9 (7.89)			

Data are n (%) or median (IQR).

**OR or β (95%CI) obtained by logistic regression, rank logistic regression and linear regression, adjusted for age, comorbidities, length of hospital stay, corticosteroid, 5: admitted to hospital, requiring HFNC or non-IMV or both, 6: admitted to hospital, requiring ECMO or IMV or both.*

mMRC, modified British medical research council; PHQ-9, patient health questionnaire version 9; GAD-7, generalized anxiety disorder version 7; ASI, Arabic scale of insomnia; PTSD, post-traumatic stress disorder.

TABLE 3 | Health-related quality of life, PCFS scales and extrapulmonary organ function at 15-month follow-up.

	Total ($n = 534$)	Non-severe ($n = 420$)	Severe ($n = 114$)	*p*	OR or β (95%CI)*	*P* for regression
Events in one-year after discharge—*n* (%)						
Non-fatal myocardial infarction or non-fatal stroke	7 (1.31)	4 (0.95)	3 (2.63)	0.3505	2.09 (0.39, 11.09)	0.3887
Heart failure hospitalization	5 (0.94)	1 (0.24)	4 (3.51)	0.0076	7.52 (0.66, 85.11)	0.1031
Arterial revascularization therapy	11 (2.06)	8 (1.90)	3 (2.63)	0.9102	0.43 (0.05, 3.74)	0.4449
New-onset venous thrombotic disease	9 (1.69)	7 (1.67)	2 (1.75)	1.0000	0.59 (0.10, 3.51)	0.5613
Exacerbation of renal disease requires dialysis or kidney transplantation	1 (0.19)	1 (0.24)	0 (0.00)	1.0000	0.00 (0.00, 161E90)	0.9413
New-onset diabetes	19 (3.56)	12 (2.86)	7 (6.14)	0.1635	2.12 (0.76, 5.91)	0.1491
New-onset AITD	10 (1.87)	9 (2.14)	1 (0.88)	0.6209	0.53 (0.06, 4.54)	0.5617
New-onset neuropsychiatric disease	3 (0.56)	1 (0.24)	2 (1.75)	0.1167	4.12 (0.29, 58.35)	0.2951
New-onset cancer	4 (0.75)	3 (0.71)	1 (0.88)	1.0000	0.00 (0.00, 6E166)	0.9524
EQ-5D-5L questionnaire						
Mobility: problems with walking	102 (19.10)	56 (13.33)	46 (40.35)	<0.0001	2.63 (1.52, 4.57)	0.0006
Personal care: problems with washing or dishing	70 (13.11)	34 (8.10)	36 (31.58)	<0.0001	2.88 (1.55, 5.36)	0.0008
Usual activity: problems with usual activity	85 (15.92)	47 (11.19)	38 (33.33)	<0.0001	2.29 (1.28, 4.09)	0.0052
Pain or discomfort	102 (19.10)	56 (13.33)	46 (40.35)	<0.0001	3.85 (2.25, 6.57)	<0.0001
Anxiety or depression	111 (20.79)	69 (16.43)	42 (36.84)	<0.0001	2.38 (1.41, 4.04)	0.0013
Quality of life	85.50 (80.00, 90.00)	89.00 (80.00, 90.50)	80.00 (70.00, 90.00)	0.0023	−4.99 (−9.02, −0.96)	0.0154
PCFS scale				0.0043	0.61 (0.39, 0.97)	0.0348
F0	348 (65.17)	291 (69.29)	57 (50.00)			
F1	13 (2.43)	10 (2.38)	3 (2.63)			
F2	13 (2.43)	9 (2.14)	4 (3.51)			
F3	95 (17.79)	66 (15.71)	29 (25.44)			
F4	65 (12.17)	44 (10.48)	21 (18.42)			

Data are n (%).

**OR or β (95%CI) obtained by logistic regression, rank logistic regression and linear regression, adjusted for age, comorbidities, length of hospital stay, corticosteroid, 5: admitted to hospital, requiring HFNC or non-IMV or both, 6: admitted to hospital, requiring ECMO or IMV or both.*

AITD, autoimmune thyroid disease; EQ-5D-5L, EuroQol 5-Dimension Questionnaire 5-level version; PCSF, post-COVID-19 functional status.

patients was still significantly higher than that of non-severe patients, with OR values of 2.55 (95% CI 1.34–4.87, **Table 2**), 2.63 (1.18–5.86) and 6.93 (2.08–23.11), respectively. However, the risk of fatigue and sleep disturbance in severe patients was not significant, and the OR values were 1.25 (95% CI 0.69–2.29, **Table 2**) and 0.81 (0.44–1.48), respectively. There was no significant difference in the proportion of cough, dyspnea, hair loss, smell disorder, or taste disorder between severe and non-severe patients ($P > 0.05$, **Table 2**).

Multivariate adjusted logistic regression analysis showed that glucocorticoid treatment during hospitalization (OR 3.58, 95%CI 1.12–11.44, $P = 0.0312$, **Figure 2**) was significantly associated with an increased risk of fatigue and GAD-7 anxiety score (OR 3.48, 95%CI 1.09–11.17, $P = 0.0358$, **Figure 2**). No significant associations were observed between fatigue syndromes and age, gender or disease severity.

Multivariate adjusted logistic regression analysis showed that gender and the presence of self-reported symptoms were significantly associated with adverse mental consequences. Compared with men, women had an OR of 2.7 (95% CI 0.93–7.28, **Figure 2**) for PTSD, an OR of 2.36 (1.07–5.20) for GAD-7 anxiety, and an OR of 2.16 (1.13–4.14) for ASI sleep disorder. Participants with self-reported symptoms showed OR values of 25.6 (95% CI 3.3–198.4) for PTSD, 18.09 (5.23–62.54) for GAD-7 anxiety, and 26.97 (9.34–77.91) for ASI sleep disorder compared with participants without self-reported symptoms. No apparent associations were observed between age or disease severity and PTSD, GAD-7 anxiety, or ASI-sleep disorder.

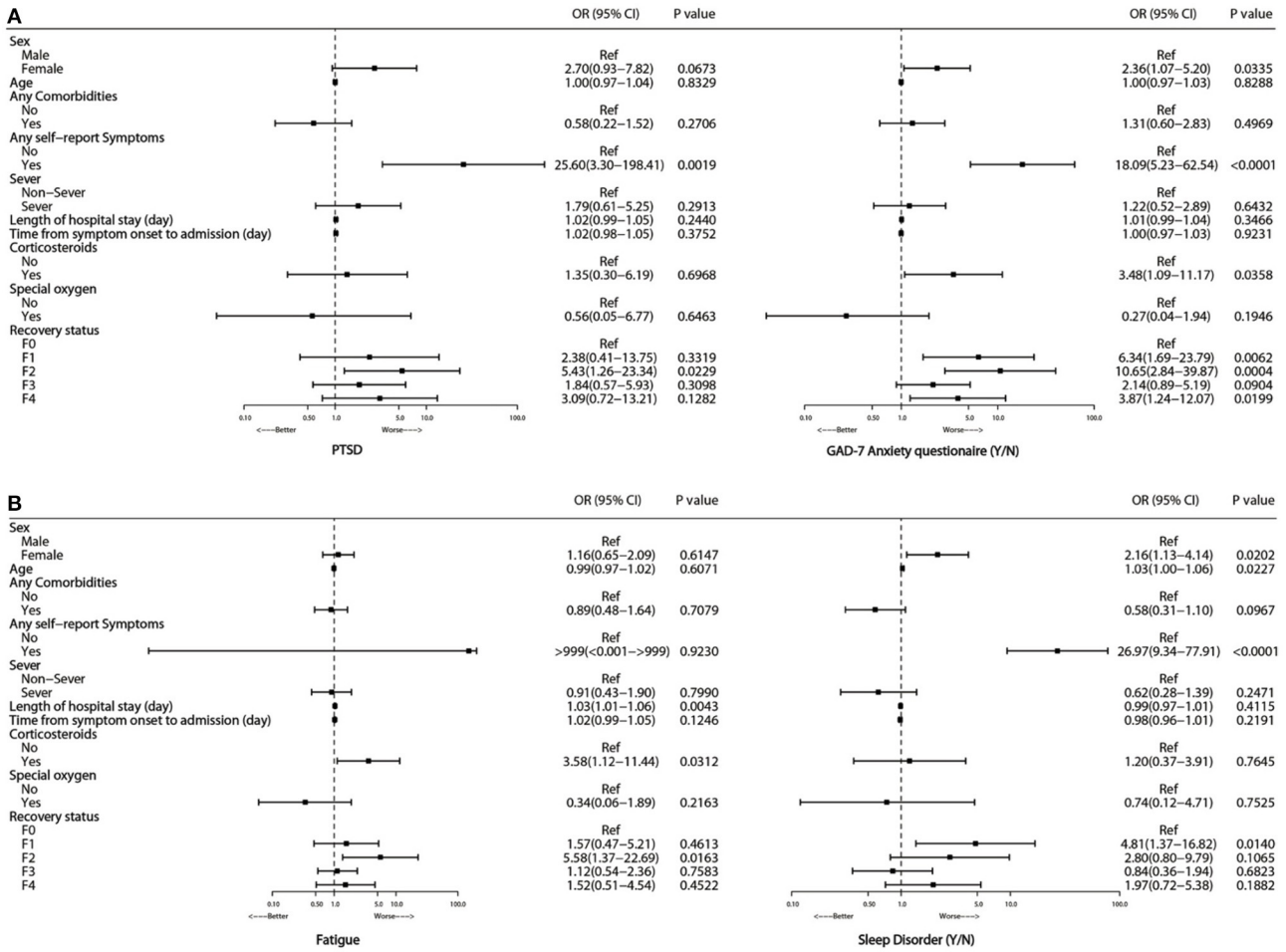

FIGURE 2 | Risk factors associated with PTSD and GAD-7 anxiety **(A)**, fatigue and sleep disorder **(B)** at 15-month follow-up.

DISCUSSION

In this study, we reported the health outcomes of patients who were hospitalized with COVID-19 at 15 months after acute infection during the first pandemic in Wuhan, China. To our knowledge, this is the longest follow-up cohort study of hospitalized COVID-19 patients.

Our data showed that the most common persistent symptoms at 15 months after onset were sleep difficulties and fatigue, followed by memory loss, chest tightness and cough. Compared with the published data of this cohort 3 months after discharge (8), most of the acute symptoms of COVID-19 patients were significantly relieved or even disappeared, and no serious respiratory complications remained. This is consistent with the data of the previous 12-month long-term follow-up study of COVID-19 (7). Our data also showed that residual psychological problems remain prominent. At the 15-month follow-up, 6.4% (34/534) of the patients had depression, 9.2% (49/534) had anxiety, 13.0% (70/534) had insomnia and 4.7% (25/534) had PTSD. These results suggested that the psychological consequences of long-term COVID-19 should be noted.

Compared to the general public, patients that were infected by COVID-19 have in fact a higher risk of developing these adverse mental and psychological symptoms. The prevalence of generalized anxiety disorder (GAD) in adult was very common both in community and in clinic. According to the review of epidemiological studies in Europe, the 12-month prevalence rate of GAD was 1.7–3.4% (17) and the lifetime prevalence was 4.3–5.9% (18). The prevalence rate of GAD in COVID-19 patients in this study was 9.2%, which was significantly higher than that of the general public. Data from a multi-country study involving 252,503 cases from 68 countries showed that the 1-year prevalence rate of mild depression was 2.8% (19). Another community survey in Taiwan, China, including 5,664 individuals aged ≥55, showed that the prevalence rate of mild depression was 4% (20, 21). In our study, the prevalence rate of depression in COVID-19 patients reached 6.4%, which was also significantly higher than that of the general public. In the sample from general adult population in the United States and Canada, the 1-year prevalence rate of PTSD is 3.5–4.7 (22, 23). The prevalence rate of PTSD in COVID-19 patients in this cohort was as high as 25% at 3 months after discharge (data not published). Although the

prevalence rate of PTSD at 15 months after onset has dropped to 4.7%, it is still in the high level when compared with that in the general public. Given all of that, COVID-19 patients still have a higher risk of adverse mental and psychological illness, even 15 months after onset.

Unlike individual-level traumatic events, the COVID-19 outbreak has been a continuing crisis for every member of society (24). Globally, the epidemic has led to an increase of about 53 million in the incidence of depression in 2020, an increase of about 27.6% (25).

Furthermore, we attempted to analyze the potential risk factors related to health outcomes and the relationship with the severity of the disease. Our data demonstrated that female COVID-19 patients were more likely to have residual symptoms, such as fatigue and sleep disorders, and a range of adverse psychological and psychiatric consequences. Patients with long-term legacy symptoms are more likely to develop PTSD. Before the outbreak, women had higher rates of depression and anxiety disorders than men worldwide (25). In China, the prevalence of any depressive disorder in women is higher than that in men, and its lifetime prevalence is 1.44 times that of men (26). After the outbreak of pandemic, an even greater difference in mental disorder prevalence was found between the two genders, which was speculated that females are more likely to be affected by the social and economic consequences of the pandemic (27–29).

In addition, our study first showed that the use of glucocorticoids during hospitalization was significantly related to an increased risk of chronic fatigue and anxiety in patients with COVID-19 after discharge. High-dose corticosteroids were administered to many critically ill patients in Wuhan (30) and were associated with higher mortality risk. Previous research on SARS patients found that high-dose corticosteroid use could lead to osteonecrosis of the femoral head (OFNH) (31). Unfortunately, we were unable to obtain specific dose and use time of each patient in this cohort which limits the conclusions we can draw from these data. Future studies are urgently needed that are specially designed to address the relation between glucocorticoid use and adverse psychological outcomes.

The underlying mechanism of long COVID-19 is complicated and cannot be simply attributed to SARS-CoV-2 infection. The pathogenesis of psychiatric symptoms and disorders that arise during the COVID-19 pandemic may include biologic and psychosocial factors.

On one hand, several retrospective studies also suggest that COVID-19 may affect the brain (32, 33). In addition, a literature review demonstrated that past viral epidemics were associated with neuropsychiatric symptoms such as demyelination, encephalopathy, and neuromuscular dysfunction, as well as mood changes and psychosis (34). The symptoms occurred during infection or following recovery from the infection in the ensuing weeks, months, or longer. Multiple studies suggest that COVID-19 may indirectly affect the central nervous system through the associated inflammatory immune response and medical interventions that are administered (32, 33, 35). Immunologic findings in patients with COVID-19 include elevated serum C-reactive protein and pro-inflammatory cytokines (e.g., IL-6) and decreased total blood lymphocyte counts (34). Critical illness and resultant intensive care unit stays commonly expose patients to extreme physiological and psychological stressors that are life-threatening and traumatic, and frequently precipitate persistent psychiatric illness (35, 36).

On the other hand, psychiatric illnesses that occur during the pandemic may stem from psychosocial factors such as (37–41): frequency and extent of exposure to individuals infected with the virus, fear of infecting family members, fear of being discriminated against, lack of access to testing and medical care for COVID-19, physical distancing, home confinement, quarantining, and loneliness, shortages of available resources (e.g., personal protective equipment), diminished personal freedoms, continuous media reporting about the pandemic and the uncertainty surrounding its eventual outcome. The role of those mentioned above social and psychological factors is particularly serious in Wuhan, where the first outbreak occurred. Thus, psychological and social intervention of this disease carries great importance for the COVID-19 patients in recovery phase. The rehabilitation of COVID-19 patients is a long-term and systematic project. Our research will help inform decision-making on care service design and priorities for these patients.

We also investigated the long-term performance of extrapulmonary organs and deaths during follow-up. For example, it has been observed that some patients have new-onset diabetes, are newly diagnosed with AITD, and have venous thromboembolic diseases, including cardiovascular and cerebrovascular events. The receptor angiotensin-converting enzyme 2 (ACE2), which modulates the invasion of SARS-CoV-2 into the body, is also expressed in many vital metabolic organs and tissues, including pancreatic β cells (42), adipose tissue (43), intestines (44), and kidneys (44); SARS-CoV-2 infection may cause pleiotropic changes in glucose metabolism, complicate the pathophysiology of existing diabetes, or cause new hyperglycemia or new diabetes (45). There have been some precedents of ketosis-prone diabetes caused by coronaviruses. A previous study showed that the incidence of high fasting blood glucose and acute new-onset diabetes in SARS coronavirus pneumonia patients is higher than that in non-SARS patients (46). Our study showed that 3.5% (19/534) of the patients had a new fasting blood glucose of >7 mmol/L or HbA1c $\geq$ 6.5% at the 15-month follow-up and had no previous history of diabetes. We deduced that COVID-19 has potential diabetic effects.

This study has several limitations. First, for the new symptoms that appeared after COVID-19, there was no further stratification to determine whether the symptoms continued after COVID-19, worsened after COVID-19 recovery, or occurred after discharge from the hospital. Second, the cases included in this study were all hospitalized COVID-19 patients, with a lack of data from outpatients. Lastly, this is a single-center study in a specific region which challenges the generalizability of the study findings. We are in urgent need of multi-center studies covering a wider range of patient cohorts over different regions especially when describing the causes of a pandemic affecting the entire world population.

In conclusion, we conducted a 15-month follow-up and reported the persistent symptoms and psychological conditions in a COVID-19 patient cohort in Wuhan. Relevant risk factors, such as female gender and use of glucocorticoids for long

COVID, were identified. All these findings were of great significance for managing COVID-19 patients during the long-term rehabilitation period.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s. The data in this study can be shared with qualified researchers who submit a proposal with a valuable research question.

AUTHOR CONTRIBUTIONS

L-LS, Y-HB, HH, and W-FX designed the study and revised the manuscript. L-LS, JW, and Y-SW drafted the manuscript. L-LS, Y-SW, and XP performed the analysis. JL, HL, Y-RJ, XZ, LL, G-CL, J-WZ, WW, Y-JW, Z-HW, H-BS, S-SC, J-LC, and Z-WX collected the data. L-LS, Y-SW, and Y-HB designed the electronic follow-up questionnaire form. All authors had full access to all the data in the study, and they took responsibility for the integrity of the data and the accuracy of the data analysis.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.854788/full#supplementary-material

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SARS-CoV-2 pre-exposure prophylaxis: A potential COVID-19 preventive strategy for high-risk populations, including healthcare workers, immunodeficient individuals, and poor vaccine responders

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The unprecedented worldwide spread of SARS-CoV-2 has imposed severe challenges on global health care systems. The roll-out and widespread administration of COVID-19 vaccines has been deemed a major milestone in the race to restrict the severity of the infection. Vaccines have as yet not entirely suppressed the relentless progression of the pandemic, due mainly to the emergence of new virus variants, and also secondary to the waning of protective antibody titers over time. Encouragingly, an increasing number of antiviral drugs, such as remdesivir and the newly developed drug combination, Paxlovid® (nirmatrelvir/ritonavir), as well as molnupiravir, have shown significant benefits for COVID-19 patient outcomes. Pre-exposure prophylaxis (PrEP) has been proven to be an effective preventive strategy in high-risk uninfected people exposed to HIV. Building on knowledge from what is already known about the use of PrEP for HIV disease, and from recently gleaned knowledge of antivirals used against COVID-19, we propose that SARS-CoV-2 PrEP, using specific antiviral and adjuvant drugs against SARS-CoV-2, may represent a novel preventive strategy for high-risk populations, including healthcare workers, immunodeficient individuals, and poor vaccine responders. Herein, we critically review the risk factors for severe COVID-19 and discuss PrEP strategies against SARS-CoV-2. In addition, we outline details of candidate anti-SARS-CoV-2 PrEP drugs, thus creating a framework with respect to the development of alternative

and/or complementary strategies to prevent COVID-19, and contributing to the global armamentarium that has been developed to limit SARS-CoV-2 infection, severity, and transmission.

KEYWORDS

COVID-19, pre-exposure prophylaxis (PrEP), high-risk population, molnupiravir, remdesivir

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has, over the past 2 years, resulted in the death of millions of people globally (1). Moreover, COVID-19 has dramatically affected and altered the lives and livelihoods of people in every corner of the world due to its effects on local, regional, and global health care systems, economies, environments, and geopolitical posturing. During this period, various reactive, adaptive, and defensive coping strategies employed by government authorities, such as regional lockdowns, the use of SARS-CoV-2 vaccines, and antiviral drugs, have been implemented, which have influenced the evolution of the pandemic.

The roll-out of several different COVID-19 vaccines by pharmaceutical companies has been considered a major milestone in the global medical effort to prevent populations from developing severe disease from SARS-CoV-2 infection. With respect to the protective effects of these vaccines, data indicates that cellular immunity induced by COVID-19 vaccines protected against severe infection, even against new SARS-CoV-2 variants (2–4). Keeton et al. reported that the T-cell responses induced by COVID-19 vaccination or previous SARS-CoV-2 infection are cross-reactive with the Omicron variant of SARS-CoV-2, despite extensive mutation and reduced susceptibility to neutralizing antibodies of the Omicron variant (2). Nonetheless, the much anticipated protective effects of COVID-19 vaccines have been found to be limited and transient for two main reasons: (1) Protective and neutralizing antibody levels wane after a few months post vaccination, and (2) The SARS-CoV-2 virus undergoes active genomic mutation, rendering some COVID-19 vaccines functionally obsolete even before they have been utilized at a population level (5–9). One multicenter prospective study, conducted by Favresse et al. observed that vaccine-associated antibody titers decline post-vaccination with the BNT162b2 mRNA COVID-19 vaccine (Pfizer, BioNTech) (Comirnaty®), with an estimated antibody half-life of 55 and 80 days for seropositive and seronegative subjects, respectively (6). It has also been reported that vaccine-associated antibody titers achieve peak levels at 1 month after the second dose of the BNT162b2 mRNA COVID-19 vaccine, and subsequently rapidly decrease over time (9). Moreover, newer variants of SARS-CoV-2 may be evolving into virions capable of vaccine-breakthrough, containing several antibody-resistant mutations, such as the Omicron SARS-CoV-2 variant, which is a heavily-mutated virus variant, and is classified as a variant of concern (VOC) by the World Health Organization (WHO) (10). Based on three-dimensional (3D) renderings of its antibody-receptor-binding domain (RBD) complex structures, Chen et al. has claimed that the Omicron variant has an 88% likelihood of evading antibodies generated by current vaccines (11). The Omicron (B.1.1.529) variant could also increase the risk of SARS-CoV-2 reinfection, which is associated with immune evasion (12). Similarly, Hoffmann et al. have reported that the Omicron variant evades neutralization by antibodies from vaccinated individuals with up to 44-fold higher efficiency than the Delta variant (13). Also, microbial dysbiosis, gut barrier integrity loss, and/or microbial translocation are also thought to be involved in the milieu of COVID-19 disease and poor host immune responses secondary to vaccination (14, 15). This indicates that alternative strategies to vaccination to combat COVID-19, geared toward supplementing and consolidating the existing defensive arsenal, are warranted.

Pre-exposure prophylaxis (PrEP) refers to the utilization of medication/drugs before risk exposure, in order to prevent disease acquisition and transmission, for people at high risk to be infected. PrEP has typically referred to the prevention of HIV infection using specific antiviral agents by a person at risk of HIV acquisition. It has been deemed a cornerstone for HIV prevention. Convergent evidence from prospective clinical trials has demonstrated the efficacy of HIV PrEP in reducing the risk of HIV acquisition, which is known to be up to 98% effective when adherence to treatment is optimal (16–21). Several HIV PrEP drugs and drug combinations have been recommended by the WHO, and these have been employed for use in people at high risk of HIV infection, as a part of the combination HIV prevention approach (22).

With respect to SARS-CoV-2, several antiviral agents have now been investigated and developed that show inhibitory effects against SARS-CoV-2 both *in vitro* and *in vivo*, including remdesivir, molnupiravir, and Paxlovid® (nirmatrelvir/ritonavir). In this review, we propose that PrEP using the preceding antiviral drugs, as well as other potentially

effective anti-SARS-CoV-2 agents, might be considered to prevent SARS-CoV-2 acquisition in high-risk populations, inspired by the unparalleled success of PrEP in preventing the acquisition of HIV.

Herein, we critically review the risk factors for severe COVID-19, discuss potentially viable SARS-CoV-2 PrEP strategies, as well as their limitations in targeted populations, thus paving the way for the development of an alternative or complementary strategy to prevent SARS-CoV-2 infection and secondary transmission.

High risk population for COVID-19

The population that has a substantially higher probability to closely interact with SARS-CoV-2-infected individuals, and who would be considered as the group with the highest risk of exposure to SARS-CoV-2 infection, would be healthcare workers. A significantly large number of healthcare workers have already been infected by SARS-CoV-2, and some of these infected individuals experienced poor outcomes, especially at the early stages of the pandemic (23–25). In October 2021, the WHO estimated that, globally, between 80,000 and 180,000 healthcare workers died due to COVID-19 between January 2020 and May 2021 (26–28). Aside from close contact, there are multiple other risk factors, such as older age, presence of comorbidities, environmental factors, and poor vaccine response, which may facilitate SARS-CoV-2 infection and produce unfavorable outcomes, including long-COVID-19.

Demographic factors

Convergent investigational observations indicate that susceptibility to and disease severity of COVID-19 are associated with older age, male gender, and ethnicity (29–31). Data from the early stages of the pandemic in US indicates that case-fatality rates increase with age, with <1% of deaths among people aged ≤ 54 years of age, 1.4–4.9% among people aged 55–74 years of age, 4.3–10.5% among people aged 75–84 years, and 10.4–27.3% among people aged ≥ 85 years old (29). Moreover, one global meta-analysis conducted in 2021, which included 59 studies and 36,470 patients, observed that males and the older population have a materially higher risk for SARS-CoV-2 infection, severe disease, and mortality (30). In concordance with these findings, another meta-analysis, which included 14 studies, 29,909 SARS-CoV-2-infected patients, and 1,445 cases of death, indicated that older age (≥65 years old) and male gender were associated with a greater risk of death from COVID-19 infection, with a pooled odds ratio (OR) of 4.59 [95% confidence interval (CI) = 2.61–8.04, ≥65 vs. <65 years old], and 1.50 (95% CI = 1.06–2.12, male vs. female) (31). Older age is unavoidably associated with various other comorbidities, poor immunity, and increased levels of circulating pro-inflammatory cytokines (32). Additional factors, such as differences in levels of circulating sex hormones between men and women, levels of ACE2 enzymes and receptors, the presence of the transmembrane serine protease 2 (TMPRSS2) enzyme, and lifestyle factors such as smoking, may also contribute to variable risks of severity and mortality of COVID-19 in afflicted persons (32, 33).

Generally, it has been considered that non-Caucasian races are associated with increased risk of SARS-CoV-2 infection, disease severity, and mortality, compared to people of Caucasian ancestry (34–36). One meta-analysis, which included 18,728,893 patients from 50 studies, observed that individuals of black and Asian ethnicity are at increased risk of SARS-CoV-2 infection compared to Caucasian individuals, and that Asian individuals are at higher risk of ICU admission and death (34). However, after adjusting for comorbidities, another meta-analysis reported that racial discrepancies observed in risk of SARS-CoV-2 infection rates may actually be attributable to higher comorbidity prevalence in certain racial groups (37).

ABO blood groups have also been found to be associated with COVID-19 susceptibility, severity, and mortality (38–43). Group A individuals showed an increased risk of becoming infected by SARS-CoV-2, compared to group O (39–43). With respect to severity and mortality, Muñiz-Diaz et al. reported that specific ABO blood groups were also seen to represent important risk factors for development of COVID-19, with the risk in Group A individuals being significantly higher than that in Group O individuals (38).

Comorbidities

A large volume of published literature has reported that various comorbidities may predispose patients with COVID-19 to an unfavorable outcome, and a higher risk of death (44–51). Hernández-Garduño, after analyzing the data of 32,583 patients, showed that the presence of either obesity, diabetes, or hypertension were strong predictors for both the acquisition of SARS-CoV-2 infection and the development of severe disease (50). COVID-19 clinical guidance issued by The American College of Cardiology indicates that case fatality rates for comorbid COVID-19 patients are substantially higher than the average population, i.e., case fatality rates for comorbid cardiovascular disease (CVD) being 10.5%, diabetes (7.3%), chronic respiratory disease (6.3%), hypertension (6.0%), and cancer (5.6%) (51).

The United States (US) Centers for Disease Control and Prevention (CDC) has released a comorbid medical condition list for COVID-19, and has issued advice stating that having one of the listed conditions may make a person more likely to become severely ill from COVID-19 (52). This updated list includes cancer, chronic kidney disease, chronic liver

disease, chronic lung diseases, dementia or other neurological conditions, diabetes, etc. (52). Similarly, several meta-analyses have now confirmed that the listed conditions do indeed predispose individuals to severe illness (53–57). For instance, Thakur et al. published a meta-analysis, which included 120 studies and 125 446 patients, which observed that the most prevalent COVID-19 comorbidities were hypertension (32%), obesity (25%), diabetes (18%), and cardiovascular disease (16%), while patients having renal comorbidities had the highest severity and mortality rates (53).

Environment

Accumulating evidence has shown that environmental and climatic factors have a significant effect on COVID-19 transmission and mortality. These factors include population density, temperature, ozone levels, sulfur dioxide levels, humidity, wind speed, and rainfall levels (58–65).

Yin et al. analyzed the data of cities in China and in the USA, and observed that a higher population density was associated with a higher percentage of morbidity related to COVID-19, indicating the importance of social distancing and travel/movement restrictions for the prevention of COVID-19 transmission (65). Sobral et al. investigated the association between climatic conditions and global SARS-CoV-2 transmission, and found that, aside from prevailing average temperature levels, countries with higher rainfall measurements showed an increase in COVID-19 transmission, with each average inch/day of rainfall equating to an additional 56.01 newly-identified COVID-19 cases/day (62). Generally, higher population density, air pollution, rainfall, and wind speed, as opposed to lower temperature, humidity and sulfur dioxide levels are associated with higher COVID-19 infection and severity rates (58–65).

Vaccine responses

The development of protective immunity after COVID-19 vaccination relies on long-term B- and T-cell memory responses to SARS-CoV-2 (66, 67). Immunosuppressed patients, such as those with immunodeficiency, organ transplant recipients, untreated HIV-infected patients, and cancer patients who have B- or T-cell deficiency are more likely to develop severe COVID-19 (68, 69). Goubet et al. reported that the lymphopenia was associated with prolonged SARS-CoV-2 RNA virus shedding and poor prognosis in cancer patients (70). In SARS-CoV-2 susceptible individuals, Fahrner et al. (67) observed a specific deficit in the TH1/Tc1 response against the receptor binding domain of the spike protein (S1-RBD), and vaccine-induced S1-RBD TH1 immunity is reduced in hematological malignancies. Fernandez Salinas et al. (71) reported that only 33% of patients with common variable immunodeficiency (CVID) showed an antibody response to the COVID-19 vaccine; moreover, CVID could not generate RBD-specific MBCs even after two vaccine doses, compared to healthy vaccinated individuals. Amodio et al. (69) reported that five patients with inborn errors of immunity (IEI) did not mount any cellular response, as is usually observed in healthy individuals, following the BNT162b2 mRNA COVID-19 vaccine, and one of these patients was also found to not be able to mount any humoral response. Thus, for these immunosuppressed populations, alternative or complementary strategies would be lifesaving.

Lessons from the PrEP strategy for HIV

Evidence indicates that HIV PrEP is extremely effective (up to 98% effective) in reducing the risk of HIV acquisition when adherence to PrEP is optimal (16–20). PrEP has been broadly utilized to prevent HIV spread for populations who have a higher risk of acquiring HIV, such as sex workers, people who engage in recreational injection drug use, and those who practice unprotected receptive anal intercourse. Two combinations of oral antiretroviral drugs have been approved and used for HIV PrEP, including the combination of tenofovir disoproxil and emtricitabine (Truvada®), and tenofovir alafenamide and emtricitabine (Descovy®). Additionally, in December 2021, the US Food and Drug Administration (FDA) approved the first injectable preparation (cabotegravir extended-release injectable suspension) for HIV PrEP, which is believed to greatly improve medication compliance as it is administered only every 2 months.

Concerns regarding long-term drug safety, cost, development of drug resistance, and risk compensation of HIV PrEP present ongoing challenges. Some adverse effects have been observed; however, cumulative evidence has revealed that HIV PrEP has an overall satisfactory safety profile (72). The main adverse effects are usually mild to moderate nausea, vomiting, and diarrhea. Kidney and liver toxicity are rare; however, regular monitoring of renal and liver functions are required (73). Other concerns exist, including cost, development of drug resistance, and risk compensation. One meta-analysis that included 18 studies and 19,491 participants demonstrated that PrEP was highly effective across populations, presented few adverse events and instances of drug resistance, and had no significant association with changes in sexual risk behavior (21). Overall, current evidence indicates that the benefit-risk profiles of available HIV PrEP regimes are strongly favorable for the targeted population at high risk of infection by HIV.

Consideration for PrEP for COVID-19

Drawing on knowledge gained from the use of PrEP for HIV disease, SARS-CoV-2 PrEP, using specific antiviral and adjuvant drugs against SARS-CoV-2, may represent a novel preventive strategy for COVID-19. However, unlike HIV, against which there is currently no available vaccine, SARS-CoV-2 PrEP probably will be given to individuals who have poor response to vaccination and have a high-risk of developing severe COVID-19. Moreover, SARS-CoV-2 PrEP differs from post-infection treatment, which relies on therapeutic interventions to be initiated after the patient has tested positive for COVID-19, as shown in Figure 1. We thus consider a few drugs (favoring oral administration as first choice) that could potentially be used as PrEP for SARS-CoV-2. The mechanisms of action of the drugs discussed are summarized in Figure 2.

Molnupiravir

Molnupiravir (MK-4482) is an orally available prodrug of beta-D-N4-hydroxycytidine (NHC), a ribonucleoside that has broad antiviral activity against RNA viruses (74). Viral mutations and lethal mutagenesis results from NHC uptake by viral RNA-dependent RNA-polymerases. Molnupiravir has been found to be effective against SARS-CoV-2, as demonstrated by Zhou et al. (75) and Kabinger et al. (76). In a randomized, placebo-controlled, double-blind phase 2/3 trial, Arribas et al. observed that molnupiravir administration does not result in clinical benefit in patients hospitalized by COVID-19 (77). However, results gleaned from studies of molnupiravir administration to non-hospitalized patients indicate a much more favorable picture. Caraco et al. (78) (Phase 2 MOVe-OUT study) observed a lower incidence of hospitalization and/or death in the molnupiravir group vs. the placebo group in specific participants (especially those >60 years of age, and those with an increased risk for severe illness). The preceding research group subsequently concluded that molnupiravir administration can reduce hospitalizations and/or death in non-hospitalized patients with COVID-19. Furthermore, results from a study by Jayk Bernal et al. (79) (Phase 3 MOVe-OUT study) indicate that (i) the rate of hospitalization or death through day 29 is ∼31% lower with molnupiravir than with placebo, and (ii) molnupiravir treatment is associated with greater reductions in mean viral load from baseline than placebo at days 3, 5 (end-of-treatment visit), and 10. Armed with the promising results gleaned from this study conducted in non-hospitalized COVID-19 patients, the authors concluded that early treatment with molnupiravir reduced the risk of hospitalization or death in at-risk and unvaccinated adults with COVID-19. Thus, proposing the use of molnupiravir in a pre- or post-exposure prophylaxis context seems reasonable upon reflection. Although the results from the MOVe-OUT trial indicate that molnupiravir is safe for human use, a theoretical risk with the use of molnupiravir has been postulated, as molnupiravir could possibly be processed by human host cells and could, conceivably, be incorporated into the host DNA, potentially leading to cellular mutations and cellular death (75).

Remdesivir

Remdesivir is an antiviral nucleoside analog pro-drug which inhibits the RNA-dependent RNA polymerase non-structural protein 12 (NSP12) in SARS-CoV-2. It was originally developed to treat Ebola virus disease (80), but has shown positive outcomes when used in SARS-CoV and MERS-CoV infections *in vitro* and in preclinical *in vivo* animal models (81–83), and was also used in the treatment of the first reported case of COVID-19 in the United States of America (with no obvious adverse effects) (84). Ebola virus, SARS-CoV, MERS-CoV, and SARS-CoV-2 genomes obviously differ; however, remdesivir has a broad-spectrum of activity, and therefore has the capacity to effectively neutralize RNA polymerase, the structure and function of which is relatively similar in all RNA viruses (85, 86). Indeed, remdesivir, after a sequence of steps that is presumably initiated by esterase-mediated hydrolysis of the amino acid ester, is ultimately metabolized into the active nucleoside triphosphate analog form, which is utilized by the viral RNA-dependent RNA polymerase (RdRp) upon its diffusion into the cell. Then, utilization of that nucleoside triphosphate analog by RdRp inhibits viral replication, as it induces delayed chain termination (87, 88). It has now been established that remdesivir has potent *in vitro* activity against SARS-CoV-2 (89), and has been used and studied in several recent randomized clinical trials (90–94). Although authors arrive at differing conclusions regarding its efficacy for the treatment of hospitalized COVID-19 patients, a clinical benefit is suspected, especially when used in the early phase of the disease. Moreover, based on the manufacturer statement regarding remdesivir efficacy in preventing SARS-CoV-2 infection, the PINETREE study (NCT 04501952) showed positive effects of remdesivir on the course of COVID-19 in outpatients who were treated early, and was also shown to be safe, and well-tolerated (95). In order to circumvent the significant limitation to the use of remdesivir imposed by the requirement of intravenous administration (which may potentially limit its widespread use during the pandemic), the orally bioavailable nucleoside prodrug GS-621763, which has been shown to be metabolized into the same active nucleoside triphosphate formed by remdesivir, has now been developed, and has shown potent antiviral activity against SARS-CoV-2 in various cell models, with a similar therapeutic efficacy to intravenous remdesivir in a murine model of SARS-CoV-2 pathogenesis (96). Overall, it therefore seems reasonable to

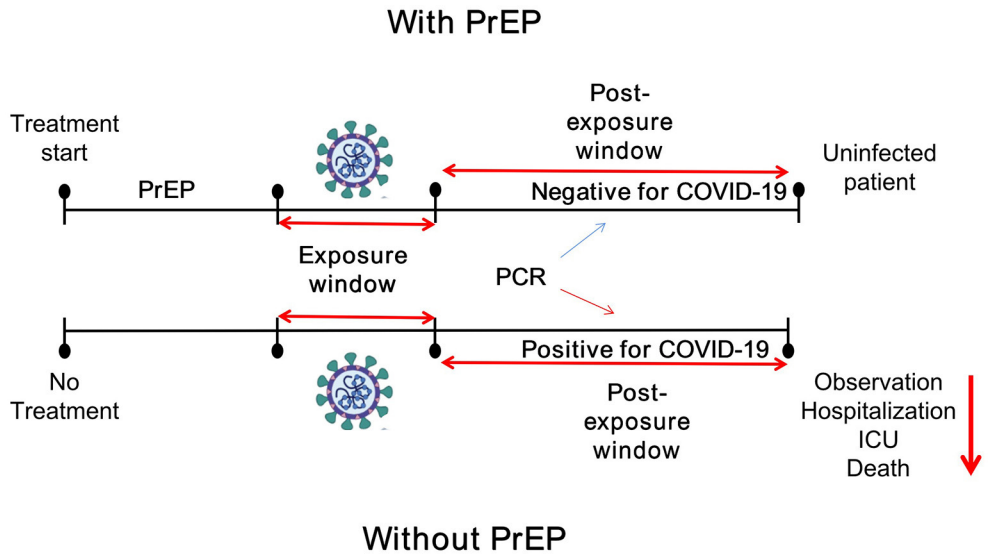

FIGURE 1
Comparison of SARS-CoV-2 PrEP strategies and post infection therapy. With an effective PrEP strategy, the patient remains negative for COVID-19 as SARS-CoV-2 cannot replicate in the patient's body. Conversely, without a PrEP strategy, any exposure to SARS-CoV-2 can result in an infection, confirmed by a positive PCR test. In the optimal scenario, the patient will develop a mild infection which will be subsequently controlled and eliminated by their immune response. ICU, intensive care unit.

actively consider the use of remdesivir or its oral prodrug as potentially useful antiviral drugs for SARS-CoV-2 pre-exposure prophylaxis.

Favipiravir

Favipiravir is a purine nucleoside analog which acts as a competitive inhibitor of RNA-dependent RNA polymerase (97). In other words, favipiravir has been shown to be a potent inhibitor of various different viral RNA-dependent RNA polymerases (RdRps), including in influenza A and B viruses, in several agents causing viral hemorrhagic fever, and also in SARS-CoV-2 *in vitro* (82, 97, 98). In a clinical context, favipiravir administration to COVID-19 patients has been shown to be capable of (i) reducing the window for viral clearance (from 11 to 4 days) and (ii) improving pulmonary inflammatory marker levels (91% of treated patients showed improvement vs. 62% in the control group) (99). Udwadia et al. in their randomized, comparative, open-label, multicenter, phase 3 clinical trial, have demonstrated that favipiravir administration can significantly shorten the time to clinical cure in COVID-19 patients (100). Importantly, Doi et al. have reported that early intervention with favipiravir is superior to late intervention in terms of viral clearance and time to defervescence. Favipiravir would thus be of potential benefit if administered in a SARS-CoV-2 PrEP context. Researchers conducting an ongoing clinical study in Canada are currently assessing the efficacy of favipiravir treatment over 25 days for the prevention of SARS-CoV-2 infection in residents and staff of nursing homes (among elderly, assisted-living patients, and healthcare professionals) (NCT04448119, Phase 2). Results and outcomes of this study are eagerly awaited.

Tenofovir-based regimens

In vitro investigations suggest that tenofovir (i) inhibits SARS-CoV-2 RdRp, although with weaker binding than remdesivir (91, 92, 101, 102) and (ii) possesses immunomodulatory effects as it demonstrates the ability to decrease both interleukin (IL)-8 and IL-10 production (103), which are both known to favor COVID-19 severity (104, 105). In addition, observations made in people living with HIV (PLWH) indicate that there is a little evidence that HIV infection increases COVID-19 risk in settings with good access to tenofovir-based antiretroviral therapy (ART) (106). The preceding intriguing observation initiated considered ruminations with respect to the potential activity of tenofovir disoproxil fumarate (TDF, now used worldwide for HIV treatment and HIV pre-exposure prophylaxis) against SARS-CoV-2. Indeed, del Amo et al. (106), in a Spanish cohort study of 77 590 PLWH taking ART, reported that the incidence (per 10,000 persons) of COVID-19 diagnosis

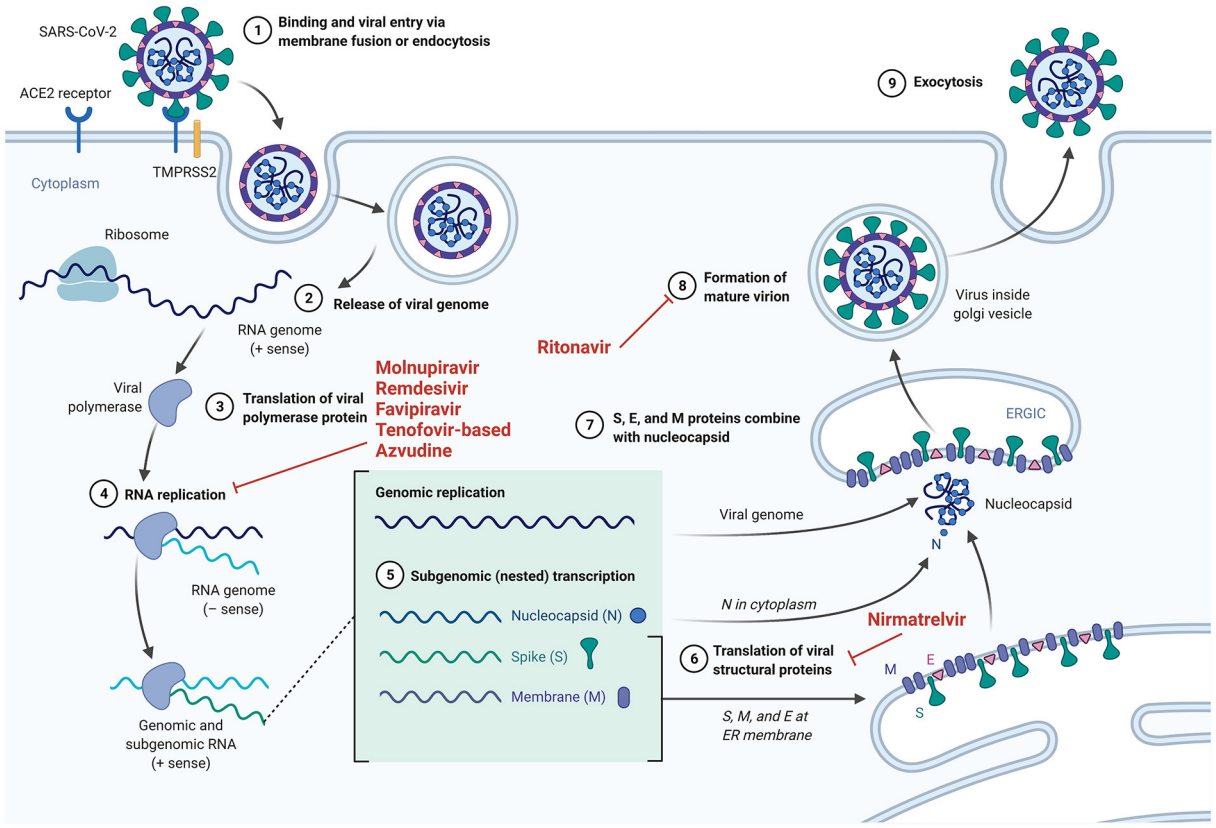

FIGURE 2
The mechanisms of action of specific drugs that could potentially serve as PrEP for SARS-CoV-2.

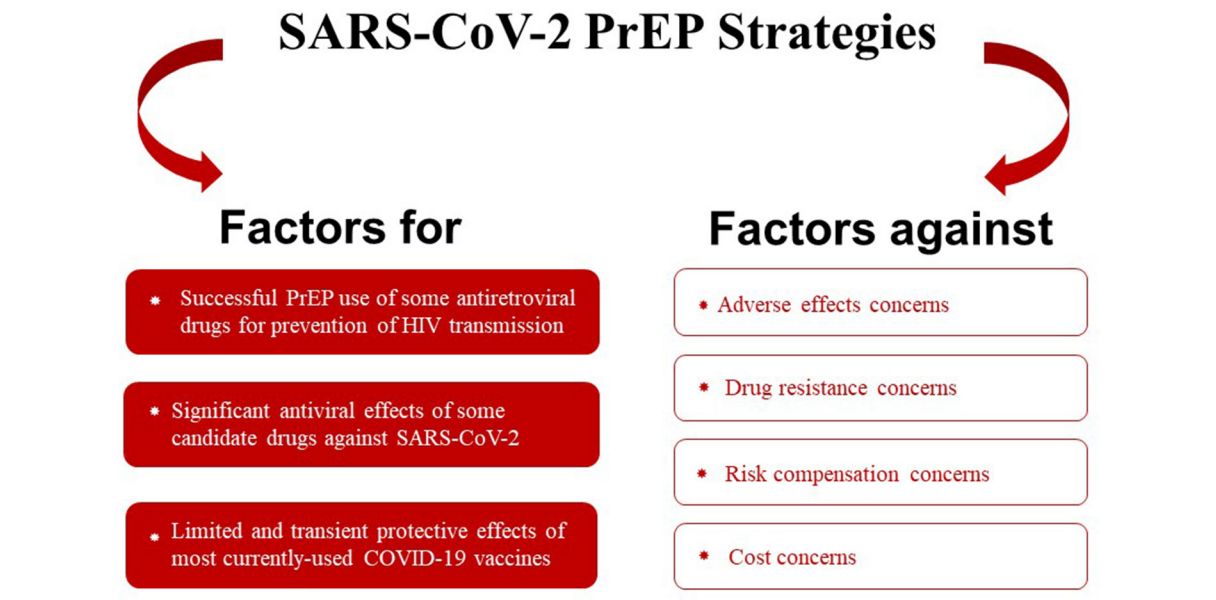

FIGURE 3
Pros and cons of SARS-CoV-2 PrEP strategies.

among patients taking TDF/FTC was 16.9 [95% confidence interval (CI), 10.5–25.9], compared to 41.7 in the general population. Furthermore, a study by Boulle et al. (107) found that PLWH taking TDF/FTC experience 59% lower mortality from COVID-19 than those taking abacavir or zidovudine (aHR, 0.41; 95% CI, 0.21–0.78). Similarly, a third cohort study by Ayerdi et al. (108) has demonstrated that HIV PrEP (tenofovir/emtricitabine) users who tested positive for SARS-CoV-2 antibodies showed higher rates of asymptomatic infection, although the difference in asymptomatic rates of SARS-CoV-2 infection was not statistically significant (42.7 vs. 21.7% for non-PrEP users; $p = 0.07$). In light of the tenofovir-based treatment safety profile and its putative anti-SARS-CoV-2 effects, the tenofovir/emtricitabine combination (the combination present in DESCOVY® and TRUVADA® for example), is currently being studied as a SARS-CoV-2 prophylactic agent. As such, we can report that (i) a clinical trial assessing the efficacy of a 12 week SARS-CoV-2 prophylaxis course of the emtricitabine/tenofovir regimen (NCT04334928) in healthcare workers in Spain is ongoing, and (ii) several additional proposed studies intend to use this specific drug combination in a preventive manner for COVID-19 (examples: NCT04519125 and NCT04405271).

Nirmatrelvir/ritonavir

Another orally administered potentially prophylactic drug is nirmatrelvir, a specific inhibitor of the SARS-CoV-2 viral 3-chymotrypsin-like cysteine (3CL) protease (109, 110). To achieve adequate drug levels, nirmatrelvir is administered together with the CYP 3A4 inhibitor, ritonavir. The role of ritonavir, well-known as a pharmacological booster, is hypothetical in Figure 2, as it is based on theoretical evidence from several researchers (111–114) showing that lopinavir and ritonavir also inhibit the coronaviral 3CL1pro protease, although coronaviruses encode a different enzymatic class of protease. Knowing that 3CL1pro protease plays an essential role in processing the polyproteins that are translated from the viral RNA, we therefore are encouraged that ritonavir could possibly also inhibit the formation of mature virions of SARS-CoV-2. In the E,PIC-HR study (NCT04960202), 5 days of therapy with nirmatrelvir/ritonavir reduced the rate of hospitalization and/or death by 88% in COVID-19 outpatients with at least one risk factor for a severe course if therapy was started early (within 5 days) after the onset of symptoms. On the 22nd of December 2021, the US FDA endorsed and authorized nirmatrelvir/ritonavir (Paxlovid®) use for the treatment of COVID-19 (115). However, due to the required combination with ritonavir, drug interactions may occur, especially in high risk populations (116). Further study of this drug combination may provide a clearer picture of its benefits when administered for SARS-CoV-2 pre- or post-exposure prophylaxis.

Azvudine

Azvudine is a safe (117) nucleoside-based broad-spectrum anti-virus clinical candidate originally developed for HIV infection treatment and prevention (118, 119). As such, azvudine was approved by China FDA for AIDS treatment on July 21, 2021 (XZXK-2021-214) in view of its efficacy in treating AIDS and its favorable safety profile during the 48-week oral treatment (120). *In vitro*, azvudine has shown significant antiviral effects against HIV (121), HCV (122), human enterovirus 71 (123), and HBV (124). Furthermore, Ren and colleagues were the first to observe a potent antiviral activity against HCoV-OC43 and SARS-CoV-2, fostering speculation with respect to its anti-COVID-19 effect. Indeed, azvudine is known to inhibit viral RNA-dependent RNA polymerase (123, 125), and in a subsequent randomized, open-label, controlled clinical trial, Ren et al. have reported in 2020 that azvudine treatment may shorten the nucleic acid negative conversion time in the mild COVID-19 context (126). They therefore requested permission for investigation using a larger sample size, to confirm their findings. Recently (in December 2021), Zhang et al. (120) have demonstrated that oral administration of azvudine was able to (i) reduce the viral load in SARS-CoV-2 infected rhesus macaques and (ii) cure all COVID-19 patients in their treatment cohort (a randomized, single-arm clinical trial; $n = 31$). They observed that all study participants demonstrated 100% viral ribonucleic acid negative conversion in 3.29 days, with a 100% hospital discharge rate in 9 days, although minor and transient side-effects (dizziness and nausea) were noted in 16.12% (5/31) of patients. It is thus valid to state that the preceding findings favor the potential utilization of azvudine in future SARS-CoV-2 pre-exposure prophylaxis strategies.

Perspectivs and challenges for PrEP for COVID-19

Formulating, investigating, and proposing preventive strategies for COVID-19, such as SARS-CoV-2 PrEP, are likely to help prevent morbidity and mortality from COVID-19 in high-risk populations. The antiviral drugs and their combinations listed in the discussion should be considered to be theoretically and hypothetically proposed strategies for SARS-CoV-2 PrEP prevention. Even though some of the proposed therapeutic methods appear to be promising, multiple challenges remain for the future development of effective SARS-CoV-2 PrEP.

Firstly, drug adverse effects or toxicity are a primary concern. For example, there are specific host DNA mutational concerns with molnupiravir use which need to be addressed (75). Remdesivir, as a lyophilized powder or injectable solution, has been associated with renal and hepatic toxicity as a consequence of the accumulation of excipient sulfobutylether-β-cyclodextrin

(SBECD) (127, 128). Moreover, most drugs listed have revealed their benefits in already-infected patients, while their efficacy and safety in preventing SARS-CoV-2 infection in uninfected and/or vaccinated individuals will warrant further studies.

Poor adherence to antiretroviral therapy against HIV has been shown to be a major determinant for the emergence of drug resistance (129). There would also be concerns regarding drug resistance development for SARS-CoV-2 PrEP. Monotherapy may well avoid drug-drug interactions; however, compared with combination therapy, monotherapy is more likely to result in the emergence of drug resistance (130, 131). Immunocompromised patients are more likely to develop high intra-host viral diversity (132–134), which further emphasizes their risk of developing drug resistance following monotherapy. Thus, further investigations should evaluate the possibility of co-administration of two or more drugs to potentially reduce the possibility of development of resistance. Thus, we believe that the US FDA-authorized nirmatrelvir/ritonavir (Paxlovid®) is one drug combination that can possibly be contemplated as an effective PrEP candidate.

A SARS-CoV-2 PrEP strategy may help prevent morbidity and mortality from COVID-19; however, it may also encourage the easing of the very effective preventive measures that attempt to decrease the spread of the virus, such as social distancing interventions and avoidance of exposure, thus potentially increasing infection risk. During the COVID-19 pandemic, risk compensation has been associated with vaccination and face mask use (135, 136). Risk compensation may also significantly impact the benefits of a SARS-CoV-2 PrEP strategy, especially if efficacy of SARS-CoV-2 PrEP in real-life is not sufficiently high.

Moreover, prior to the implementation of a SARS-CoV-2 PrEP strategy, the specific criteria for the likelihood of acquisition of infection after exposure to SARS-CoV-2 should also be studied and clearly defined, including the required exposure time for infection to occur (since the probability of being infected would increase when the exposure time exceeds specific time thresholds), the occurrence of new epidemic cases in the family or at the workplace, the physical distance from the potential infective spreader, and the duration of infection of the potential infective spreader (suspected infection or documented infection by PCR or rapid testing). Similarly, the follow-up of users of PrEP and the criteria evaluating the outcome of PrEP for COVID-19 remain to be clarified. A polymerase chain reaction (PCR) test performed 5 days after PrEP medication in parallel with a blood test evaluating toxicity of the drug(s) is recommended.

Furthermore, the PrEP concept excludes parenteral therapy, and should be available for high-risk patients at home, preferably before potential exposure. The dosage and the duration of treatment will depend on each drug used. Cost efficacy of PrEP should be considered with particular diligence and gravity, as such a preventive COVID-19 strategy, if effective, may circumvent ICU admission (where costs are known to be prohibitive), and extended hospital stays.

The PrEP for COVID-19 proposed in the preceding discussion involves the administration of the drugs listed above to only high-risk populations, particularly patients with an immunocompromised status, such as common variable immunodeficiency, lymphopenia, patients with organ transplants, and lymphoma. Additionally, the potential SARS-CoV-2 PrEP candidate drugs described herein have specific merit for use in patients who respond poorly to COVID-19 vaccination and who are more likely to develop severe COVID-19. Nevertheless, the entire SARS-CoV-2 PrEP remains a theoretical construct, and significant merits of and limitations to our proposed SARS-CoV-2 PrEP strategies exist (Figure 3). However, for selected categories of patients, PrEP for COVID-19 can likely represent a potentially viable course of action that should be carefully and impartially examined and studied.

Based on the perceived risk benefit ratio, we consider that potential SARS-CoV-2 PrEP strategies should be evaluated in the context of future SARS-CoV-2 infection waves in large populations.

Individual population health policies adopted by countries around the world have the potential to significantly challenge the PrEP strategy proposed in this article. For example, free rapid antigen testing kits are now widely available to individuals in countries such as Canada. This diagnostic test is not based the presence of SARS-CoV-2 in the test sample, but is based on specific parts of the SARS-CoV-2 virion (such as the nucleocapsid), and can thus potentially result in false positive results. The PrEP strategy that we propose is evaluated through PCR testing, which requires the presence of the virus in the test sample, and is therefore more accurate diagnostically. In Canada, based on a positive test with rapid antigen kits, pharmacists can initiate Paxlovid on the day of diagnosis, even without the approval of a physician. It is, thus, particularly difficult to initiate, follow-up, and/or evaluate the efficacy of PrEP in such a context. Another example is China with its dynamic zero COVID-19 policy. Indeed, this policy is ambitious; however, it does not favor implementation of strategies such as our proposed SARS-CoV-2 PrEP strategy as currently, (i) known cases are closely monitored, following stringent protocols, (ii) quarantine measures are largely implemented, (iii) and PCR tests are considered the gold standard. Perhaps, a PrEP strategy could be implemented if and when the prevailing COVID-19 situation in China becomes totally under control; however, the evaluation of its efficacy will remain difficult, as other associated measures aiming to reduce the exposure to SARS-CoV-2 (facemask use, decontamination measures) are ubiquitously and universally maintained.

Conclusion

HIV PrEP has been demonstrated to be an effective infection prevention strategy, with a significantly favorable benefit-risk profile for the prevention of HIV transmission to people at high risk. Based on this model, we propose the development of SARS-CoV-2 PrEP for use in high-risk populations, including healthcare workers who can induce secondary transmission, immunodeficient individuals, and poor vaccine responders. Much progress has been made in discerning the risk factors for acquiring COVID-19, which include close contact, demographic factors, presence of certain comorbidities, environmental factors, and vaccine response. Emergent drugs with beneficial effects are paving the way for development of possible PrEP strategies which could be utilized to prevent COVID-19 infection in high-risk populations. However, several challenges exist for the development of strategies for SARS-CoV-2 PrEP, such as drug toxicity and patient safety concerns, emergence of drug resistance, and the cost of drugs. We believe that collaborative efforts at conducting comprehensive assessments for ethical considerations related to SARS-CoV-2 PrEP, the benefit-risk profiles of SARS-CoV-2 PrEP, and strategic planning of implementation of SARS-CoV-2 PrEP in selected populations should be a research priority. Based on current evidence, we consider that PrEP for COVID-19 could be a potentially useful and practical adjunct to COVID-19 vaccination to prevent SARS-CoV-2 acquisition in selected at-risk patients.

Author contributions

JO and SZ wrote the first draft edition of the manuscript. VH and XL provided critical revisions of this manuscript. J-PR and YC conceived and designed the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Humoral response to SARS-CoV-2 mRNA vaccination in previous non-responder kidney transplant recipients after short-term withdrawal of mycophenolic acid

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Seroconversion rates after COVID-19 vaccination are significantly lower in kidney transplant recipients compared to healthy cohorts. Adaptive immunization strategies are needed to protect these patients from COVID-19. In this prospective observational cohort study, we enrolled 76 kidney transplant recipients with no seroresponse after at least three COVID-19 vaccinations to receive an additional mRNA-1273 vaccination (full dose, 100 μg). Mycophenolic acid was withdrawn in 43 selected patients 5–7 days prior to vaccination and remained paused for 4 additional weeks after vaccination. SARS-CoV-2-specific antibodies and neutralization of the delta and omicron variants were determined using a live-virus assay 4 weeks after vaccination. In patients with temporary mycophenolic acid withdrawal, donor-specific anti-HLA antibodies and donor-derived cell-free DNA were monitored before withdrawal and at follow-up. SARS-CoV-2 specific antibodies significantly increased in kidney transplant recipients after additional COVID-19 vaccination. The effect was most pronounced in individuals in whom mycophenolic acid was withdrawn during vaccination. Higher SARS-CoV-2 specific antibody titers were associated with better

neutralization of SARS-CoV-2 delta and omicron variants. In patients with short-term withdrawal of mycophenolic acid, graft function and donor-derived cell-free DNA remained stable. No acute rejection episode occurred during short-term follow-up. However, resurgence of prior anti-HLA donor-specific antibodies was detected in 7 patients.

KEYWORDS

SARS-CoV-2, kidney transplantation, variants of concern, delta variant, omicron variant, SARS-CoV-2 vaccination

Introduction

Kidney transplant recipients (KTR) are at high risk for severe COVID-19 infection with an overall reported 28-day probability of COVID-19 related death of 21.3% and a twofold higher risk of death in KTR compared to non-transplanted patients (1–3). Response to vaccination is significantly impaired in KTR compared to healthy cohorts even after three doses of an mRNA vaccine (4–13). Furthermore, vaccine-induced SARS-CoV-2 specific antibodies wane over time in KTR and healthy cohorts alike, facilitating breakthrough infections with higher viral load (14–16). With the surge of the highly transmissive immune-escaping B.1.1.529 (omicron) variant, KTR remain at risk for COVID-19 disease. A fourth vaccine dose has been recommended recently in several countries for the elderly and immunocompromised, however, seroconversion rates in KTR with low or no antibody response after three vaccine doses after an additional fourth vaccine dose remain low and range between 42 and 50% (17–21).

Neutralizing antibodies are considered a strong predictor of protection from symptomatic COVID-19 disease (22–26). We and others showed that lower anti-spike antibodies in KTR are concomitant with lower or even absent neutralization of variants of concern such as the B.1.617.2 (delta) or B.1.1.529 (omicron) variant (13, 27–29). Therefore, seropositivity in commercially available assays testing for antibodies to the wild-type spike antigen may result in an overestimation of actual protection against viral variants (13, 22, 27, 30).

To enhance vaccination responsiveness and to better protect KTR from COVID-19 disease, adaptive immunization strategies for KTR are urgently needed. One attempt to enhance seroconversion in KTR is through modulation of immunosuppression as especially patients treated with mycophenolic acid (MPA) have shown significantly impaired seroconversion rates when compared to KTR with other immunosuppressive maintenance regimens (31–33).

In this study, we aimed to determine the effect of an additional full elasomeran dose (100 μg), formerly known as mRNA-1273, in non-responder KTR with at least 3 previous vaccine doses of any COVID-19 vaccine. In KTR with triple immunosuppressive therapy including a calcineurin inhibitor (CNI), MPA and corticosteroids (CS), MPA was withdrawn in those with stable graft function and no prior rejection in the past 12 months to investigate the efficacy of short-term MPA withdrawal on COVID-19 vaccine immunogenicity.

Materials and methods

Study design

We enrolled 76 KTR with an anti-spike S1 IgG antibody index $\leq$ 10 after at least three COVID-19 vaccinations to participate in this prospective observational cohort study between January and February 2022 at the Department of Nephrology, University of Heidelberg, Germany. The cut-off $>$ 10 was identified as we previously showed that an anti-spike S1 IgG antibody index $>$ 10 significantly correlated with the presence of wild-type SARS-CoV-2 neutralizing antibodies (13, 14). An additional mRNA-1273 vaccine dose (full dose, 100 μg) was administered to the identified patients. Serum for analysis of humoral responses to vaccination was drawn immediately before and with a median (IQR) of 27 (27–30) days after vaccination. Patients with a history of prior SARS-CoV-2 infection and/or detectable anti-nucleocapsid antibodies were excluded from the study. Further, we excluded 7 patients with PCR-confirmed breakthrough infections during follow-up from analysis (**Figure 1**).

Patients were stratified according to current immunosuppressive maintenance therapy. Summarized, short-term withdrawal of MPA was discussed in patients with a triple immunosuppressive maintenance therapy (CNI, MPA, and CS) in case graft function was stable (defined as S-creatinine $\leq$ 2.5 mg/dl and proteinuria $\leq$ 2 g/l) and no graft rejection the past 12 months, an anti-spike S1 IgG antibody index $\leq$ 10 after at least three COVID-19 vaccinations and no prior SARS-CoV-2 infection. Decision on short-term withdrawal of MPA was based on shared decision-making after

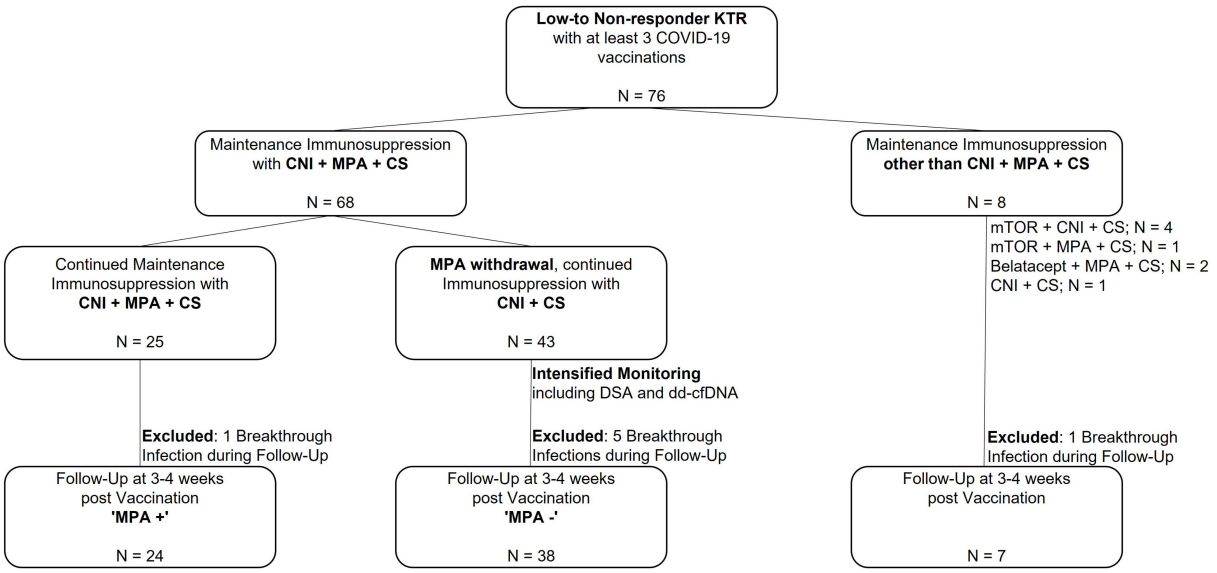

FIGURE 1
Study flow chart to assess humoral responses to an additional COVID-19 vaccination in 76 non-responder kidney transplant recipients after an additional mRNA-1273 vaccine dose. A total of 76 kidney transplant recipients (KTR) with different immunosuppressive regimens with no seroconversion after at least 3 COVID-19 vaccine doses were included in this trial. Short-term withdrawal of mycophenolic acid (MPA) during vaccination was evaluated in 68 KTR with maintenance immunosuppression consisting of a calcineurin inhibitor (CNI), MPA and corticosteroids (CS). In 25 KTR, triple immunosuppressive therapy was maintained ("MPA + "), whereas MPA was paused in 43 KTR ("MPA-"). These 43 KTR received intensified monitoring including testing for donor-specific HLA antibodies (DSA) and donor-derived cell-free DNA (dd-cfDNA) prior to and after withdrawal of MPA. In addition, humoral response was assessed in 8 KTR with immunosuppressive maintenance therapy other than CNI, MPA and CS. Breakthrough infections during the 4 weeks post vaccination surveillance period occurred in all three groups with 1 breakthrough infection in the group where maintenance immunosuppression with CNI, MPA and CS was maintained, 5 breakthrough infections in the group where MPA was withdrawn temporarily, and 1 breakthrough infection in the group with maintenance immunosuppression other than CNI, MPA and CS. Thus, follow-up for humoral response was reduced to 69 KTR. CNI, calcineurin inhibitor; CS, corticosteroids; KTR, kidney transplant recipients; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; N, number.

detailed information of the patient and performed according to our department's standard operating procedure for MPA withdrawal upon infection/vaccination. In 43 patients, MPA was consecutively withdrawn 5-7 days prior to vaccination and remained paused for additional 4 weeks after vaccination (**Figure 1**). Donor-specific anti-HLA antibodies (DSA) and donor-derived cell-free DNA (dd-cfDNA) were determined in addition to routine transplant laboratory prior to MPA withdrawal and at follow-up. The formation of *de novo* DSA was evaluated including prior DSA testing available from post-transplant routine laboratory.

Humoral response to COVID-19 vaccination was assessed by determination of anti-spike S1 IgG, surrogate neutralizing, and anti-receptor-binding domain (anti-RBD) antibodies. In addition, IgG antibodies targeting the SARS-CoV-2 full spike, the spike S1 and S2 subunits, and the nucleocapsid protein were measured. Neutralization of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants of concern was determined in all KTR after additional COVID-19 vaccination using a live-virus assay.

The study was approved by the ethics committee of the University of Heidelberg and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants. The study is registered at the German Clinical Trial Register (DRKS00024668).

Assessment of humoral responses after COVID-19 vaccination with three commercially available tests

Anti-Spike S1 IgG and anti-nucleocapsid antibodies were determined by using the SARS-CoV-2 Total Assay (Siemens, Eschborn, Germany) and the Elecsys anti-SARS-CoV-2 assay (Roche, Mannheim, Germany), respectively. The anti-SARS-CoV-2 IgG spike assay was calibrated with two different calibrators containing low and high concentrations of the spike protein. After calibration, the system calculated an index as cut-off, values < 1.0 were reported as negative and values ≥ 1.0 were reported as positive according to the manufacturer's instructions. An index value of 1 corresponds to 21.8 binding antibody units (BAU) per milliliter according to the World Health Organization's international standard for anti-SARS-CoV-2 immunoglobulin (34, 35). Surrogate neutralizing

antibodies were measured using a surrogate virus neutralization assay (Medac, Wedel, Germany). The assay mimics the virus-host interaction by direct protein-protein interaction using purified RBD from the viral spike and the ACE-2 host cell receptor (36). IgG antibodies against the SARS-CoV-2 full spike, the spike S1 and spike S2 subunits, and the RBD protein were detected using a bead-based multiplex assay for the Luminex platform (LabScreen Covid Plus, One Lambda, Inc., West Hill, CA, United States). This assay further determines IgG antibody reactivity against the spike S1 of four common cold coronaviruses (HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43) (37). All assays have been described in previous works and were performed according to the manufacturer's instructions (38–41).

Live-virus neutralization against the B.1.617.2 (delta), and the B.1.1.529 (omicron) variant

Neutralization titers were determined in twofold serial dilution experiments using VeroE6 cells, as described previously (13, 14, 27, 42–47). Virus stocks were produced by isolation and amplification of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variant from nasopharyngeal and oropharyngeal swabs of PCR-confirmed SARS-CoV-2 positive patients (27, 48). B.1.617.2 (delta) variant was amplified in VeroE6 cells. Stocks of B.1.1.529 (omicron) were produced in Calu-3 cells to avoid rapid cell culture adaptation. Virus titers of stocks were determined by plaque assay and Tissue Culture Infectious Dose (TCID) 50 assay in VeroE6 cells. To validate virus stocks, genome sequencing was performed. For the neutralization assays, twofold serial dilutions of vaccine sera were incubated with 6 × 104 TCID 50 of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variant. Virus replication was determined by immunostaining for the viral nucleocapsid protein using an in-cell ELISA. Data were normalized to a no-serum (100%) and a mock-infected (0%) control. The serum dilution that results in 50% reduction of normalized signal gives the inhibitory dilution 50 (ID_{50}).

Determination of donor-specific anti-HLA antibodies (DSA)

In all patients in whom MPA was paused prior to vaccination, we screened for the development of *de novo* DSA or an increase of previously detected DSA. DSA of IgG isotype against mismatched donor HLA were determined by Luminex technology using the LABScreen Single Antigen kit of One Lambda, Inc. (West Hill, CA, United States). DSA with MFI ≥ 500 were considered positive as the incidence of graft loss has shown to be higher in patients with *de novo* DSA or non-DSA at an MFI ≥ 500 (49).

Quantification of donor-derived cell-free DNA (dd-cfDNA)

dd-cfDNA constitutes a marker of graft injury and has been shown to significantly discriminate biopsy-confirmed rejection from no-rejection (50–53). Venous blood was drawn into 10 mL cell-free DNA BCT tubes (Streck, Omaha, NE, United States) and processed within 7 days. cfDNA was extracted using the Circulating Nucleic Acid kit (Qiagen, Redwood City, CA, United States) and amplified using the AlloSeq cfDNA assay (CareDx, Brisbane, CA, United States), a single multiplex PCR including index adapters and PCR primers for 202 single nucleotide polymorphisms (SNPs). Differences in SNPs loci are used to compute the amount of dd-cfDNA relative to the total amount of cfDNA from a sample. PCR products were sequenced on a MiSeq system (Illumina, San Diego, CA, United States). Data was analyzed using the AlloSeq cfDNA software (CareDx) which reports the percentage of donor-derived cfDNA. All steps were performed according to the manufacturers' instructions. dd-cfDNA was measured in 40 patients before and in all 43 patients at 4 weeks follow-up after withdrawal of MPA.

Reactogenicity

Reactogenicity after additional COVID-19 vaccination was assessed in all 76 KTR using a 12-item questionnaire to inquire about any adverse events following vaccination as described previously (**Supplementary Methods**) (38, 39, 46).

Statistics

Data are given as median and interquartile range (IQR) or number (N) and percent (%). For continuous variables, the Mann–Whitney *U* test and the Wilcoxon matched-pairs rank test were applied for unpaired or paired variables, respectively. Fisher's exact test was applied for categorial variables. A multiple linear regression analysis was performed to differentiate predictors of maximum anti-S1 IgG antibody levels in KTR with immunosuppressive maintenance therapy consisting of CNI, MPA, and CS. To describe the correlation of different commercially available assays to neutralization titers obtained by live-virus neutralization assays, we calculated Spearman's rho as a non-parametric measure of rank correlation. Statistical analysis was performed using GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, CA, United States) and statistical significance was assumed at a P-value < 0.05.

Results

Study population

We prospectively enrolled 76 KTR with no seroresponse after at least three prior COVID-19 vaccinations before administration of an additional dose with mRNA-1273 (100 μg). Five patients (7%) had four vaccinations, 71 patients (93%) 3 vaccinations prior to inclusion into the study. Median (IQR) age was 57 (47–63) years and 29/76 (38%) participants were females. Baseline characteristics including transplant-related data, cause of nephropathy and comorbidities are given in Table 1.

Humoral immune responses in kidney transplant recipients as determined by commercially available assays

After vaccination, 24/69 (35%) KTR showed seroconversion with anti-spike S1 IgG antibodies above the predefined cut-off. Anti-spike S1 IgG index, % inhibition for surrogate neutralizing antibodies, and MFI for anti-RBD antibodies before vaccination increased from a median (IQR) of 0.12 (0.10–0.98) to 1.92 (0.10–47.18), from 21.6 (13.7–29.8) to 35.7 (15.4–93.2), and from 272 (0–4876) to 8,009 (206–18,149) after vaccination, respectively ($P < 0.001$ for all, Figure 2). When comparing KTR with immunosuppressive maintenance therapy consisting of CNI, MPA, and CS ($N = 62$) and stratifying for MPA withdrawal, 18/38 (47%) KTR in whom MPA was withdrawn showed seroconversion compared to 3/24 (13%) with continued immunosuppressive maintenance therapy including MPA ($P = 0.006$). Anti-S1 IgG index after vaccination was with a median (IQR) of 4.30 (0.22–78.8) significantly higher in patients with prior MPA withdrawal compared to the 0.20 (0.10–3.94) in those without MPA withdrawal ($P = 0.006$, Figure 2). Correspondingly, surrogate neutralizing and anti-RBD antibodies were significantly higher in patients where MPA was withdrawn compared to those without MPA withdrawal ($P = 0.002$ and $P < 0.001$, respectively, Figure 2). Patients with breakthrough infections ($N = 7$) were excluded from the analysis.

In a multiplex bead-based assay, we determined antibodies targeting different areas of the spike protein (full spike, spike S1, spike S2) and antibodies targeting the nucleocapsid protein. In all KTR, spike-specific antibodies increased from 1,200 (0–9,558) to 15,921 (1,179–21,411), from 362 (0–3,547) to 4,948 (100–14,873), and from 0 (0–630) to 1,362 (0–6,126) for the full spike, the spike S1 and the spike S2 after additional vaccination, respectively ($P < 0.001$ for all, Figure 3). No significant differences in antibodies against the nucleocapsid protein were seen before and after vaccination ($P = 0.46$, Figure 3). When again stratifying results for patients where MPA was withdrawn prior to vaccination, antibodies against the full spike, the spike S1 and spike S2 subunits after additional vaccination were significantly higher in these patients compared to those who remained on maintenance therapy with MPA ($P < 0.001$ for antibodies against the full spike and the spike S1, $P = 0.003$ for antibodies against the spike S2, Figure 3). No significant differences were seen in antibodies against the

TABLE 1 Baseline characteristics.

All study participants, N	76
Age at enrollment (years), median (IQR)	57 (47–63)
Sex (female), N (%)	29 (38)
BMI (kg/m^2), median (IQR)	24.8 (21.9–28.8)
Vaccination related data[1]	
Homologous mRNA vaccination, N (%)	51 (67)
Heterologous mRNA vaccination, N (%)	13 (17)
Heterologous vaccination including a viral vector	12 (16)
vaccine, N (%)	5 (7)
More than three previous vaccine doses	
Transplant-related data	
First transplant, N (%)	68 (89)
Time since transplantation (years), median (IQR)	4.7 (2.2–9.8)
Rejection during the past 12 months, N (%)	2 (3)
S-Creatinine prior to Vaccination (mg/dl)	1.5 (1.3–1.8)
S-Creatinine after Vaccination (mg/dl)	1.4 (1.2–1.7)
Immunosuppressive maintenance therapy	
CNI + MPA + CS, N (%)	68 (89)
Tacrolimus vs. Cyclosporine A, N (%)	51 (75) vs. 17 (25)
mTOR + CNI + CS, N (%)	4 (5)
mTOR + MPA + CS, N (%)	1 (1)
Belatacept + MPA + CS, N (%)	2 (3)
CNI + CS	1 (1)
Cause of end-stage kidney disease	
Vascular, N (%)	4 (5)
Diabetes, N (%)	7 (9)
Glomerular disease, N (%)	31 (41)
PKD, N (%)	15 (20)
Systemic, N (%)	2 (3)
Reflux/chronic pyelonephritis	6 (8)
Other/Unknown, N (%)	11 (14)
Comorbidities	
Arterial Hypertension, N (%)	57 (75)
Diabetes, N (%)	11 (14)
CAD, N (%)	18 (24)
Chronic lung disease, N (%)	11 (14)
Chronic liver disease, N (%)	5 (7)
Malignancy, N (%)	18 (24)

BMI, body-mass index; CAD, coronary artery disease; CNI, calcineurin inhibitor; CS, corticosteroids; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; N, number; PKD, polycystic kidney disease.

[1]Homologous mRNA vaccination: 49 KTR received three doses with BNT162b2; 2 KTR received four doses with BNT162b2; Heterologous mRNA vaccination: 5 KTR received two doses with BNT162b2 followed by one dose of mRNA-1273; 6 KTR received two doses of mRNA-1273 followed by one dose of BNT162b2; 2 received three doses of BNT162b2 followed by one dose of mRNA-1273; Heterologous vaccination including a viral vector vaccine: 7 received two doses of ChAdOx1 followed by one dose with BNT162b2; 1 received two doses of ChAdOx1 followed by one dose of mRNA-1273; 2 received one dose of ChAdOx1 followed by two doses of BNT162b2; 1 received one dose of ChAdOx1 followed by one dose of mRNA-1273 and one dose of BNT162b2; 1 received three doses with BNT162b followed by one dose of Janssen COVID-19 vaccine.

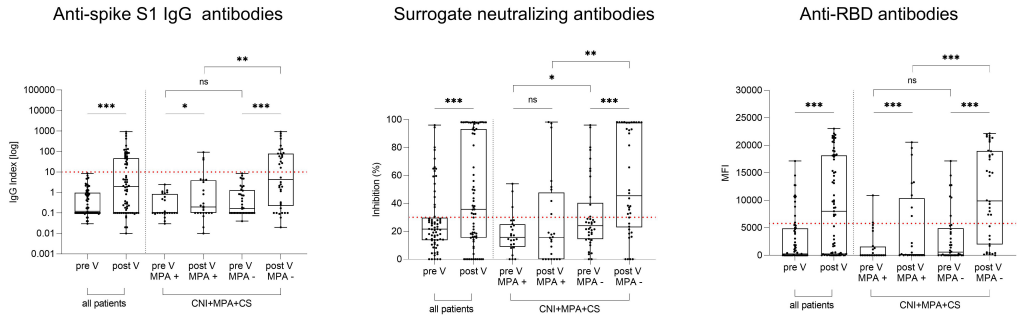

FIGURE 2
Anti-spike S1 IgG, surrogate neutralizing, and anti-receptor-binding domain antibodies in 69 kidney transplant recipients before and after an additional mRNA-1273 vaccine dose. Anti-spike S1 IgG (left panel), surrogate neutralizing (middle panel) and anti-RBD (right panel) antibodies in 69 KTR before and after additional COVID-19 vaccination. Results were stratified for 62 patients with triple immunosuppressive therapy consisting of a calcineurin inhibitor (CNI), mycophenolic acid (MPA), and corticosteroids (CS) according to temporary MPA withdrawal during vaccination (MPA + vs MPA -). KTR with breakthrough infections (N = 9) were excluded from all analyses. The dashed red line indicates the cut-off for detection of antibodies for each assay. CNI, calcineurin inhibitor; CS, corticosteroids; MFI, mean fluorescence intensity; MPA, mycophenolic acid; V, vaccination; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ns, non-significant.

nucleocapsid protein between the two groups after vaccination (P = 0.84, Figure 3). In addition, antibodies against 4 common cold coronaviruses were determined by this multiplex assay. We did not detect any significant differences in antibodies against the spike S1 of the HCoV-229E, the HCoV-HKU1, the HCoV-NL63, and the HCoV-OC43 before and after vaccination in all KTR and when stratified according to MPA withdrawal (Supplementary Figure 1).

In patients with maintenance immunosuppressive therapy consisting of CNI, MPA, and CS, a multiple linear regression analysis, including age, gender, time since transplantation, S-creatinine levels at time of vaccination, and MPA withdrawal upon vaccination was performed to identify possible confounders of maximum anti-S1 IgG levels (Supplementary Table 1). Besides MPA withdrawal (β: 100.7; 95% CI: 10.7; 190.7; P = 0.03), no other parameter examined was associated with higher anti-S1 IgG antibody concentrations (Supplementary Table 1). With the exception of a greater incidence of end-stage kidney disease caused by diabetes, no significant differences in baseline characteristics were detected when comparing KTR in whom MPA was paused during vaccination to those who remained on triple immunosuppressive maintenance therapy including MPA (Supplementary Table 2). KTR that underwent MPA withdrawal and did not seroconvert successfully were transplanted more recently than KTR with MPA withdrawal that showed seroconversion (P = 0.04; Supplementary Table 3).

Neutralizing antibody response against the B.1.617.2 (delta) and B.1.1.529 (omicron) variants

Neutralization of the SARS-CoV-2 delta and omicron variants was determined with all KTR serum samples taken after vaccination using a live-virus assay. Neutralization titers were above 1:10 in 33/69 (48%) KTR for the delta variant, and in 13/69 (19%) KTR for the omicron variant. Neutralizing antibody titers for the delta variant were a median (IQR) ID_{50} of 0 (0–1:80) and significantly higher compared to the median (IQR) ID_{50} of 0 (0–0) for the omicron variant ($P < 0.001$, Figure 4A). When comparing patients where MPA was withdrawn prior to vaccination to those who remained on immunosuppressive maintenance therapy including MPA, the former exhibited significantly higher neutralization titers against both, the delta and omicron variant (P = 0.04 for delta and P = 0.02 for omicron, Figure 4B). A higher anti-S1 IgG antibody index correlated with higher neutralization titers of the delta and omicron variants (Figure 4C).

Monitoring of patients with mycophenolic acid withdrawal

S-creatinine and proteinuria remained stable in KTR in whom MPA was withdrawn during vaccination with a median (IQR) S-creatinine of 1.4 mg/dl (1.3–1.8) and proteinuria of 18.7 g/molCrea (10.6–28.7) before vaccination compared to 1.4 mg/dl (1.3–1.6) and 19.6 g/molCrea (11.9–34.9) 4 weeks after vaccination, respectively (P = 0.5 and P = 0.13).

In addition, donor-specific anti-HLA antibodies (DSA) and donor-derived cell-free DNA were determined in all 43 patients with MPA withdrawal prior to and 4 weeks after withdrawal (Supplementary Table 4). In 29 patients, we did not detect any formation of *de novo* DSA. Two patients showed decreasing DSA reactivities below the cut-off of ≤ 500 during study period from a maximum MFI of 872 to 109 (DPB1*04:01) and 540–340 (C*07:02). In 7 patients we detected an increase of the MFI values of present DSA or a resurgence of previously detected

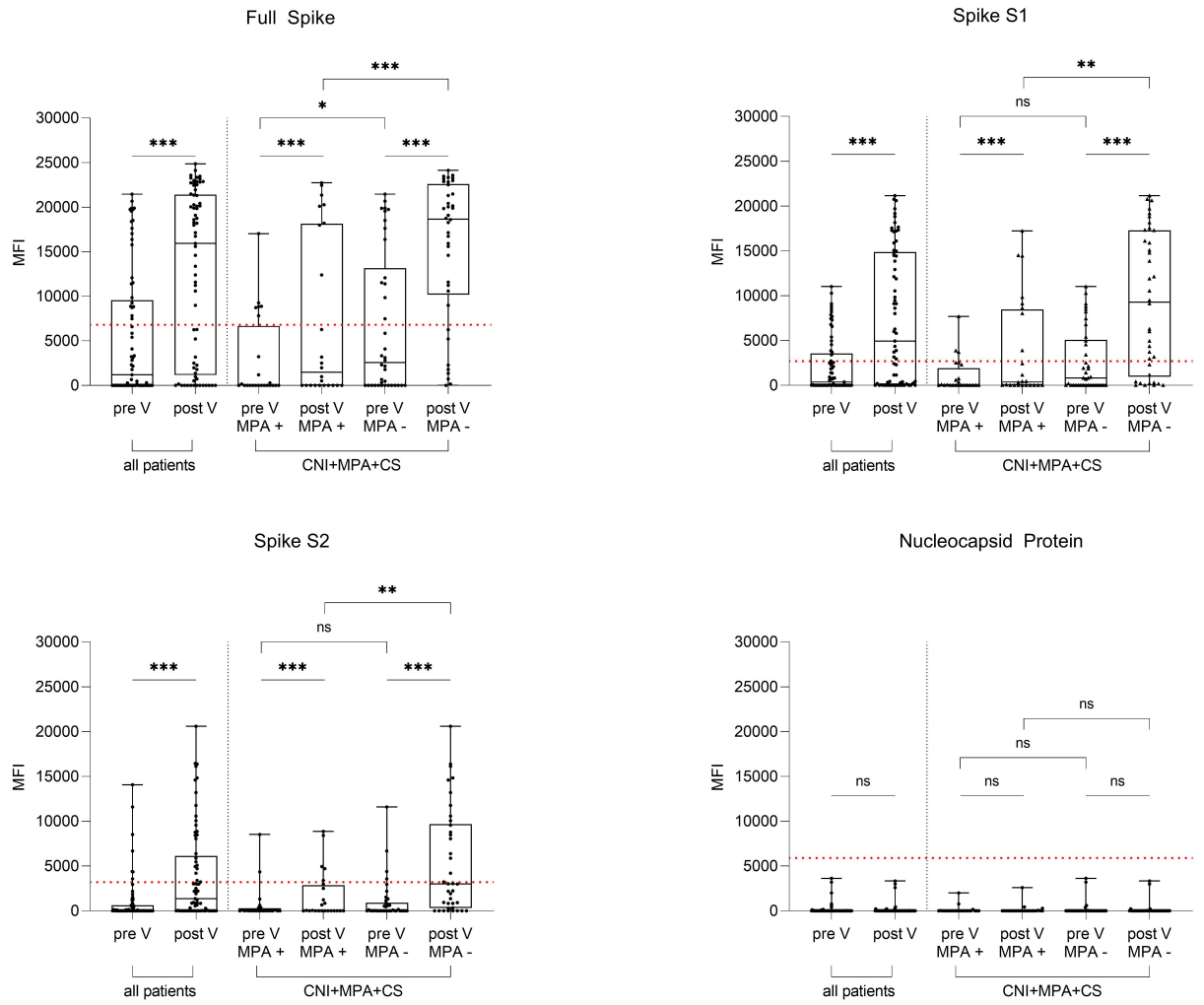

FIGURE 3
IgG antibodies against the full spike, the spike S1 and S2 subunits and the nucleocapsid protein in 69 kidney transplant recipients before and after an additional mRNA-1273 vaccine dose. IgG antibodies targeting the SARS-CoV-2 full spike (upper left panel), the spike S1 (upper right panel) and S2 subunits (lower left panel), and the nucleocapsid protein (lower right panel) were determined in 69 kidney transplant recipients (KTR) before and after additional vaccination using a multiplex bead-based assay. Results were stratified for 62 patients with triple immunosuppressive therapy consisting of a calcineurin inhibitor (CNI), mycophenolic acid (MPA), and corticosteroids (CS) according to temporary MPA withdrawal during vaccination (MPA + vs MPA -). KTR with breakthrough infections ($N = 9$) were excluded from all analyses. The dashed red line indicates the cut-off for each respective target. CNI, calcineurin inhibitor; CS, corticosteroids; KTR, kidney transplant recipients; MFI; mean fluorescence intensity; MPA, mycophenolic acid; V, vaccination; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, non-significant.

DSA during MPA withdrawal. DSA could not be evaluated in two patients due to unavailable donor DNA for HLA typing and in three patients due to lacking of Luminex beads with the relevant HLA specificities. dd-cfDNA levels remained stable in all study participants with a median% of 0.14 (0.10–0.19) before and 0.14 (0.11–0.22) after MPA withdrawal ($P = 0.11$). In 42/43 (98%) KTR, dd-cfDNA remained below 0.5%, a cut-off that is strongly associated with likely risk for allograft injury (50, 54). dd-cfDNA only increased slightly in one patient from 0.51% to 0.65% without any corresponding changes in S-creatinine, proteinuria, or DSA levels (patient 32, **Supplementary Table 4**).

Reactogenicity

Vaccination was overall well-tolerated in all KTR. Any side effect was reported by 43/76 (57%) KTR with local reactions being the most frequent reported in 35/76 (46%, **Supplementary Figure 2**). Side effects were distributed evenly in patients where MPA was withdrawn compared to those with continued maintenance immunosuppression including MPA with slightly more patients reporting use of medication in the group where MPA was withdrawn (**Supplementary Figure 2**).

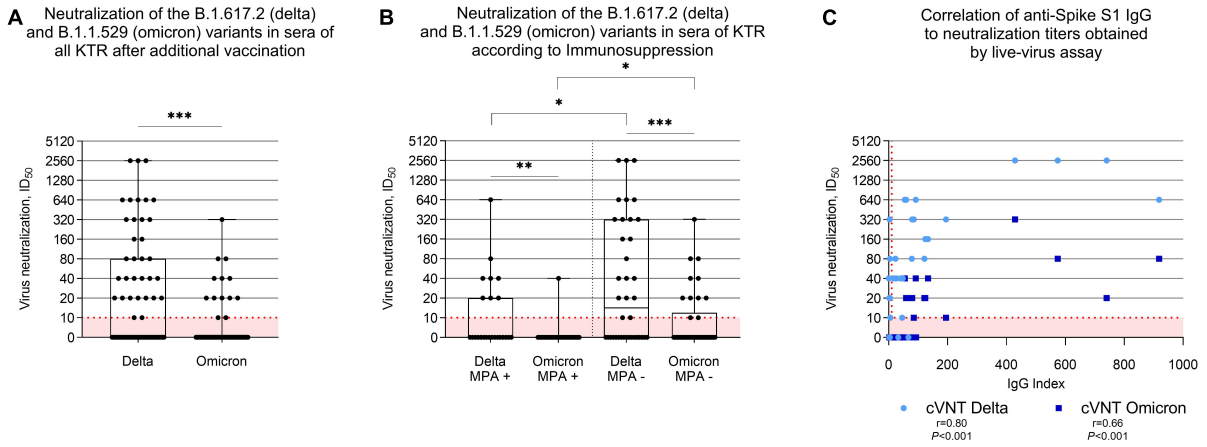

FIGURE 4
Neutralization of the SARS-CoV-2 B.1.617.2 (delta) and the B.1.1.529 (omicron) variants by antibodies in sera of 69 kidney transplant recipients after an additional mRNA-1273 vaccine dose. **(A)** Vaccine-induced cross-neutralization of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants by antibodies in sera of 69 kidney transplant recipients (KTR) after an additional mRNA-1273 vaccine dose as determined by using a live-virus assay. The dashed red line indicates the cut-off for detection which is the 1:10 dilution in this assay. **(B)** Cross-neutralization of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants by antibodies in sera of 62 KTR with maintenance immunosuppressive therapy consisting of a calcineurin inhibitor (CNI), mycophenolic acid (MPA), and corticosteroids (CS) stratified according to temporary MPA withdrawal during additional vaccination. **(C)** Correlation analysis of anti-S1 IgG results obtained by a commercially available assay with cross-neutralization titers of the B.1.617.2 (delta) and the B.1.1.529 (omicron) variants by sera of kidney transplant recipients taken after an additional mRNA-1273 vaccine dose. KTR with breakthrough infections ($N = 9$) were excluded from the analyses. cVNT, conventional virus neutralization test; ID_{50}; inhibitory dilution 50; KTR, kidney transplant recipients; MPA, mycophenolic acid; r; Spearman's rho; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

Discussion

In this study we found that a temporary halt of MPA prior to an additional COVID-19 vaccine booster enhanced seroconversion rates and lead to higher antibody levels for those KTR who had no prior seroresponse after at least three COVID-19 vaccinations. The reactogenicity profile was acceptable and showed mostly the typical expected local adverse events. After vaccination, 24/69 (35%) KTR showed seroconversion with anti-spike S1 IgG antibodies above the predefined cut-off. Correspondingly, KTR with higher anti-spike S1 IgG antibody levels exhibited higher levels of neutralizing antibodies targeting the B.1.617.2 (delta) and B.1.1.529 (omicron) variant. The 35% seroconversion rate we found is lower compared to other studies examining the effect of a fourth vaccine dose in KTR with seroconversion rates ranging between 42 and 50% (17–21). Lower seroconversion rates in our study cohort may be attributed to including only previous non-responder KTR in our trial. Of note, 18/38 (47%) KTR in whom MPA was paused surpassed the cut-off and showed significantly higher anti-spike S1 IgG antibodies compared to those who remained on triple immunosuppressive therapy. MPA withdrawal remained an independent variable associated with higher anti-spike S1 IgG antibodies when stratifying for age, gender, time since transplantation and S-Creatinine levels. In KTR with MPA withdrawal during vaccination we did not find any significant changes in S-creatinine, proteinuria or dd-cfDNA, indicative of no acute rejection (50–54). Although these findings may indicate immune quiescence, we detected resurgence in pre-existing DSA in 7 patients and the development of *de novo* DSA in one patient in whom MPA was withdrawn.

A few studies have examined the effect of MPA withdrawal to enhance vaccination responsiveness in small cohorts (55, 56). After showing a dose-dependent effect of MPA on antibody levels after two COVID-19 vaccinations, Kantauskaite et al. examined the effect of temporary MPA dose reduction by 25–50% in 24 KTR receiving a third mRNA vaccination matched to 24 KTR without changes in immunosuppressive maintenance therapy (31, 55). The authors found significantly higher antibody levels in patients with MPA reduction 3 weeks prior until 1 week after third vaccination, however, patients were not followed-up on graft function or development of DSA (55). Schrezenmeier et al. applied a fourth mRNA vaccine dose to 29 KTR during temporary halt of MPA and observed seroconversion in 76% of patients (56). Although the authors did not compare seroconversion rates to patients who remained on triple immunosuppressive therapy, their results are much in line with what we present in our current study. Higher seroconversion rates in their study cohort may apply to the fact that 52% in their study cohort received a heterologous vaccination protocol and median time since transplantation with 9.9 years (± SD 5.9) was longer than for our study cohort, both factors that have shown to influence seroconversion rates (57). Those KTR that failed to seroconvert despite MPA withdrawal in our study cohort were transplanted more recently compared to those that seroconverted successfully. This is

consistent with present literature arguing that progressive dose reduction of immunosuppression with longer time since transplantation influences vaccine responsiveness in KTR (13, 33, 57, 58). Notably, Schrezenmeier et al. also followed-up on graft function, development of DSA and changes in dd-cfDNA and did not detect any differences when comparing pre-MPA withdrawal levels to post-MPA withdrawal levels (56). The resurgence of DSA in 7 of our patients may be attributed to the fact that we applied a lower cut-off (MFI $\leq$ 500), nevertheless we also detected HLA antibodies with MFI $\geq$ 1000 in 3 patients which is the cut-off Schrezenmeier et al. applied (56). Although no patient had a biopsy-confirmed rejection during study period, we think that MPA withdrawal in future trials may thus only be considered in patients without any prior DSA or current DSA to enhance safety.

Several studies showed reduced vaccine-elicited neutralization against omicron compared to SARS-CoV-2 wild-type even in healthy cohorts (59–62). Kumar et al. recently reported in a study cohort of 60 solid organ transplant recipients that only 55.0% and 18.3% of patients exhibited neutralizing antibody activity against delta and omicron 1 month after a third mRNA vaccine dose, respectively (28). In addition, first real-world data indicate a significantly reduced three-dose vaccine efficacy (95% CI) against infection with delta or omicron of 70.6% (31–87.5%) and 29.4% (0.3–50.0%) in immunocompromised individuals compared to 93.7% (92.2–94.9%) and 71.6% (69.7–73.4%) in the general population, respectively (63). Benotmane et al. recently showed in a cohort of 67 KTR with weak humoral responses after a third vaccine dose that 66% of patients were able to mount neutralizing antibodies against the delta variant after a fourth vaccine dose (21). Our results show even lower percentage of patients exhibiting neutralizing antibody activity against delta (48%) and omicron (19%) which again may be due to a selection bias only including non-responder KTR. After an additional, in most instances fourth mRNA vaccine dose in our study cohort of previous non-responder KTR, the 45/69 (65%) of patients that remained anti-spike S1 IgG seronegative and the concomitant reduced neutralization against the B.1.1.529 (omicron) variant remains distressing. This is in concordance with recently published results by Karaba et al. who reported that neutralization against the omicron variant did not increase significantly after additional vaccination in a cohort of 25 solid organ transplant recipients (SOTRs) with low seroresponse after three vaccinations, leaving SOTRs at high risk for omicron infection (64).

For KTR that fail to seroconvert even after adapted immunization protocols, pre-exposure prophylaxis with monoclonal antibodies remains an option although recent data suggests resistance of the newly surging BA.2 omicron sublineage to most available monoclonal antibodies (61, 65). The combination of Cilgavimab/Tixagevimab (Evusheld) has shown to retain partial neutralizing activity against the omicron variant *in vitro* and al Jurdi et al. recently demonstrated that SOTRs that received a pre-exposure prophylaxis with Evusheld at increased dosing of 300 mg of each antibody had significantly fewer breakthrough infections with omicron compared to SOTRs without pre-exposure prophylaxis and SOTRs that received the initially recommended dose of 150 mg of each antibody (66–68). As the COVID-19 pandemic continues to evolve and new and challenging variants of concern arise, the development of other safe and effective monoclonal antibodies that retain neutralization against the current SARS-CoV-2 variants remains a key aspect to safely protect immunocompromised patients who remain seronegative even after adapted immunization protocols.

There are several limitations to our study: this was a non-randomized single-center trial including 76 KTR with no vaccine response after at least three COVID-19 vaccinations. Larger, randomized multi-center trials and longer follow-up periods are needed to validate our results and evaluate clinical relevance and outcomes of MPA withdrawal before adapting vaccination protocols. Another limitation of our study is the lack of data on cellular immunity. Although neutralizing antibodies are seen as highly predictive of protection from symptomatic SARS-CoV-2 infection, our data do not fully reflect the immune response following COVID-19 vaccination (22). Further, although an increase in reactivities of DSA in some patients of our trial occurred during MPA withdrawal, we cannot eliminate the possibility that vaccination itself may have led to an alloimmune response. In this study, we aimed to investigate to what extent a reduction of immunosuppression (MPA withdrawal) is associated with an improved vaccination response without being associated with adverse events. Therefore, DSA and dd-cfDNA as early indicators of rejection were only measured in patients in whom MPA was withdrawn. In addition, serum MPA levels were not measured in either group to assess patient adherence, which could confound the results.

In conclusion, our data show a significant improvement in humoral immune response after an additional vaccine dose in previous non-responder KTR with at least three vaccine doses. Higher anti-S1 IgG antibody levels were associated with better neutralization of the B.1.617.2 (delta) and B.1.1.529 (omicron) variants. The effect was most pronounced in KTR where MPA was withdrawn 5–7 days prior to vaccination and remained paused for additional 4 weeks. Thus, MPA withdrawal or dose reduction seem reasonable approaches to enhance seroconversion rates. For safety reasons, this may be applied in patients without current or previous DSA.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the University of Heidelberg. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LB and CSp analyzed and interpreted the data and drafted the manuscript. LB, TK, JB, MBu, CN, FK, MR, MT, MS, KK, and CSp collected and managed the data. LB, PS, CSü, TT, and CSp performed experiments on humoral response. MBa, HK, and RB performed experiments on live virus neutralization. CM, AB, PS, MZ, CSü, RB, and TT supervised the project and revised the manuscript. All authors critically reviewed the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.958293/full#supplementary-material

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Kidney health in the COVID-19 pandemic: An umbrella review of meta-analyses and systematic reviews

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Background: This umbrella review aims to consolidate evidence from systematic reviews and meta-analyses investigating the impact of the coronavirus disease—2019 (COVID-19) on kidney health, and the associations between kidney diseases and clinical outcomes in COVID-19 patients.

Methods: Five databases, namely, EMBASE, PubMed, Web of Science, the Cochrane Database of Systematic Reviews and Ovid Medline, were searched for meta-analyses and systematic reviews from January 1, 2020 to June 2, 2022. Two reviewers independently selected reviews, identified reviews for inclusion and extracted data. Disagreements were resolved by group discussions. Two reviewers independently assessed the methodological quality of all included reviews using ROBIS tool. A narrative synthesis was conducted. The characteristics and major findings of the included reviews are presented using tables and forest plots. The included meta-analyses were updated when necessary. The review protocol was prospectively registered in PROSPERO (CRD42021266300).

Results: A total of 103 reviews were identified. Using ROBIS, 30 reviews were rated as low risk of bias. Data from these 30 reviews were included in the narrative synthesis. Ten meta-analyses were updated by incorporating 119 newly available cohort studies. Hospitalized COVID-19 patients had a notable acute kidney injury (AKI) incidence of 27.17%. AKI was significantly associated with mortality (pooled OR: 5.24) and severe conditions in COVID-19 patients (OR: 14.94). The pooled prevalence of CKD in COVID-19 patients was 5.7%. Pre-existing CKD was associated with a higher risk of death (pooled OR: 2.21) and disease severity (pooled OR: 1.87). Kidney transplant recipients were susceptible to SARS-CoV-2 infection (incidence: 23 per 10,000 person-weeks) with a pooled mortality of 18%.

Conclusion: Kidney disease such as CKD or recipients of kidney transplants were at increased risk of contracting COVID-19. Persons with COVID-19 also

had a notable AKI incidence. AKI, the need for RRT, pre-existing CKD and a history of kidney transplantation are associated with adverse outcomes in COVID-19.

Systematic review registration:
www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021266300, identifier: CRD42021266300.

KEYWORDS

COVID-19, acute kidney injury, kidney transplant, renal replacement therapy, chronic kidney disease

Introduction

The coronavirus disease—2019 (COVID-19) pandemic has caused huge challenges in healthcare globally. According to the World Health Organization (WHO), as of June 6, 2022, more than 529 million patients had been diagnosed worldwide with over 6 million COVID-19 related deaths (1). The broad clinical spectrum of COVID-19 ranges from an asymptomatic response to mild upper respiratory tract infection to critical illness with acute respiratory distress syndrome (2, 3).

Although respiratory symptoms are the dominant feature, accumulative evidence suggests that acute kidney injury (AKI) is prevalent among patients with COVID-19, particularly among critically ill patients (4–6). The presence of AKI in COVID-19 patients, particularly those with severe disease, is associated with a poor prognosis (7). A large prospective cohort study of 20,133 hospitalized COVID-19 patients noted that the mortality risk was 1.28-fold higher among CKD patients as compared to non-CKD patients (8). Recently, a rapidly growing evidence base has suggested that the presence of AKI, CKD, and other kidney impairments were associated with the poor prognosis of COVID-19 patients (1, 9–13).

Despite our understanding of COVID-19 and kidney diseases, the considerable number of studies has inevitably resulted in substantial heterogeneity in study designs and variability of risk estimates and occasionally conflicting data. When focusing on large number of meta-analyses and systematic reviews published, the highest level of evidence, the variety of evidence quality and the duplication of patient data are problematic and might hinder the identification and application of evidence-based strategies in medical practice.

Given the paucity of current knowledge, the purpose of this umbrella review of meta-analyses and systematic reviews was to summarize and consolidate evidence addressing the following two research questions: (1) what is the incidence/prevalence of AKI, CKD, and kidney transplant in COVID-19 patients? (2) what is the impact of these kidney diseases on the clinical outcomes in patients with COVID-19?

Methods

This umbrella review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (14). The review protocol was prospectively registered in the Prospective Register of Systematic Reviews (PROSPERO, CRD42021266300).

Objectives

To consolidate evidence to determine (1) the incidence/prevalence of AKI, CKD, and kidney transplant in COVID-19 patients and (2) the association between these kidney disorders and outcomes in patients with COVID-19.

Study design

Meta-analyses and/or systematic reviews assessing the associations between AKI, CKD, kidney transplant, and COVID-19 were included. The diagnosis was based on 2012 Kidney Disease: Improving Global Outcomes (KDIGO) AKI and 2012 KDIGO CKD definitions. Notably, we focused on patients with a history of CKD before being diagnosed with COVID-19, instead of patients developing CKD after COVID-19- induced AKI.

The study set no restrictions on the age, sex, and ethnicity of the participants investigated, and no restriction was applied to the original recruitment locations or settings. We limited the included reviews to those published in the English language. If multiple meta-analyses and/or systematic reviews on the same research question were identified, the most recent reviews with the largest number of studies and effect sizes were included. We also assessed the quality of the included studies, synthesized the results of the included studies,

and provided sufficient details of the characteristics of the included studies.

Search strategies

Five databases, namely, EMBASE, PubMed, Web of Science, the Cochrane Database of Systematic Reviews and Ovid Medline, were systematically searched from January 1, 2020 to June 2, 2022, to identify systematic reviews and/or meta-analyses of observational studies examining the associations of kidney health with COVID-19. Appropriate free-text terms and medical subject headings (MeSH) were used to research kidney risk factors, kidney diseases, and COVID-19. The search strategy used the following terms/keywords: ("2019-nCoV" OR "Coronavirus" OR "COVID-19" OR "SARS-CoV-2" OR "2019-nCoV" OR novel coronavirus) AND (renal or kidney or nephron*) AND (meta-analysis or systematic review).

Eligibility criteria

The different results from the databases were exported into EndNote X9, and duplicates were removed. Two reviewers (ZYL and LJ) independently completed the title and abstract screening in duplicate. The full texts of potentially eligible articles were scrutinized independently by the same two investigators (ZYL and LJ) to identify reviews for inclusion. Studies were included in this review if they met the inclusion criteria as follows: (1) they were systematic reviews or meta-analyses; and (2) they included observational studies which reported the associations between kidney diseases and COVID-19. Studies were excluded for the following reasons: (1) the study only reported the management or therapeutic strategy; (2) the study was an abstract only; or (3) the study was not published in English. Disagreements were resolved through discussion to reach a consensus.

Data extraction

Two researchers (LJ and YC) independently performed the data extraction. For each eligible review, we extracted the following information: (1) name of the first author; (2) number of included studies; (3) publication year; (4) number of total participants; (5) population inclusion criteria; (6) exposures; (7) number of exposed groups; (8) controls; (9) number of controlled groups; (10) outcomes; (11) type of effect model; (12) odds ratios, risk ratios, or hazard ratios; (13) estimates; (14) 95% confidence intervals (-CIs -); (15) I^2; heterogeneity (Q-test, P-value); and (16) publication bias (Egger's test, P-value). Disagreements were resolved by group discussion.

Quality assessment

Two reviewers (WW and LY) critically assessed the methodological quality of all included reviews using Risk of Bias in Systematic Review (ROBIS) (15). The quality of the included reviews was assessed in the following three phases: (1) relevance of the review, (2) identifying concerns within the systematic review process under the following four domains: study eligibility criteria, identification and selection of studies, data collection and study appraisal, and synthesis and findings and (3) judging risk of bias. Each included review was given a "low," "high" or "unclear" risk of bias score. Disagreements were resolved by group discussion.

Data synthesis

Eligible systematic reviews and meta-analyses formed the unit of analysis. A narrative synthesis was conducted. The characteristics and major findings of the included reviews are presented using tables and forest plots.

Update of eligible reviews

An update of an included review was necessary if meeting the following criteria: (1) The review was rated as low risk of bias using ROBIS tool; (2) there were new eligible primary studies not yet included in the existing review. If more than one reviews on the same topic were eligible, we updated the most recent review. The pooled percentages were used to meta-analyze the incidence and prevalence of outcomes. The pooled ORs with 95% CIs were used to assess the associations between exposures and clinical outcomes. A random-effects model was used to allow for heterogeneity. $P < 0.05$ was considered statistically significant. The statistical analyses were conducted in Stata, version 16.0 (Stata Corp).

Results

Literature search

Overall, the searches identified 522 studies in the five databases. After the removal of duplicates, and reviewing the titles and abstracts, 126 studies were selected for full-text screening. After applying the inclusion and exclusion criteria, 103 reviews that addressed the research questions were identified. The process of the literature search is summarized in Figure 1. The full reference list is provided in Supplementary Table 1.

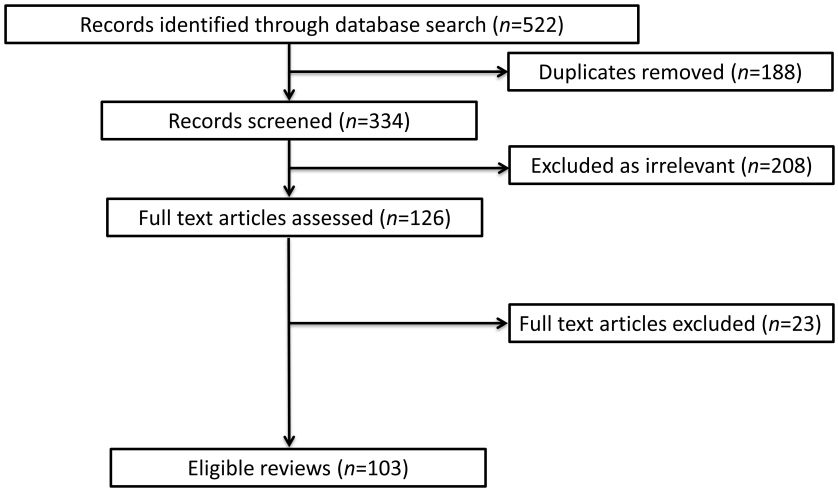

FIGURE 1
The flowchart of the study search and selection process.

Methodological quality

Using ROBIS tool, the methodological quality of 103 included reviews was assessed. Thirty reviews (29.1%) were rated as low risk of bias, and 73 reviews (70.9%) were rated as high risk of bias. Fifty-five reviews (53.4%) did not establish the methods prior to the conduct of the review. Forty-four reviews (42.7%) did not examine if the pooled results were robust through sensitivity analysis or funnel plot. Seventy-three reviews (70.9%) did not address the bias of included primary studies. The full assessments are provided in Supplementary Table 2.

Update of eligible reviews

One hundred and nineteen primary studies were incorporated for review update. The list was presented in Supplementary Table 3.

Characteristics of the reviews at low risk of bias

Data from 30 reviews rated as low risk of bias were included in the narrative synthesis. The number of studies in the included reviews ranged from 6 (16) to 348 (17). Among these reviews, the earliest date of literature search was March 1, 2020 (16), and the last date of literature search was July, 2021 (18). The characteristics of these 30 reviews are shown in Table 1.

Acute kidney injury in COVID-19

The incidence of AKI in COVID-19 patients

Of the reviews reporting the incidence of AKI in COVID-19 patients, six were rated as low risk of bias. The findings are summarized in Table 2.

The overall incidence of AKI in general COVID-19 patients

Five reviews at low risk of bias reported the incidence of AKI in general COVID-19 patients. All these five reviews included hospitalized patients. The largest of which (Chan et al.) included COVID-19 patients from 17 countries (Austria, Brazil, Canada, China, Denmark, France, Germany, India, Iran, Italy, Japan, Korea, Malaysia, Spain, Turkey, United Kingdom, and United States) and suggested that the incidence of AKI in general hospitalized COVID-19 patients was 20.4% (95% CI: 12.7–28.4%) (41).

The incidence of AKI in severe or critically ill COVID-19 patients

Four reviews at low risk of bias reported the incidence of AKI in severe or critically-ill COVID-19 patients. The review by Chang et al. focused on 12,437 COVID-19 patients admitted to the ICU in seven countries (the USA, China, UK, Italy, Spain, France, and Mexico) and reported that the incidence of AKI was 32% (95% CI: 13–58%) (36). Hansrivijit et al. included a total of 31 studies from three countries (China, the USA, and Spain) and found that the prevalence of AKI was higher in critically ill COVID-19 patients (19.9%) than in general COVID-19 patients (7.3%) (25).

TABLE 1 The characteristics of 30 reviews at low risk of bias.

First author	Publish year	Last date of search	Number of included studies	Sample size	References
Izcovich, A.	2020	April 28, 2020	207	75,607	(19)
Mesas, A. E.	2020	July 27, 2020	60	51,225	(20)
Wang, B.	2020	March 1, 2020	6	1,558	(16)
Luo, L.	2020	July, 2020	124	NA	(21)
Lim, M. A.	2020	April 11, 2020	15	3,615	(22)
Oltean, M.	2020	June 4, 2020	12	204	(23)
Ssentongo, P.	2020	July 9, 2020	25	65,484	(24)
Hansrivijit, P.	2020	April 24, 2020	26	5,497	(25)
Zhou, S.	2020	June 16, 2020	58	13,452	(26)
Zhang, T.	2020	April 10, 2020	16	3,975	(27)
Papadopoulos, V. P.	2020	January 7, 2021	41	NA	(28)
Zhou, Y.	2020	April 26, 2020	52	21,164	(29)
Fu, E. L.	2020	May 29, 2020	142	49,048	(30)
Lee, A. C.	2021	May 25, 2020	36	22,573	(31)
Kremer, D.	2021	January 18, 2021	74	5,559	(32)
Mirjalili, H.	2021	January 10, 2020	10	11,755	(33)
Zhang, L.	2021	September 29, 2020	34	344,431	(34)
Du, P.	2021	October 22, 2020	17	7,611	(35)
Chang, R.	2021	May 1, 2020	28	12,437	(36)
Schlesinger, S.	2021	October 10, 2020	22	17,687	(37)
Menon, T.	2021	November, 2020	20	4,350	(38)
Liu, Y. F.	2021	April 13, 2020	36	6,395	(25)
Li, Y.	2021	May 2020	40	NA	(39)
Dessie, Z. G.	2021	August 31, 2020	42	423,117	(40)
Chan, K. W.	2021	October 5, 2020	74	NA	(41)
Chung, E. Y.	2021	February 22, 2021	348	1,139,979	(17)
Taylor, E. H.	2021	February 21, 2021	58	44,305	(42)
Ho, Q. Y.	2021	September 5, 2020	23	1,373	(43)
Shi, Q.	2021	July, 2021	56	79,104	(18)
Cai, X.	2021	January 30, 2021	38	42,779	(44)

Two reviews at low risk of bias examined the association between the severity of COVID-19 and the prevalence of AKI. Liu et al. conducted the largest review (n = 36 studies) and reported that the incidence of AKI was significantly increased in the severe group compared with the non-severe group (OR: 11.02, 95% CI: 6.54–18.57) (45).

The incidence of AKI in children and adolescents with COVID-19

One review rated as low risk of bias included three studies focused on pediatric COVID-19 patients and reported that the incidence of AKI was 16.11% (41).

The incidence of AKI in patients with COVID-19 and pre-existing CKD

One review at low risk of bias by Chung et al. focused on new-onset AKI in patients with COVID-19 and CKD. The incidence of AKI was 73 per 1,000 person-weeks (95% CI: 60–87) (17).

The incidence of AKI in patients with COVID-19 from different regions

Notably, substantial difference was observed in the reported AKI incidence across regions. Chan et al. performed subgroup analyses and suggested that the incidences of AKI in Guangdong, Hong Kong, Hubei, Istanbul, Madrid, Michigan, New Delhi, New York, North Zealand, and Pennsylvania were 1.74, 3.72, 4.25, 29.17, 11.42, 44.79, 40.63, 33.07, 11.71, and 49.33%, respectively (41). In addition, Fu, E. L. included 49,048 hospitalized COVID-19 patients and reported that the incidence of AKI was 28.6% (95% CI: 19.8–39.5) in the USA and Europe (n = 20 studies) and 5.5% (95% CI: 4.1–7.4) in Asia (n = 62 studies) (30).

TABLE 2 The incidence of AKI in COVID-19 patients.

Study	Number of included studies	Population	Incidence of AKI (95% CI)	I^2 (p-value)	References
Zhou, S., 2020	58	All COVID-19 patients	9% (4.2–15.2%)	NA	(26)
Hansrivijit, P., 2020	26	All COVID-19 patients	8.4% (6.0–11.7%)	88.9%	(25)
		Critically ill COVID-19 patients	19.9% (11.8–31.5%)	48.4%	
		Hospitalized COVID-19 patients	7.3% (5.0–10.4%)	89.5%	
Fu, E. L., 2020	142	COVID-19 patients in Asia	5.5% (4.1–7.4%)	94%	(30)
		COVID-19 patients in the USA and Europe	28.6% (19.8–39.5%)	97%	
Chan, K. W., 2021	74	All COVID-19 patients	20.4% (12.07–28.74%)	99.72% (<0.001)	(41)
		COVID-19 patients with kidney transplant history	35.99% (26.20–45.79)	NA	
		Pediatric COVID-19 patients	16.11% (5.14–27.08)	NA	
Chung, E. Y., 2021	348	Patients with COVID-19 and CKD	7.3% (6–8.7%)	NA	(17)
Chang, R., 2021	28	COVID-19 patients admitted to ICU	32% (13–58%)	96.49 % (<0.01)	(36)

Reviews eligible for update

One review was considered eligible for update (41), thereby, 59 newly published studies were added. Fifty-eight studies only included hospitalized patients and 1 study included both hospitalized patients and non-hospitalized patients. The pooled incidence of AKI in hospitalized COVID-19 patients was 27.17% (95% CI: 23.84–30.5%; Figure 2), while Kang et al. reported the incidence of AKI was 0.37% (95% CI: 0.25–0.55%) in all COVID-19 patients in Korean (46).

Risk factors for AKI in COVID-19 patients

Of the included reviews that investigated the risk factors for AKI in COVID-19 patients, three were rated as low risk of bias. These findings suggested that advanced age, male sex, smoking, obesity, comorbidities (cardiovascular disease, coronary artery disease, diabetes, CKD, hypertension, pneumopathy, heart failure, and cancer), mechanical ventilation, and the use of vasopressors were potential risk factors for AKI (Figure 3) (30, 41, 44).

The incidence of urgent renal replacement therapy in COVID-19

Four reviews at low risk of bias reported the incidence of urgent RRT (patients received RRT for AKI) in COVID-19 patients (Table 3), the largest by Fu et al. suggested that the incidence of RRT was 2.2% (95% CI: 1.5–3.3%) in China, and 7.7% (95% CI: 5.1–11.4%) in Europe and the USA (30). Regardless of region, Zhou et al. reported the rate of urgent-start RRT as 3.4% (95% CI: 1.9–5.4%) (26). One review rated as low risk of bias, by Brienza et al., reported that the incidence of RRT in the severe and non-severe group was 7.5 and 0.3%, respectively (OR: 14.75, 95% CI: 3.4–64.8) (47). Only one review by Chan et al. included three studies based on pediatric COVID-19 patients and suggested that the rate of RRT was 5.54% (41).

Reviews eligible for update

We considered one review eligible for update (41). Twenty newly published studies were added. Figure 4 shows the incidence of urgent RRT in COVID-19 patients (6%, 95 CI: 5–7%).

The predictive value of AKI and the effect of urgent RRT on poor outcomes in COVID-19 patients

Seven reviews at lower risk of bias investigated the associations between AKI and poor outcomes in COVID-19 patients, including AKI and mortality ($n = 6$), and AKI and disease severity in COVID-19 patients ($n = 3$). A summary of the findings is shown in Table 4.

AKI and mortality in COVID-19 patients

Of the reviews that examined the associations between AKI and mortality in COVID-19 patients, six reviews were rated as low risk of bias. The largest review by Chan et al. ($n = 74$ studies) reported that the presence of AKI was associated with an 8-fold increased risk of death in COVID-19 patients (OR: 8.33, 95% CI: 5.45–14.94) and AKI stages 1, 2, and 3 were associated with 6.5-, 23.6-, and 93.8-fold increased risks of death (41). Zhou et al. also reported that the mortality rate among people with AKI and COVID-19 was 72.3% (95% CI: 47.1–92.0%) (26). Of note, in patients with COVID-19 admitted to the ICU, AKI was significantly associated with a much elevated risk of death (OR: 12.47, 95% CI: 1.52–102.7) (36). Notably, the

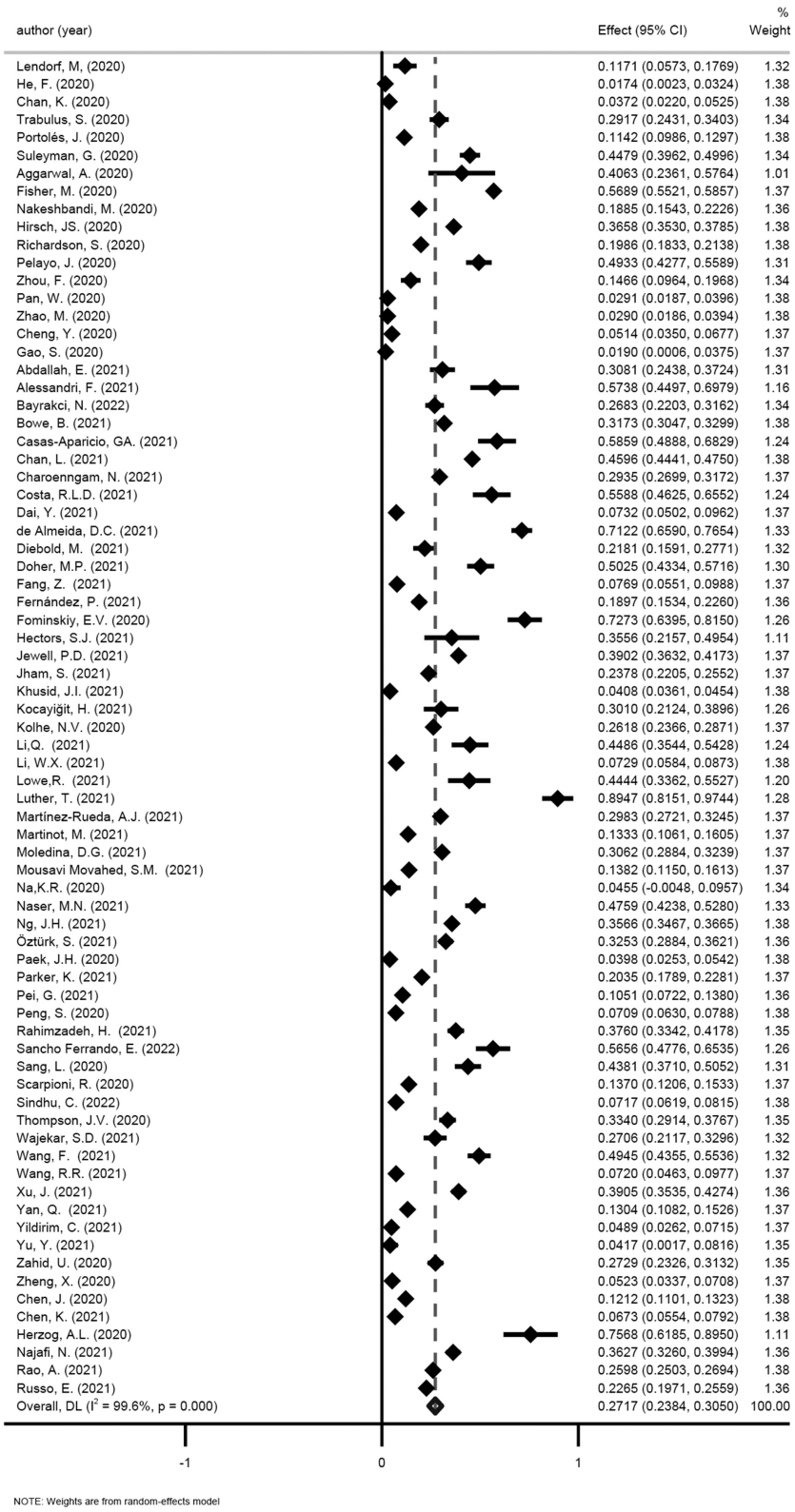

FIGURE 2
Meta-analysis of incidence of AKI in a random effect model.

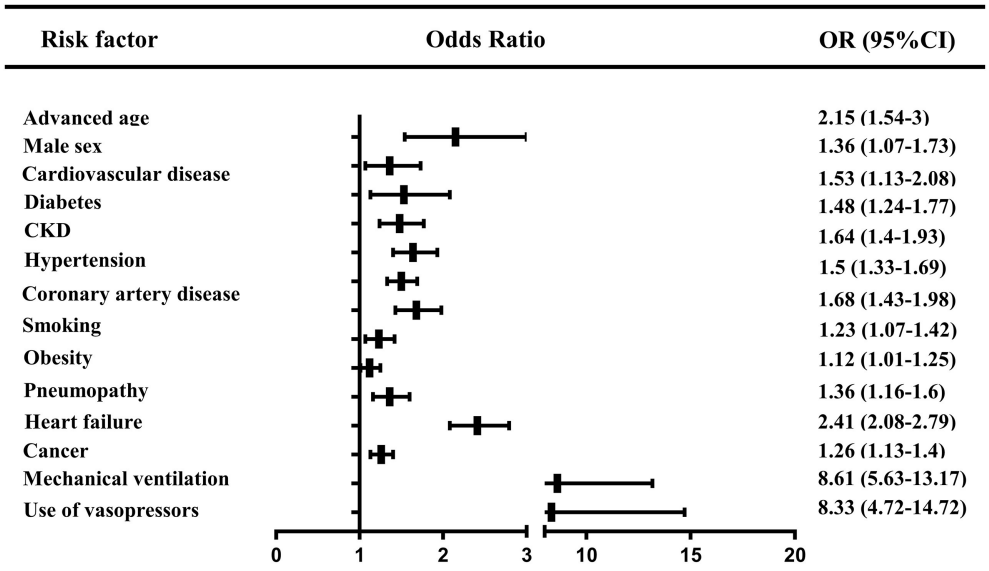

FIGURE 3
The risk factors for AKI in COVID-19 patients. CKD, chronic kidney disease.

TABLE 3 The incidence of urgent RRT in COVID-19 patients.

Study	Number of included studies	Population	Incidence of urgent RRT (95% CI)	I^2 (p-value)	References
Fu, E. L., 2020	142	COVID-19 patients in China	2.2% (1.5–3.3%)	92%	(30)
		COVID-19 patients in the USA and Europe	7.7% (5.1–11.4%)	80%	
Zhou, S., 2020	58	All COVID-19 patients	3.4% (1.9–5.4%)	NA	(26)
Hansrivijit, P., 2020	26	All COVID-19 patients	3.6% (1.8–7.1%)	82.2%	(25)
Chan, K. W., 2021	74	All COVID-19 patients	2.97% (1.91–4.04%)	93.52% (<0.001)	(41)
		COVID-19 patients with kidney transplant history	12.65% (0.72–24.58)	NA	
		Pediatric COVID-19 patients	5.54% (−1.14 to 12.21)	NA	

above results were not adjusted for confounders (such as age, sex, and comorbidities).

AKI and severity of COVID-19

Three reviews at low risk of bias examined the predictive value of AKI for severe conditions in COVID-19 patients. The review by Chan et al. had the largest number of included studies ($n = 74$), concluding that AKI was associated with a higher rate of ICU occupancy (OR: 17.58, 95% CI: 10.51–29.38) (41). In addition, Lim et al. reported that AKI was relevant to severe conditions (diagnosed according to the severity categories proposed by WHO) in COVID-19 patients (OR: 8.12, 95% CI: 4.43–14.86) (22). Similarly, in children and adolescents with COVID-19, Shi, Q. reported that AKI increased the risk of ICU occupancy (OR: 55.02, 95% CI: 6.26–483.35) (18). However, it is worth highlighting that above ORs were from univariate analysis without the adjustment for confounders (such as age, sex, and comorbidities).

Urgent RRT dependent AKI and poor outcomes of COVID-19 patients

Two reviews at low risk of bias examined the associations between RRT and poor outcomes (defined as mortality and severity or critical conditions) in COVID-19 patients. The review by Chan, K. W. with the largest number of included studies showed that the application of RRT was associated with an 18.7-fold increased risk of death and a 34-fold increased risk of critical conditions (diagnosed according to the severity categories proposed by WHO) (41). Similarly, the above results were not adjusted for any confounders.

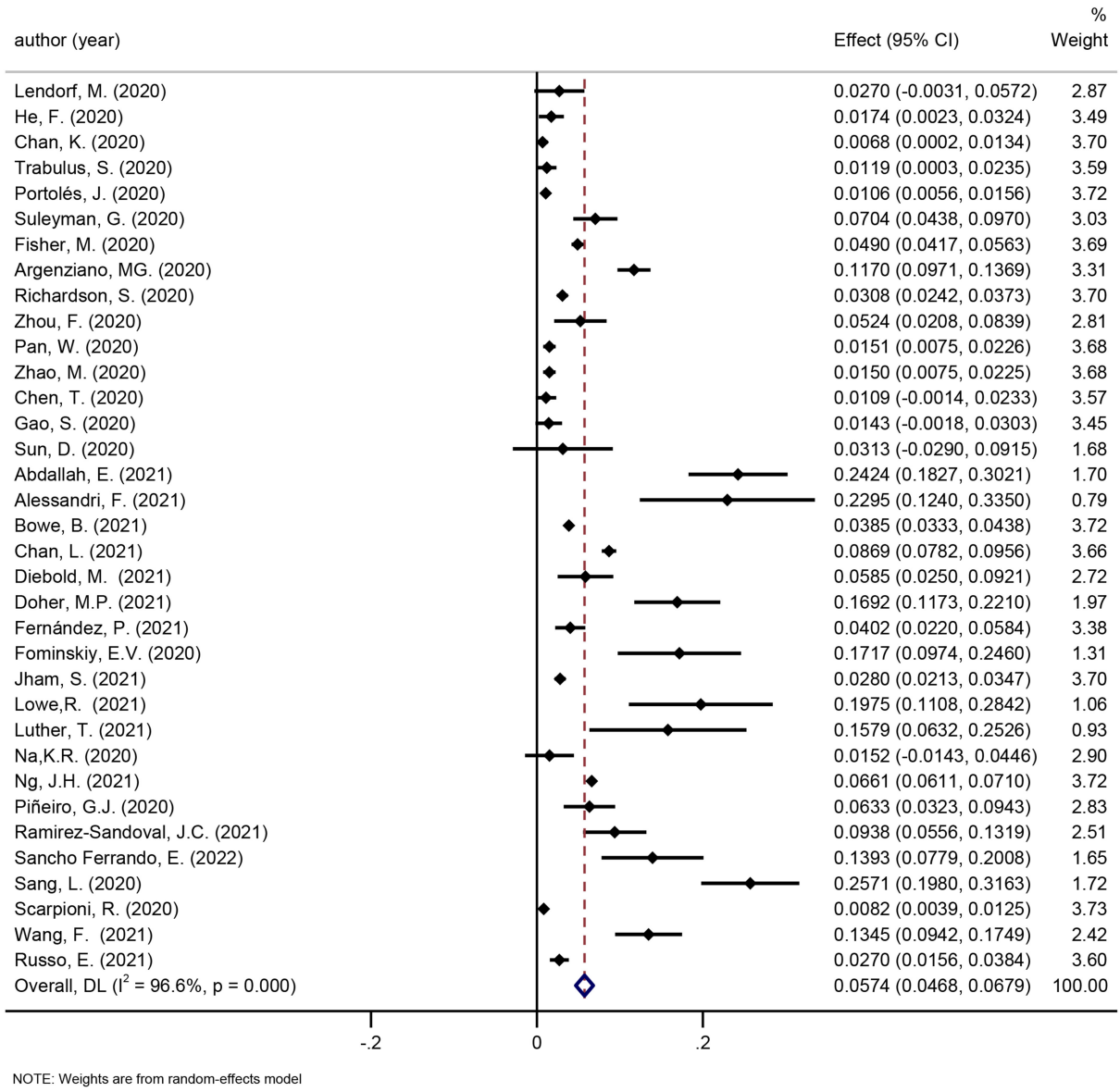

FIGURE 4
Meta-analysis of incidence of urgent RRT in a random effect model.

Reviews eligible for update

We considered one review eligible for update (41). Incorporating the results in the meta-analysis did not alter the significance of the associations between AKI and poor outcomes in COVID-19 patients. The updated meta-analysis showed that AKI was significantly associated with mortality and disease severity in COVID-19 patients (OR: 5.24 and 14.94, respectively; Figures 5A,B). Figure 6 shows that urgent RRT significantly predicted death in COVID-19 patients (OR: 14.21, 95% CI: 4.45–45.35).

Chronic kidney disease in the COVID-19 pandemic

The incidence of COVID-19 in CKD patients

Among reviews reporting the incidence of COVID-19 in CKD patients, two reviews were rated as low risk of bias. Notably, the CKD category included in many reviews was unclear and might not be uniform across studies. The largest review by Chung et al. (n = 348 studies) reported that the incidence of COVID-19 in CKD patients was 66 per 1,000

TABLE 4 AKI and poor outcomes in COVID-19 patients.

Study	Number of included studies	Exposure	Outcome	Metric	Effects (95% CI)	I^2 (p-value)	References
Hansrivijit, P., 2020	26	AKI	Mortality	OR	13.33 (4.05–43.91)	85%	(25)
Lim, M. A., 2020	15	AKI	Mortality	RR	13.38 (8.15–21.95)	24% (0.25)	(22)
		AKI	Severity	RR	8.12 (4.43–14.86)	0% (0.73)	
		AKI	ICU admission	RR	5.9 (1.32–26.35)	0% (0.49)	
Zhou, Y., 2020	52	AKI	Mortality	OR	45.79 (36.88–56.85)	17% (0.31)	(29)
		AKI	Severity	OR	6.97 (3.53–13.75)	0% (0.501)	
Papadopoulos, V. P., 2020	41	AKI	Mortality	OR	7.52 (1.96–28.9)	NA	(28)
Dessie, Z. G., 2021	42	AKI	Mortality	OR	1.87 (1.48–2.26)	86.53% (<0.001)	(40)
Chang, R., 2021	28	AKI	Mortality	OR	12.47 (1.52–102.7)	81.15% (0.005)	(36)
Chan, K. W., 2021	74	AKI	Mortality	OR	9.03 (5.45–14.94)	89.7% (<0.001)	(41)
		AKI	Severity	OR	17.58 (10.51–29.38)	63% (0.004)	

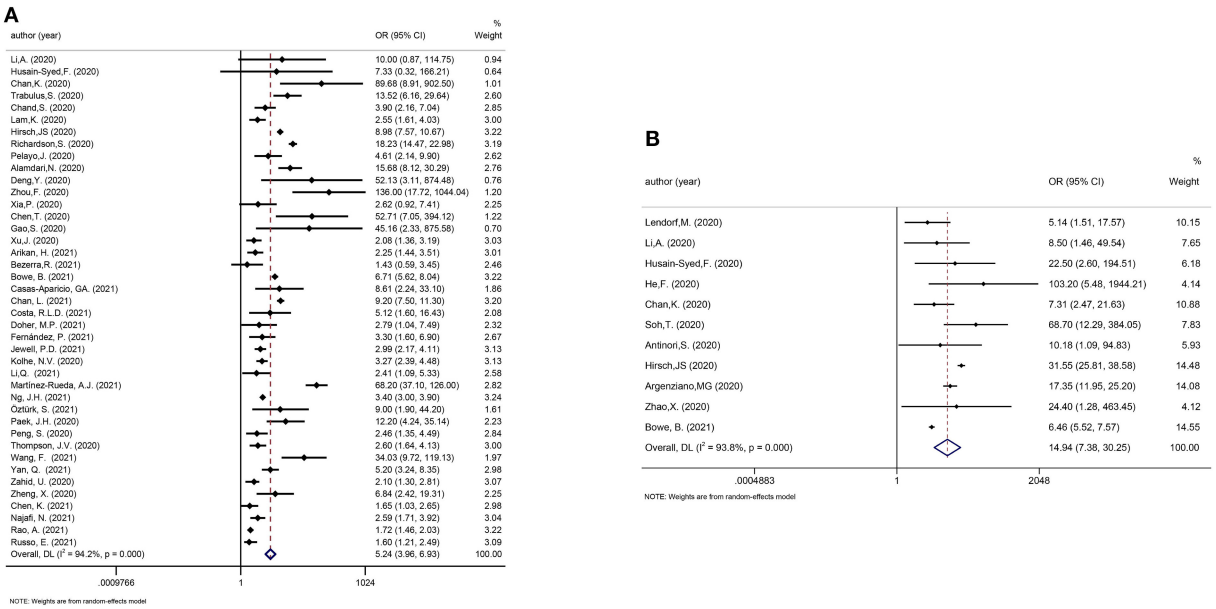

FIGURE 5
(A) Meta-analysis of AKI and mortality in a random effect model. **(B)** Meta-analysis of AKI and disease severity in a random effect model.

person-weeks (95% CI: 58–75), and the incidence of COVID-19 varied between predialysis CKD patients and chronic dialysis patients (16 and 105 per 1,000 person-weeks, respectively) (17). Mirjalili et al. included only Iranian cases and reported that the proportion of SARS-CoV-2 infection in CKD patients was 5.0% (95% CI: 1.9–12.4%) (33).

Pooled prevalence of CKD in COVID-19 patients

Five reviews at low risk of bias suggested that CKD was a common comorbidity in COVID-19 patients. Zhou et al. included the largest number of studies (n = 37 studies), and suggested that the pooled CKD prevalence in all COVID-19 patients was 3.52% (95% CI, 1.98–5.48%) (29). Three reviews at low risk of bias summarized the prevalence of CKD in severe COVID-19 patients. Lee et al. reported that CKD was a common comorbidity in severe COVID-19 patients (8.46%, 95% CI: 3.72–18.1%) (31). Chang et al. reported that the prevalence of CKD in COVID-19 patients admitted to ICU was 9% (95%CI: 4–18%) (36). Zhou et al. suggested that CKD prevalence was higher in severe COVID-19 patients than in non-severe COVID-19 patients (OR: 3.42, 95% CI 2.05–5.61) (29). Two reviews at low risk of bias summarized the prevalence of CKD in deceased

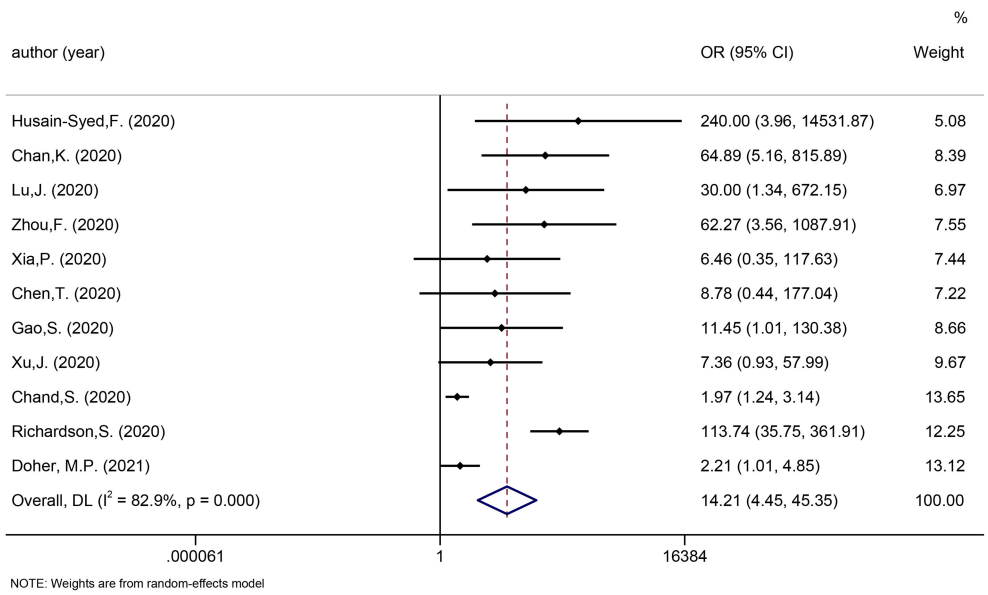

FIGURE 6
Meta-analysis of urgent RRT and mortality in a random effect model.

TABLE 5 Pooled prevalence of CKD in COVID-19 patients.

Study	Number of included studies	Population	Pooled prevalence of CKD (95% CI)	I^2 (p-value)	References
Zhou, Y., 2020	52	All COVID-19 patients	3.52% (1.98–5.48%)	93% (<0.01)	(29)
		Severe COVID-19 patients	6.13% (2.81–10.64%)	84% (<0.01)	
		Deceased COVID-19 patients	6.36% (2.34–12.17%)	81% (<0.01)	
Lee, A. C., 2021	36	Deceased COVID-19 patients	9.028% (4.641–16.83%)	90% (<0.01)	(31)
		Severe COVID-19 patients	8.317% (3.479–18.585%)	95% (<0.01)	
Menon, T., 2021	20	All COVID-19 patients	4% (2–8%)	95% (<0.01)	(38)
Mirjalili, H., 2021	10	All COVID-19 patients	5% (1.9–12.4%)	NA	(33)
Chang, R., 2021	28	Severe COVID-19 patients	9% (4–18%)	96.97% (<0.01)	(36)

COVID-19 patients. Lee et al. reported that the proportion of CKD patients among non-survivors with COVID-19 was 9.05% (95% CI: 5.57–15.0%) (31). Zhou et al. showed that CKD was more common in deceased patients than in survivors (OR: 6.46, 95% CI: 3.40–12.29) (29). The pooled prevalence of CKD in COVID-19 patients is shown in Table 5.

Reviews eligible for update

Two reviews were considered eligible for updating (17, 29). The incidence of COVID-19 in the dialysis population was 0.89% (95%CI: 0.83–0.95%; Figure 7A) and the pooled prevalence of CKD in COVID-19 patients was 5.66% (95%CI: 5.08–6.23%; Figure 7B).

CKD and poor outcomes in COVID-19 patients

Of the included reviews focusing on CKD and mortality in COVID-19 patients, 12 reviews were rated as low risk of bias. Chung performed a review that included the largest number of studies ($n = 348$) and reported that overall mortality in patients with CKD and COVID-19 was 32 per 1,000 person-weeks (95% CI: 30–35). In different subgroups, the mortality in people with CKD5D and COVID-19 was 30 per 1,000 person-weeks (95% CI: 26–35) (17). Izcovich et al. reported a significant association between pre-existing CKD and mortality in COVID-19 patients (OR: 2.27, 95% CI: 1.69–3.05) (19). A review by Zhou, S. reported that end-stage renal disease (ESRD) was significantly associated with an increased risk of mortality in patients with

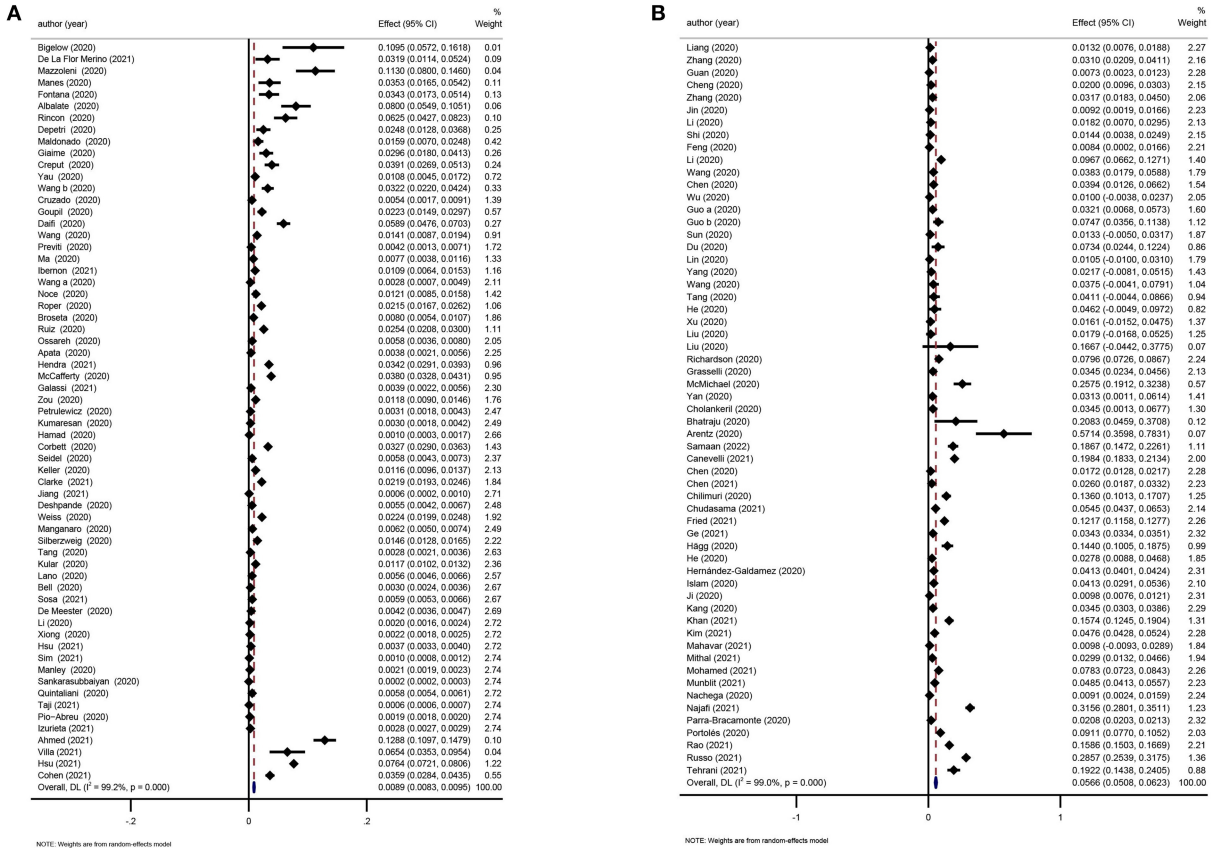

FIGURE 7
(A) Meta-analysis of incidence of COVID-19 in the dialysis population in a random effect model. **(B)** Meta-analysis of prevalence of CKD in COVID-19 patients in a random effect model.

COVID-19 (OR 1.81, 95% CI 1.44–2.27, $p < 0.00001$) (26). In the diabetic population with COVID-19, Schlesinger et al. found that pre-existing CKD could significantly predict the death (OR: 1.93, 95% CI: 1.28–2.90) (37).

Ten reviews rated as low risk of bias explored the associations between CKD and the severity of COVID-19. In the largest review by Izcovich et al. ($n = 207$ studies), the pooled effect showed that CKD was significantly related to an increased risk of severe COVID-19 (diagnosed according to the severity categories proposed by the WHO; OR: 2.21, 95% CI: 1.51–3.24) (19). The major findings are summarized in Table 6.

Reviews eligible for update

We considered one review eligible for updating (21). Evidence incorporating recent studies and the meta-analysis showed that CKD was a risk factor for death and disease deterioration in COVID-19 patients (OR: 2.21 and 1.87, respectively; Figures 8A,B).

The incidence of COVID-19 in kidney transplant recipients

Limited reviews have assessed the effects of COVID-19 in kidney transplant recipients (KTRs). Chung et al. included 120,281 KTRs and suggested that the incidence of COVID-19 in KTRs was 23 per 10,000 person-weeks (95% CI: 18–30) (17).

Adverse events in kidney transplant recipients with COVID-19

Four reviews at low risk of bias reported adverse events in KTRs with COVID-19, including AKI, urgent-RRT and mortality (Supplementary Table 4). Three reviews at low risk of bias reported the incidence of AKI in KTRs with COVID-19. The largest review by Kremer et al. included 5,559 KTRs ($n = 74$ studies) with COVID-19 and the pooled incidence of AKI was 50% (95% CI: 44–56%) (32). Two reviews at low risk of bias analyzed the application of RRT in KTRs with COVID-19. The largest review by Ho et al. ($n = 74$ studies, 1,373 KTRs with COVID-19) demonstrated that the rate of RRT was 12.4% (8.3–18%) (43). Kremer et al. reported that the mortality rate in KTRs

TABLE 6 CKD and poor outcomes in COVID-19.

Study	Number of included studies	Exposure	Outcome	Metric	Effects (95% CI)	I^2 (p-value)	References
Zhou, Y., 2020	52	CKD	Mortality	OR	6.46 (3.40–12.29)	1% (0.4)	(29)
		CKD	Severity	OR	3.42 (2.08–5.61)	0% (0.43)	
Izcovich, A., 2020	207	CKD	Mortality	OR	2.27 (1.69–3.05)	NA	(19)
		CKD	Severity	OR	2.21 (1.51–3.24)	NA	
Luo, L., 2020	124	CKD	Mortality	OR	3.07 (2.43–3.88)	72.9% (<0.001)	(21)
		CKD	Severity	OR	2.2 (1.27–3.80)	77.4% (<0.001)	
Mesas, A. E., 2020	60	CKD	Mortality	OR	3.2 (2.52–4.06)	75.8% (<0.001)	(20)
Ssentongo, P., 2020	25	CKD	Mortality	RR	3.25 (1.13–9.28)	84% (<0.01)	(24)
Zhou, S., 2020	58	CKD	Mortality	OR	1.97 (1.56–2.49)	65% (<0.00001)	(26)
		ESRD	Mortality	OR	1.81 (1.44–2.27)	0% (0.62)	
Zhang, T., 2020	16	CKD	Severity	OR	1.26 (0.7–2.28)	31% (0.18)	(27)
Wang, B., 2020	6	CKD	Severity	OR	2.51 (0.93–6.78)	0% (0.501)	(16)
		CKD	ICU admission	OR	2.94 (0.4–21.69)	NA	
Li, Y., 2021	40	CKD	Mortality	OR	1.57 (1.27–1.93)	62.2% (0.01)	(39)
Lee, A. C., 2021	36	CKD	Mortality	OR	8.86 (5.27–14.89)	NA	(29)
		CKD	Severity	OR	1.92 (1.65–2.23)	NA	
Zhang, L., 2021	34	CKD	Mortality	OR	8.91 (3.83–20.73)	61% (0.05)	(40)
		CKD	Severity	OR	3.2 (1.87–5.49)	0% (0.46)	
Mirjalili, H., 2021	10	CKD	Mortality	OR	0.552 (0.367–0.829)	0% (0.719)	(33)
Schlesinger, S., 2021	22	CKD	Mortality	RR	1.44 (0.96–2.15)	83%	(37)
		CKD	Severity	RR	1.93 (1.28–2.9)	81%	
Du, P., 2021	17	CKD	Severity	OR	3.59 (1.9–6.76)	19%	(35)
Liu, Y. F., 2021	36	CKD	Severity	OR	3.28 (2–5.37)	0% (0.72)	(25)
Menon, T., 2021	20	CKD	Mortality	OR	5.58 (3.27–9.54)	0% (0.84)	(38)

with COVID-19 was 23% (95% CI: 20–27%). Ho et al. included 412 KTRs with COVID-19, and suggested that the proportion of critical cases was 27.7% (95% CI: 21.5–34.8%) (43).

Reviews eligible for update

We considered one review eligible for updating (32). Twenty-three recent studies examined mortality in KTRs with COVID-19. The pooled mortality rate was 18% (95% CI: 14–22%; Figure 9).

Discussion

This umbrella review provides a comprehensive overview of existing evidence of the association between kidney diseases and COVID-19. A total of 103 systematic reviews and meta-analyses were identified, among which 30 reviews were rated as low risk of bias. We found that COVID-19 patients had a notable higher AKI incidence, varying by geographic location and disease severity. Advanced age, male sex, smoking, obesity, comorbidities (cardiovascular disease, diabetes, CKD, hypertension, pneumopathy, and cancer), mechanical ventilation, and the use of vasopressors were potential risk factors for COVID-19-associated AKI. It is important to note that many of these factors place patients at risk for other forms of AKI. The incidence of AKI, the need for RRT and pre-existing CKD were independently associated with adverse outcomes such as death and a severe disease among COVID-19 patients. KTRs are susceptible to SARS-CoV-2 infection and are at increased risk of developing a severe form of infection.

A number of studies have shown that kidney impairment is prevalent among COVID-19 patients, particularly among critically ill patients. A meta-analysis involving COVID-19 patients from 17 countries suggested that the overall incidence of AKI was 20.4% (41). In this umbrella review, the pooled incidence of AKI in COVID-19 patients was 27%. Focusing on COVID-19 patients admitted to the ICU, the incidence of AKI was 32% (36). The incidence of AKI was significantly increased in the those with severe COVID-19 group compared to those with non-severe disease (diagnosed according to the severity categories proposed by WHO), as well as in non-survivors than in survivors (45). There was also substantial heterogeneity across regions (41). The reported AKI incidence was 28.6%

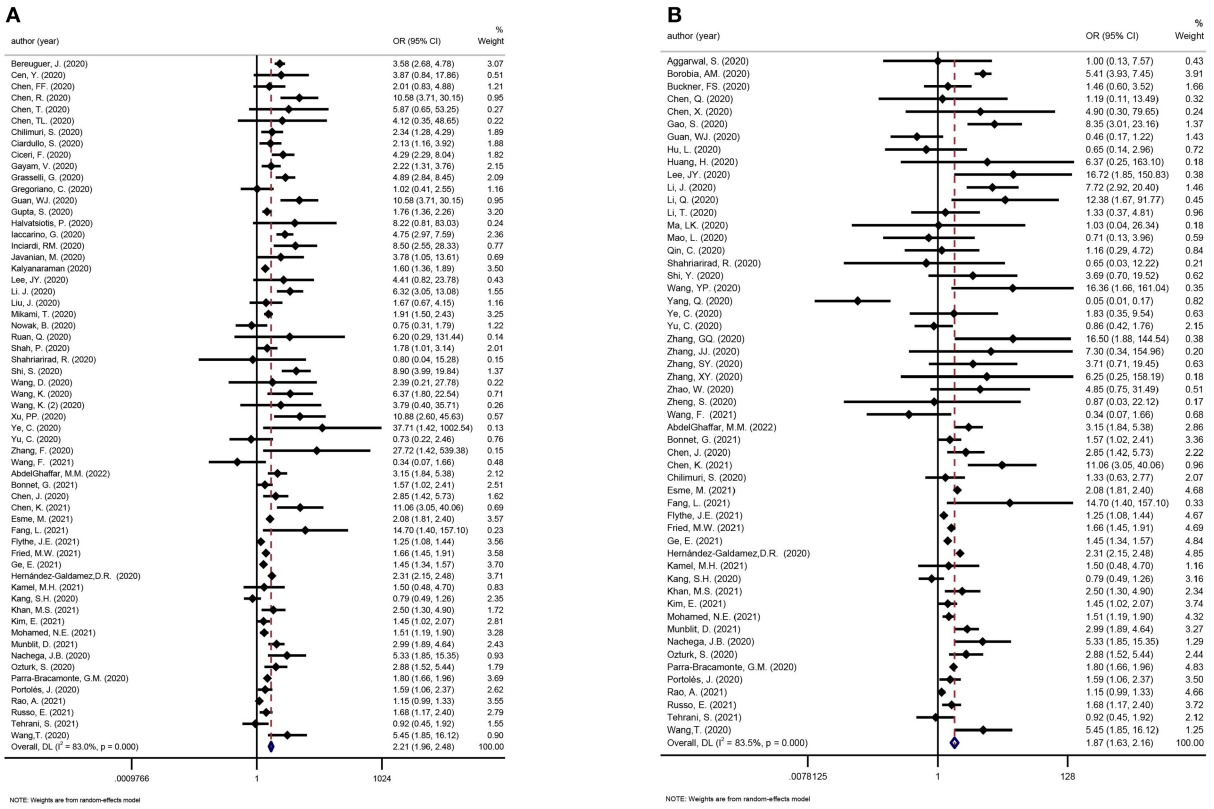

FIGURE 8
(A) Meta-analysis of CKD and mortality in a random effect model. **(B)** Meta-analysis of CKD and disease severity in a random effect model.

in the USA/Europe compared with 5.5% in China (30). In children, meta-analyses showed the incidence of AKI among pediatric COVID-19 patients was 11.9–16.11% (41, 48). The widespread application of a standardized AKI definition has facilitated comparisons across COVID-19 studies. However, as AKI is a syndrome that encompasses a multitude of clinical scenarios and pathophysiological processes, it is not surprising that the reported AKI incidence shows some variation. While the difference could partially be explained by the heterogeneities in region or patient population, other possibilities might also contribute, such as different AKI diagnostic criteria used (biochemical or coded diagnosis), hospital setting (academic center or regional hospital) and the specific definitions of relevant terms (such as baseline creatinine) (49, 50). As the pandemic goes on, the health-care community gained better understanding on the disease and are more sophisticated in treatment which might contribute to a drop of AKI and RRT rates over time (51, 52). More importantly, different research groups recently reported Omicron demonstrated lower replication in lower airway organoids, lung cells and gut cells. Whether SARS-CoV-2 variants have altered virulence on kidneys awaits further verification (53–55).

In addition to direct pathogenic mechanisms, baseline comorbidities, organ crosstalk and COVID-19 systemic effects may contribute to AKI (56). Evidence suggests that advanced age, male sex, coronary artery disease, diabetes, CKD, hypertension, elevated levels of C-reactive protein, and decreased levels of serum albumin are potential risk factors for AKI in COVID-19 patients (30, 41), which is consistent with findings reported in general hospitalized patients (57, 58). Pre-existing CKD seems to be a particularly strong risk factor for AKI. A systematic review and meta-analysis showed the new-onset AKI incidence to be 73 per 1,000 person-weeks among COVID-19 patients with CKD (17). For severely ill COVID-19 patients, critical care interventions might also be related to an increased risk of AKI, such as mechanical ventilation and the use of vasopressors (44). Observational studies can suggest an association but not causation. Since AKI patients could have an increased likelihood of being ventilated or prescribed vasopressors, caution should be exercised when interpreting these risk factors. Some additional risk factors for AKI and AKI severity, such as apolipoprotein L1 genetic variation and use of renin-angiotensin-aldosterone system inhibitors (59, 60), have been suggested by recent studies but have not yet been assessed on a meta-analysis level.

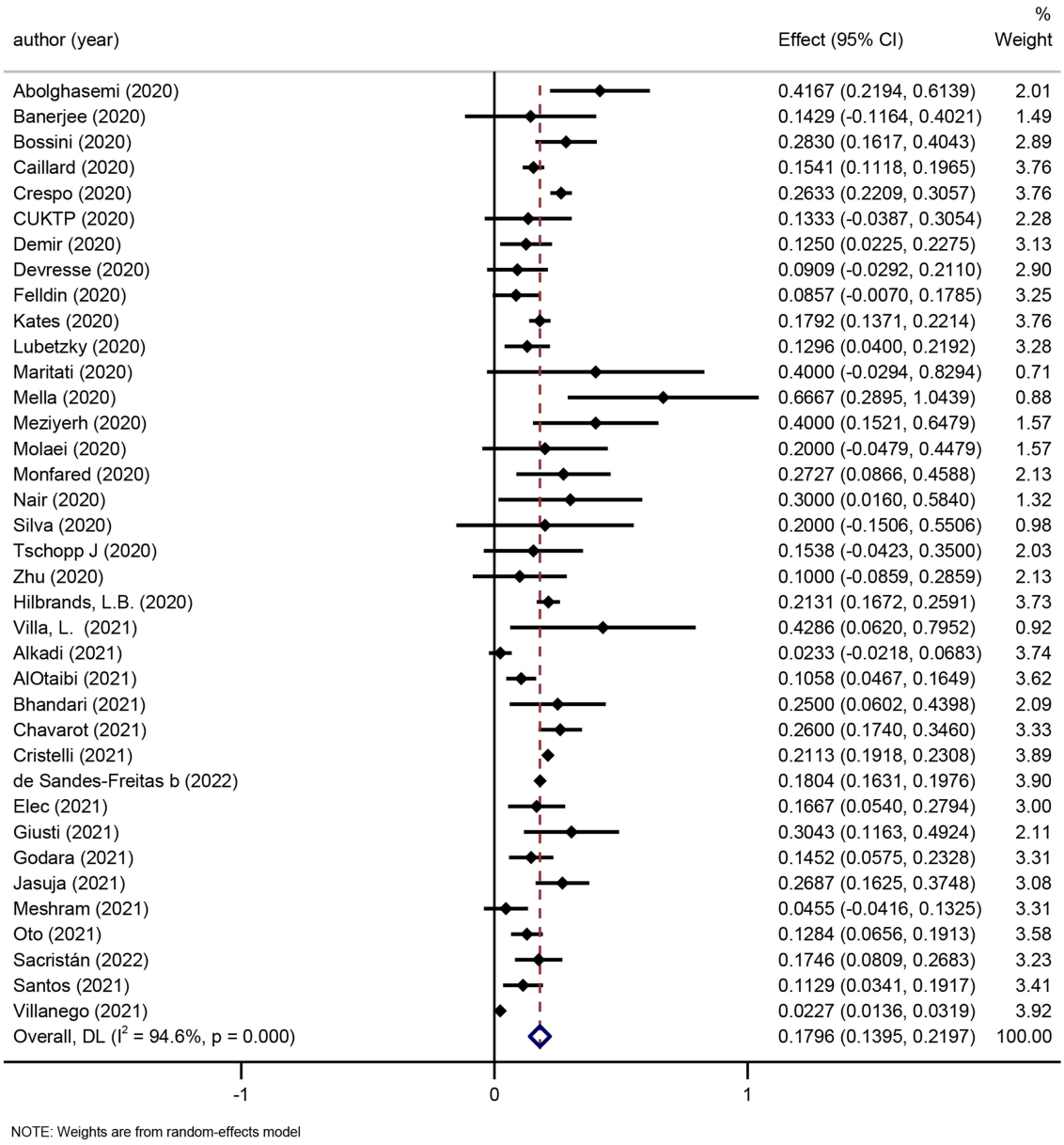

FIGURE 9
Meta-analysis of mortality in KTRs with COVID-19 in a random effect model.

A growing number of studies have investigated the molecular mechanisms of COVID-19-induced AKI (61–65). First, the infection of SARS-CoV-2 might cause direct tubular injury. Compared with lung tissue, the kidney expresses relatively high levels of ACE2. Therefore, SARS-CoV-2 could bind to ACE2 and subsequently causes acute tubular necrosis (62). Second, also of considerable interest is the indirect tubular injury by SARS-CoV-2. Several evidence have shown that SARS-CoV-2 could attract the macrophage to infiltrate into the kidney and cause cytokine storm (63). In addition, organ crosstalk also contributes to the development of AKI, such as lung–kidney axis and cardiovascular–kidney crosstalk (64, 65).

Apart from being a target of the virus, the kidneys also seem to have a substantial influence on the outcomes of the disease. AKI has long been recognized as associated with poor outcomes. Even in non-ICU hospitalized patients with AKI, the mortality rate could reach 10–20% (66, 67). A strong and graded relationship between AKI severity and increased mortality was observed in COVID-19 patients. A meta-analysis enrolling 74 cohorts revealed that AKI was related to an 8-fold increased risk

of death in COVID-19 patients. From AKI stage 1 to stage 3, the odds ratios of mortality were 6.5, 23.6, and 93.8, respectively (41). For critically ill patients in the ICU, AKI could predict an even higher risk of death (OR: 12.47, 95% CI: 1.52–102.7) (36). Meanwhile, AKI was also shown to be associated with COVID-19 severity and ICU occupancy in both adults and children (18, 22, 41). Patients with AKI who require RRT are among the most severely ill individuals in the ICU. This umbrella review indicated that AKI was significantly associated with mortality and disease severity in COVID-19 patients (OR: 5.24 and 14.94, respectively). Furthermore, the pooled rate of urgent-start RRT was to be 6% in all COVID-19 patients in this umbrella review. The application of RRT was a strong predictor of poor outcomes in COVID-19, predicting an 18.7-fold increased risk of death and a 34-fold increased risk of a critical condition (41). In Non-COVID associated AKI, prior data demonstrate that AKI was associated with an increased cost of USD $1,795 per admission, and USD $42,077 if RRT was needed (68). Affecting a large number of patients, AKI increases the risk of adverse outcomes and resource utilization, which warrants an improved strategy for the prevention, recognition and management of AKI in COVID-19 patients.

As a major chronic health burden, CKD prevalence in the general population is estimated to be between 9 and 12% (69, 70). Evidence from this umbrella review suggests that the overall CKD prevalence in COVID-19 patients is only 5.66%, which is considerably low compared to general population. CKD was more common in severely ill COVID-19 patients, with an odds ratio of 1.87 in the severe vs. non-severe group and 2.21 in the deceased vs. survivor group. Of note, CKD is a wide-spectrum clinical syndrome defined by either functional or structural abnormalities in the kidneys for more than 3 months (71). It was not clear in many of the studies if the COVID-19 patients were accurately screened for CKD as per the definition; therefore, the reported prevalence needs to be further validated. COVID-19 disproportionately affects people with chronic diseases such as CKD. The incidence of COVID-19 in people with pre-existing CKD was 66 per 10,000 person-weeks. The incidence was higher in the chronic dialysis subgroup than in the non-dialysis CKD subgroup (105 vs. 16 per 10,000 person-weeks), which may be attributable to the greater exposure to SARS-CoV-2 at health facilities when undergoing maintenance hemodialysis. In comparison, another study involving home-based dialysis patients reported a COVID-19 incidence similar to that in the general population (72). In CKD patients, COVID-19 infection was related to an increased risk of death (incidence rate ratio 10.26) compared with CKD patients without COVID-19 (58). Disrupted immune activation of both the innate and adaptive immune systems might contribute to susceptibility to infection and disease exacerbation in CKD patients (69, 73). Both CKD and ESRD were associated with increased mortality in COVID-19 patients (19, 26). Pre-existing CKD also significantly predicts the death in the diabetic COVID-19 population (37). In addition to mortality, there is also an incremental increase in the likelihood of severe COVID-19 and hospitalization in CKD patients compared with those without CKD (19, 74).

Although it remains unclear how CKD patients are more likely to contract COVID-19 and suffer from severe conditions, several reasons could partially explain these findings. First, comorbidities that accompany CKD might contribute to the development of COVID-19. In current studies, CKD was associated with multiple comorbidities, such as cardiovascular diseases and type 2 diabetes mellitus (75). A large population-based study provided robust evidence that patients with chronic heart failure had much higher risk of hospitalization of pneumonia than general population (76). Additionally, most of the patients with type 2 diabetes mellitus have abnormal immune functions, such as decreased $CD3^+$T and NK T cells, and imbalance of $CD4^+$ /$CD8^+$ T cells, which may aggravate SARS-CoV-2 infection (77). Second, the proportion of older patients is much higher in CKD groups, which has been proved in multiple studies. Older age is a recognized risk factor for severe COVID-19. Li et al. found age older than 50 years was a feature of severe COVID-19 pneumonia (78). A retrospective study illustrated that the median age of deceased patients was 68 years ago, significantly older than recovered groups (79). Moreover, other clinical characteristics of CKD patients, such as hemodynamic instability, anemia, and electrolyte abnormality, are also possibly involved in COVID-19 development and progression.

In a state of immunocompromise, KTRs are susceptible to infections (80). This umbrella review suggested that the incidence of COVID-19 in KTRs was higher than that in the general population (23 per 10,000 person-weeks vs. 2–6 per 10,000 person-weeks) (81, 82). Because of less kidney function reserve, the use of calcineurin inhibitors and other mechanisms, AKI commonly develops in KTRs, and the external insult from COVID-19 makes these patients even more vulnerable to AKI. The pooled incidence of AKI in KTRs with COVID-19 was dramatically high (21), and AKI was associated with increased mortality in KTRs (83). Application of RRT and graft loss were relatively common in KTRs (84, 85). The pooled mortality rate was 18% in KTRs with COVID-19. KTRs are at increased risk of developing severe forms of SARS-CoV-2 infection, reflecting an increased susceptibility to COVID-19 and perhaps delayed viral clearance.

Strength and limitations

Umbrella reviews consolidate the highest level of evidence, but there is an intrinsic limitation that they can only focus on existing meta-analyses or systematic reviews. The present umbrella review covered topics on the incidence/prevalence, aggravating factors and prognosis of AKI, CKD, and kidney

transplant patients with COVID-19, while other important issues that have not yet been assessed at the meta-analysis level, such as AKI non-recovery and risk of CKD progression during post-acute COVID-19, may have been overlooked. Second, with emerging evidence, the published meta-analyses could quickly become outdated. We therefore updated five meta-analyses by incorporating 119 newly available cohort studies to guarantee that conclusions are up-to-date. The emergence of variants such as Omicron, and worldwide vaccine application are both potential major modifiers of COVID-19 epidemiology (53), however related studies focusing on kidney outcomes are scarce. Third, based on our research questions, only observational studies were available. The results were more indicative of association rather than causality, and should thus be interpreted with caution. As the included studies only performed univariate analyses, the ORs pooled were not adjusted for confounders, which is an unavoidable limitation inherited from the source studies. Fourth, notable heterogeneity existed in our sourcing reviews. For example, the criteria of COVID-19 disease severity were different across many papers looking at the same outcome. The quality of the included reviews also varied, with 73 reviews rated as high risk of bias. As such, we exclusively focused results from reviews rated as low risk of bias. It's worth mentioning that some reviews published early in the pandemic included pre-print studies to account for the rapid emerging evidence base, but results of pre-prints are subject to change after peer-review and might be a potential source of bias. Nevertheless, as we have updated these meta-analyses and enrolled only peer-reviewed articles, the pre-prints are unlikely to bias our final results. At last, therapeutic options for kidney disease patients with COVID-19 were not analyzed, as this was beyond the scope of the present umbrella review.

Conclusion

To conclude, our umbrella review found that patients with fundamental kidney disease such as CKD and a history of kidney transplantation, were at increased risk of the development and progression of COVID-19. Persons infected by SARS-CoV-2 also had a notably high AKI incidence, with advanced age, male sex, coronary artery disease, diabetes, CKD, and hypertension being risk factors. AKI and the need for RRT were independent predictors of adverse outcomes in COVID-19. Specific observations on different SARS-CoV-2 variants and vaccination strategies, as well as follow-up studies on mid-/long-term kidney and patient outcomes in the post-acute phase of COVID-19 are needed.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LY, JL, and WW were responsible for study design, literature research, study selection, and manuscript drafting. YZ was responsible for study design, statistical analysis, and manuscript drafting. CY and YP were responsible for data extraction. JK and JZ were responsible for manuscript revision. LZ and LM were responsible for data verification and manuscript revision. PF and TC were responsible for the study design and manuscript revision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.963667/full#supplementary-material

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Follow-up of young adult monozygotic twins after simultaneous critical coronavirus disease 2019: A case report

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Background: The influence of the host genome on coronavirus disease 2019 (COVID-19) susceptibility and severity is supported by reports on monozygotic (MZ) twins where both were infected simultaneously with similar disease outcomes, including several who died due to the SARS-CoV-2 infection within days apart. However, successive exposures to pathogens throughout life along with other environmental factors make the immune response unique for each individual, even among MZ twins.

Case presentation and methods: Here we report a case of a young adult monozygotic twin pair, who caught attention since both presented simultaneously severe COVID-19 with the need for oxygen support despite age and good health conditions. One of the twins, who spent more time hospitalized, reported symptoms of long-COVID even 7 months after infection. Immune cell profile and specific responses to SARS-CoV-2 were evaluated as well as whole exome sequencing.

Conclusion: Although the MZ twin brothers shared the same genetic mutations which may be associated with their increased risk of developing severe COVID-19, their clinical progression was different, reinforcing the

role of both immune response and genetics in the COVID-19 presentation and course. Besides, post-COVID syndrome was observed in one of them, corroborating an association between the duration of hospitalization and the occurrence of long-COVID symptoms.

KEYWORDS

COVID-19, monozygotic twins, SARS-CoV-2, immunity, genetic variants

Introduction

The ongoing global pandemic of coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 virus has already affected the health of millions of people worldwide, with a significant number of deaths (1). Although older patients and those with comorbidities who are infected are more subject to unfavorable outcomes, reports of young people without chronic diseases who died from COVID-19 support the existence of genetic and immunological risk factors (2, 3). Also, several reports of identical twins deceased due to COVID-19 within days apart give further support to the influence of the host genome on COVID-19.

The first worldwide case of death from COVID-19, within 3 days apart, in one pair of adult unvaccinated MZ was reported in April 2020 in the United Kingdom. Aged 37, both twin sisters worked as nurses and had the same underlying health condition. Recently (2022), France's famous twin Bogdanoff's brothers died of COVID-19 6 days apart. Aged 72, the brothers had not been vaccinated against COVID-19 either.

The identification of genetic variants related to immune response, associated with higher susceptibility to the infection or severe COVID-19 has been the focus of numerous studies around the world (2, 4–8). Currently, genome-wide association studies (GWAS) have identified some genetic variants, including rare loss-of-function variants in genes involved in type I interferon (IFN) pathways (3, 9, 10) or missense variants that affect the activity of transmembrane serine protease 2 (3, 11, 12), that contribute to susceptibility or severe COVID-19, respectively. Here, we investigated a case of simultaneous critical COVID-19 in young adult MZ brothers in 2021, before being vaccinated. We present a comprehensive assessment of their innate and adaptive immunity, genetic profiling, and systemic biomarkers.

Case presentation

In June 2021, a 31-year-old Brazilian monozygotic twin brother pair (ID 01 and ID 02) started with cough and fever on the 26th. Both tested positive for SARS-CoV-2 infection on June 29th. During the following days symptoms worsened with dyspnea. From June 30th to July 4th, azithromycin was used. Blood oxygen saturation reached a critical level of 80% and the twin brothers were admitted directly to the intensive care unit on July 8th. They were intubated due to pulmonary involvement and were extubated after 5 days of mechanical ventilation (July 13th). The Gamma variant was the only SARS-CoV-2 variant identified in the region at the end of June 2021 (13) and it is known that this variant was associated with increased mortality risk and severity of COVID-19 cases in younger age groups, particularly in the unvaccinated population at the time (14). The twins received the same supportive ICU measures (sedation and proning). Also, at the hospital, both twins received the same treatment: dexamethasone (from July 9th to July 19th), to inhibit inflammation in the lungs. Due to detected resistant bacterial infections in both twins after extubation, they were treated with meropenem (from July 13th to July 20th). ID 01 was discharged on the 22nd of July and ID 02 7 days later. Both required respiratory physiotherapy for at least 3 months after hospital discharge. Seven months after the COVID-19 episode, ID 02 complained of persistent muscle fatigue, commonly associated with the post-COVID syndrome. The twins lived apart but worked at the same company as realtors. They did not have any known health conditions or comorbidities. The entire timeline of main events is presented in **Figure 1**.

Blood samples from the twins were collected in February 2022 (7 months after COVID-19 diagnosis and 4 months after getting a second dose of Pfizer-BioNTech COVID-19 vaccine) at our Research Center (HUG-CELL) for global immune profiling and genetic investigation. Peripheral blood mononuclear cells (PBMCs), plasma, and serum were obtained to perform the immunological assays and DNA for whole-exome sequencing (WES). Complementary clinical laboratory analyses were also performed in whole blood samples.

Surface immunophenotyping of PBMC was performed by flow cytometry (**Table 1**). The twins displayed normal frequencies of CD3+, CD4+, and CD8+ T-cells, monocytes, NK cells, and lymphocytes B as expected in healthy donors (15). The type I/III IFN production by PBMCs after toll-like receptor (TLR) stimulus (double-stranded RNA Poly I:C), was evaluated for 1, 4, and 8 h. Although there was heterogeneity in IFN or IFN-induced gene expression, the twins presented an early and strong (FC = 20 or higher) mRNA expression of at least two of the five types of I/III IFN analyzed. Thus, no failure in the innate IFN

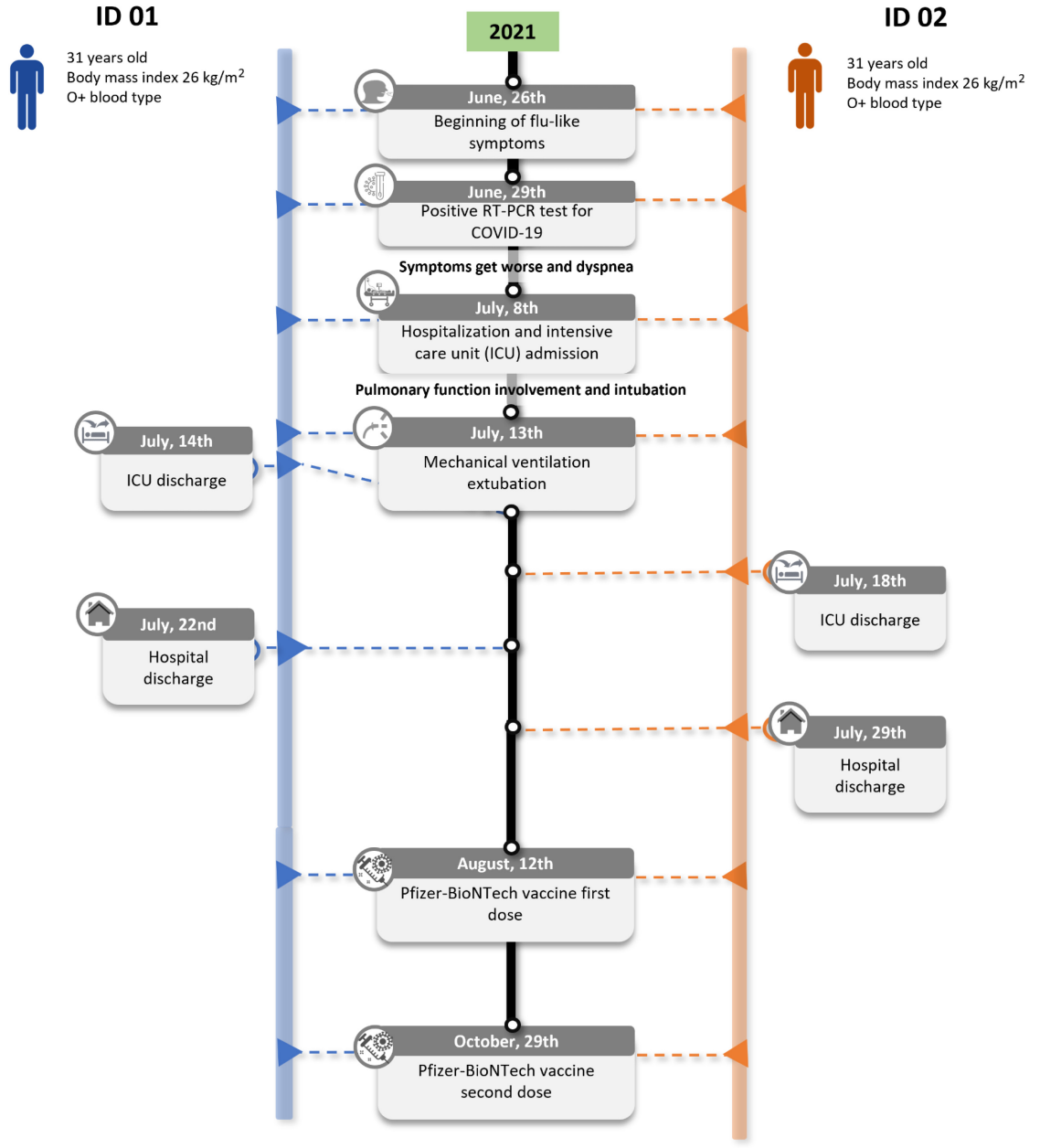

FIGURE 1
Clinical timeline of the major events of the twins' case.

response was observed. The production of interferon-gamma (IFN-γ) and interleukin-2 (IL-2) by PBMC, after stimulation by SARS-CoV-2 peptides, was also evaluated. Similar results were observed in both twins, for CD4 + T lymphocyte responses. ELISA serological assays were performed for SARS-CoV-2 IgA, IgG, and IgM for the receptor-binding domain (RBD) and nucleocapsid protein (NP) to assess their humoral immune response. The antibody profiles of SARS-CoV-2 IgA, IgM, and IgG were virtually identical between the MZ twin brothers. The global immune profiling of the twins is presented in Figure 2.

Hematologic and systemic parameters of the post-COVID phase (Table 2) revealed great similarity between the MZ twins, except for a very slight increase in creatine phosphokinase (an enzyme specific to muscle tissues, which may increase after muscle injuries) and ferritin (an acute phase reactant that can increase its serum concentration during inflammation), presented by ID 02. These findings might be related to the fatigue reported only by this twin. Likewise, both presented mild

TABLE 1 PBMCs immunophenotyping of the twin volunteers, 7 months after COVID-19 episode.

ID	CD3 + T-cells	CD4 + T-cells	CD8 + T-cells	Monocytes	NK cells	B-cells
01	56.2	63.5	31.1	7.7	8.9	9.3
02	57.5	57.7	35.3	8.0	8.8	7.7
Healthy*	45–70	25–60	5–30	10–30	5–10	5–15

*Reference parameters values for individuals in the same age range (15).

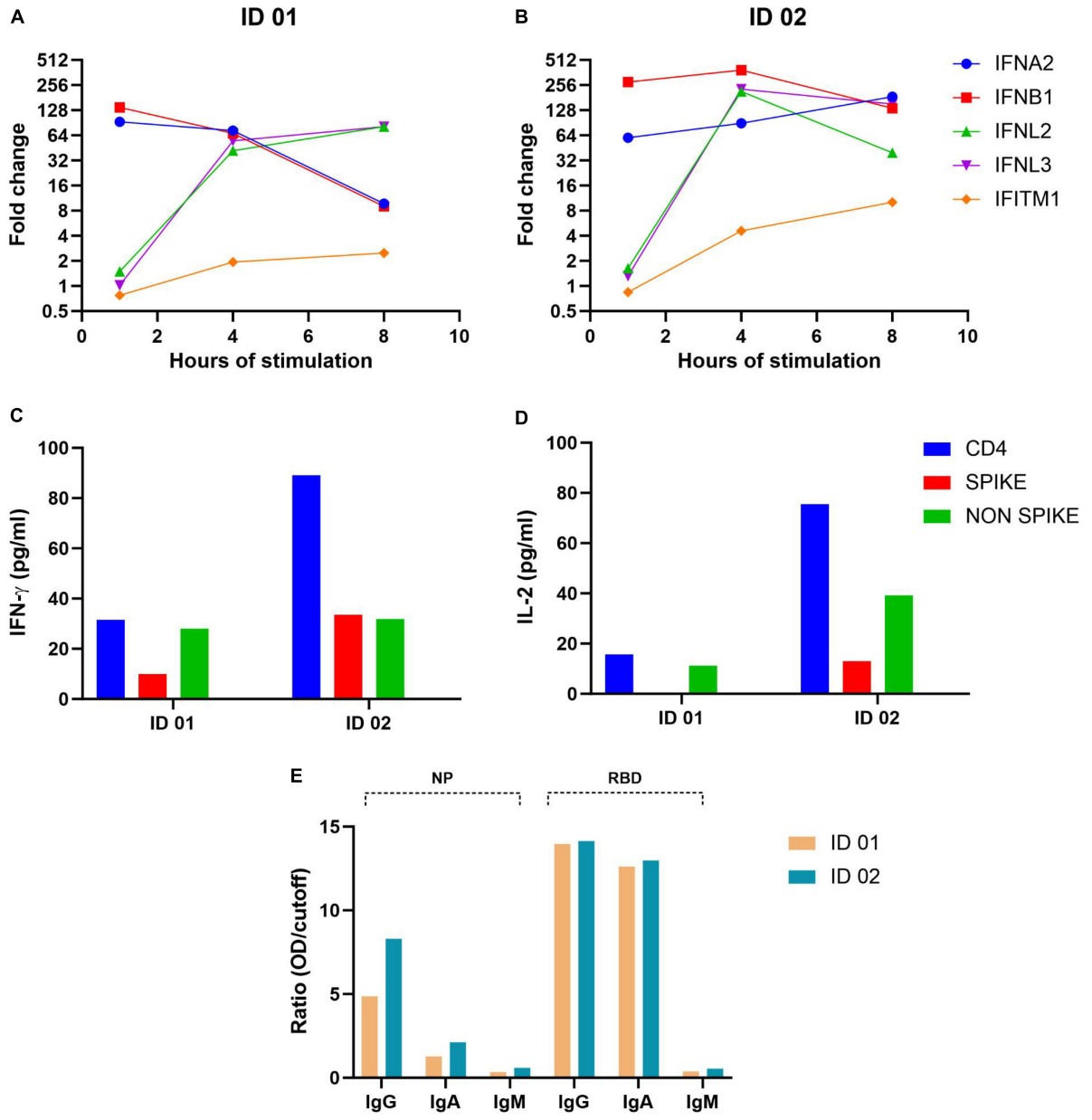

FIGURE 2
Global immune profiling of the twin volunteers, 7 months after the COVID-19 episode. **(A,B)** Type I/III IFN production by PBMCs after toll-like receptor (TLR) stimulus. **(C,D)** IFN-γ and IL-2 production by PBMC when stimulated by SARS-CoV-2 peptides. **(E)** Serological assays for SARS-CoV-2 IgA, IgG, and IgM through ELISA for the receptor-binding domain (RBD) and nucleocapsid protein (NP).

TABLE 2 Blood test parameters of the volunteers, 7 months after the COVID-19 episode.

Variables	ID 01	ID 02	Reference values
Erythrogram			
Erythrocyte/red blood cell (RBC) count (millions/mm^3)	4.64	4.57	4.30–5.70
Hemoglobin concentration (g/dL)	14.8	14.1	13.5–17.5
Hematocrit (%)	42.0	42.0	39.0–50.0
Mean cell/corpuscular volume—MCV (μ^3)	90.5	91.9	81.2–95.1
Mean cell hemoglobin—MCH (%)	31.9	30.9	26.0–34.0
Mean cell hemoglobin concentration—MCHC (pg)	35.2	33.6	31.0–36.0
RBC distribution width—RDW (%)	11.6	11.3	8.0–15.5
Erythrocyte sedimentation rate—ESR (mm/1 h)	**12**	**18**	0–6
Leukogram			
Leukocyte/white blood cell (WBC) count/mm^3	4,310	4,840	3,500–10,500
Neutrophil count/mm^3	2,030.01	2,574.88	1,700.00–8,400.00
Eosinophil count/mm^3	349.11	208.12	50.00–420.00
Basophil count/mm^3	60.34	48.40	0.00–105.00
Lymphocyte count/mm^3	1,560.22	1,669.80	900.00–3,150.00
Monocyte count/mm^3	310.32	338.80	140.00–1,260.00
Coagulation parameters			
Platelet count/mm^3	311,000	304,000	140,000–400,000
Prothrombin time (s)	10.2	10.6	9.6–12.0
Activated partial thromboplastin time—APTT (s)	32.5	32.1	22.7–32.5
D dimer (μg/mL)	0.15	0.17	0–0.50
Homeostasis parameters			
C-reactive protein—CRP (mg/dL)	0.12	0.14	0–0.60
Ferritin (ng/mL)	331.0	**512.1**	25.0–400.0
Lactate dehydrogenase—LDH (U/L)	193	198	0–250
Parameters of tissues' functions			
B-type natriuretic peptide–BNP (pg/mL)	<5	<5	<100
Troponin T (ng/mL)	<0.003	0.003	0.000–0.030 (negative)
Creatine phosphokinase—CPK (U/L)	186	**211**	0–190
Glutamic-oxaloacetic transaminase—GOT (U/L)	34	28	0–50
Glutamic pyruvic transaminase—GPT (U/L)	47	33	0–50
Urea (mg/dL)	27	28	10–50
Creatinine (mg/dL)	0.8	0.9	0.7–1.2

The parameters out of the reference values were highlighted in red.

changes in erythrocyte sedimentation rate, a parameter that may be increased in different inflammatory conditions.

WES was performed in peripheral blood DNA with the Illumina NovaSeq platform at HUG-CELL facilities. The identical twins do not carry any rare variants in genes associated with inborn errors (IE) of Toll-like receptor 3 (TLR3) and IFN regulatory factor 7 (IRF7) dependent type I IFN immunity, which underlies life-threatening COVID-19 pneumonia (5, 16). Also, we did not detect any copy number variation (CNV) in IE genes. The Neanderthal-derived genetic variant rs35044562 (17) was not detected in the twins. However, we detected two rare missense variants (with a mean CADD score > 20), one in the *BTK* gene (NM_000061:exon8:c.G684A:p.M228I) carried in homozygosity and one in the *NFKB2* gene (NM_002502:exon22:c.T2531C:p.V844A) carried in the heterozygous state, which may be linked to their increased risk of developing severe COVID-19. In addition, we analyzed the genotypes and haplotypes (**Supplementary Table 1**) of the HLA cluster in the MHC region by using a hla-mapper (version 4) (18) to optimize read alignment along the MHC region. Interestingly, the twins present the alleles HLA-A*02:01 and HLA-E*01:01 (both carried in the heterozygous state), which were associated with the high severity of COVID-19.

Discussion

Twin studies are important to investigate the contribution of genetics vs. the environment, in the susceptibility or resistance to infectious diseases as well as their pathomechanisms. Moreover, the study of the monozygotic ones may represent a powerful approach to further explore the immunological factors that contributed to the host defense. Beyond the host genotype, the individual immune response plays a determining role in

the success or failure against SARS-CoV-2 (15). The immune repertoire which is also somatically defined by mutations occurring at later stages of development could justify different disease courses, and/or outcomes even in monozygotic twins (19, 20).

The genetic causes responsible for the clinical variability associated with COVID-19 remain the subject of investigation. Worldwide genomic studies of large cohorts of individuals with different clinical manifestations have been published, suggesting the involvement of different genetic variants responsible for greater susceptibility or resistance to SARS-CoV-2. GWAS identified a potential effect of variants in the *SLC6A20*, *LZTFL1*, *CCR9*, *FYCO1*, *CXCR6,* and *XCR1* genes as responsible for greater susceptibility to SARS-CoV-2 (2, 19) in addition to variants in the genes *REXO2*, *C11orf71*, *NNMT,* and *CADM1*, involved in the immune response (21). Additionally, variants in many genes involved with the innate immune response seem to be involved in the susceptibility/predisposition to severe cases of COVID-19 such as those involved in the IFN and TLRs pathways, as well as the *ACE2* and *TMPRSS2* genes involved in virus entry into the cell (3, 7, 22). Variants in the genes *IL1B, IL1R1, IL1RN, IL6, IL17A, FCGR2A*, and *TNF* which encode cytokines would also have a possible relation with disease susceptibility and cytokine storm development. The Human Leukocyte Antigen (HLA) gene cluster and genes associated with the Major Histocompatibility Complex (MHC) are important candidates for the mechanisms of innate and adaptative immunity and susceptibility to COVID-19 infection and manifestation (23).

Interestingly the identified heterozygous *NFKB1* missense variant (NM_002502:exon22:c.T2531C:p.V844AI) and the hemizygous missense variant in the *BTK* gene (NM_000061:exon8:c.G684A:p.M228I) are the central hubs that connect proinflammatory signaling pathways for survival, proliferation, cytokine production, and lymphocyte development. Interestingly, variants in both genes have been reported in primary antibody immunodeficiencies (24, 25). However, since these variants were not studied at the protein or functional level, their pathogenicity is yet to be determined. Genetic variant in chromosome 3, previously associated with high severity cases of COVID-19 and inherited from Neanderthals (rs35044563), was not detected in both volunteers (17). Regarding the HLA complex, the twins present the alleles HLA-A*02:01 and HLA-E*01:01 (both carried in the heterozygous state), which were associated with high severity of COVID-19 (13).

The global immune profiling assays, after 7 months of the COVID-19 episode, revealed great similarities between the MZ twins. It is known that the failure to elicit a strong type I IFN response contributes to severe COVID-19 (14). Infections trigger massive T cell expansion, leading to the skewing of the TCR repertoire due to antigen−specific T cell expansion. A low clonotype sharing between MZ twins with rheumatoid arthritis that were mismatched for SARS-CoV-2 infection suggests an immune repertoire reshaping might be induced after COVID-19 (26). However, clonality and alterations of T-cell receptors' repertoires were partly associated with immune activation mediated by IFN type I and III (27) and here both twins displayed early and strong I/III IFN responses. The production of cytokines IFN-γ and IL-2 by T lymphocytes, when stimulated by SARS-CoV-2 peptides, was expressive. IL-2 and IFN-γ, which play a critical role in the activation of macrophages and other immune cells related to viral clearance, were found to be specific biomarkers of SARS-CoV-2 cellular response (28). The virus-specific antibody responses showed a vigorous IgA and IgG similar response in both twins. Since these analyses were done post-vaccination, it is not clear whether it was the viral infection or the vaccines that stimulated the production of these antibodies but it is clear there is no deficient humoral response. Taken together, regarding the immune response, all parameters analyzed were practically identical among the MZ twins.

Although both twins required intensive care and mechanical ventilation for 5 days, ID 02 required longer hospitalization and presented long-term symptoms consistent with long COVID. After 7 months of follow-up, twin ID 02 reported persistent muscle weakness and fatigue, while twin ID 01 referred to a return to his usual state of health. Muscle dysfunction (intense fatigue) is among the most reported symptoms of the post-COVID syndrome (17). The laboratory values obtained at 7 months demonstrated that twin ID 02 had an elevation in ferritin and CPK but otherwise had similar hematological and functional parameters relative to twin ID 01. Importantly, the twins were not on any medications or supplements. The CK levels at admission are reported to be higher in those subjects who later experience more severe outcomes and were associated with a worse prognosis (20) while severe to critical COVID-19 patients showed higher ferritin levels compared to mild to moderate COVID-19 patients (29). Thus, the slightly abnormal ferritin and CPK from ID 02 even after 7 months post-hospitalization might play a role in the pathogenesis of post-acute sequelae of COVID-19.

Conclusion

This case study on two young-adult monozygotic twins simultaneously infected with SARS-CoV-2, both requiring ICU care but with different periods of clinical progression suggests the contribution of both immune response and the genetics in the COVID-19 presentation and course. Besides, the post-COVID syndrome was observed in one of them, corroborating an association between the duration of hospitalization and the occurrence of long-COVID symptoms.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by CAAE 34786620.2.0000.5464. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MC and MVRS: data curation, investigation, formal analysis, visualization, and writing—original draft. FS: data curation and investigation. VC and EC-N: writing—review and editing. MN: conceptualization, formal analysis, investigation, methodology, software, and writing—review and editing. MOS and EC: formal analysis, investigation, methodology, software, and writing—review and editing. JO and GS: methodology and writing—review and editing. KS: investigation, visualization, and writing—review and editing. JK: funding acquisition, resources, and writing—review and editing. MZ: conceptualization, funding acquisition, project administration, writing—original draft, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1008585/full#supplementary-material

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Comparison of vaccine-induced antibody neutralization against SARS-CoV-2 variants of concern following primary and booster doses of COVID-19 vaccines

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The SARS-CoV-2 pandemic has, as of July 2022, infected more than 550 million people and caused over 6 million deaths across the world. COVID-19 vaccines were quickly developed to protect against severe disease, hospitalization and death. In the present study, we performed a direct comparative analysis of four COVID-19 vaccines: BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna), ChAdOx1 (Oxford/AstraZeneca) and Ad26.COV2.S (Johnson & Johnson/Janssen), following primary and booster vaccination. We focused on the vaccine-induced antibody-mediated immune response against multiple SARS-CoV-2 variants: wildtype, B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and B.1.1.529 (Omicron). The analysis included the quantification of total IgG levels against SARS-CoV-2 Spike, as well as the quantification of antibody neutralization titers. Furthermore, the

study assessed the high-throughput ACE2 competition assay as a surrogate for the traditional pseudovirus neutralization assay. The results demonstrated marked differences in antibody-mediated immune responses. The lowest Spike-specific IgG levels and antibody neutralization titers were induced by one dose of the Ad26.COV2.S vaccine, intermediate levels by two doses of the BNT162b2 vaccine, and the highest levels by two doses of the mRNA-1273 vaccine or heterologous vaccination of one dose of the ChAdOx1 vaccine and a subsequent mRNA vaccine. The study also demonstrated that accumulation of SARS-CoV-2 Spike protein mutations was accompanied by a marked decline in antibody neutralization capacity, especially for B.1.1.529. Administration of a booster dose was shown to significantly increase Spike-specific IgG levels and antibody neutralization titers, erasing the differences between the vaccine-induced antibody-mediated immune response between the four vaccines. The findings of this study highlight the importance of booster vaccines and the potential inclusion of future heterologous vaccination strategies for broad protection against current and emerging SARS-CoV-2 variants.

KEYWORDS

COVID-19, SARS-CoV-2, vaccines, antibodies, immunity, neutralization, booster, omicron

Introduction

At the end of 2019, a highly transmissible, pathogenic and novel coronavirus emerged, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), causing the Coronavirus Disease 2019 (COVID-19) pandemic. As of July 2022, the SARS-CoV-2 pandemic has led to ∼550 million confirmed cases and caused over 6 million deaths across the world (1). Under the pressure of the COVID-19 pandemic, multiple effective vaccines were quickly developed to protect against severe disease, hospitalization and death (2–5). By July 2022, more than 12 billion COVID-19 vaccine doses had been administered globally (1).

The vaccination program against SARS-CoV-2 in Denmark started in late December 2020 with the rollout of the two mRNA-based vaccines, BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna), and shortly thereafter an adenoviral vector-based vaccine, ChAdOx1 (Oxford/AstraZeneca). In March 2021, the Danish Health Authority decided to exclude ChAdOx1 from the vaccination program due to a possible link between the vaccine and a rare syndrome, now designated vaccine-induced immune thrombotic thrombocytopenia (VITT) (6, 7). Recipients of one dose of ChAdOx1 were offered heterologous vaccination with a second dose of an mRNA vaccine (BNT162b2 or mRNA-1273). Due to the risk of an equivalent link between Ad26.COV2.S (Johnson & Johnson/Janssen), another adenoviral vector-based vaccine, and VITT (7, 8), the Danish Health Authority decided to only administer Ad26.COV2.S through a voluntary system outside of the Danish national vaccination program.

The majority of COVID-19 vaccines were developed as two dose regimens (one dose for Ad26.COV2.S) and made on the basis of the original Wuhan-Hu-1 sequence of the SARS-CoV-2 Spike (S) protein (9). Since the end of 2020, a series of novel variants of concern (VOCs) have emerged, including B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and B.1.1.529 (Omicron), causing new waves of infections worldwide.

Currently, B.1.1.529 has become the dominant SARS-CoV-2 strain globally with a greater number of mutations than previous VOCs and several divergent sub-lineages (10). These mutations include 15 clustered in the receptor-binding domain region of the S protein, which is the main target of neutralizing antibodies after SARS-CoV-2 infection and vaccination. Nine of these mutations map to the angiotensin-converting enzyme 2 (ACE2) receptor-binding motif enhancing the binding affinity of ACE2 to the receptor-binding domain of B.1.1.529 (11). This leads to significantly increased transmissibility, unprecedented abilities to evade immunity by displaying almost complete resistance toward the majority of monoclonal antibodies and a substantial loss of neutralizing potency. Consequently, this reduces the efficacy of COVID-19 vaccines (12–14).

Along with documentation of waning immunity over time post-vaccination (15, 16), several studies have shown that prior SARS-CoV-2 infection and primary COVID-19 vaccination was insufficient for protection against infection

with B.1.1.529. This was demonstrated by non-quantifiable neutralization titers *in vitro*, and higher rates of reinfection and vaccine breakthrough cases (13, 14, 17–19). Vaccine efficacy 3–4 months after two doses of BNT162b2 has been shown to drop from 74.4% against B.1.617.2 to 15.4% against B.1.1.529 and similar observations were shown for mRNA-1273 and ChAdOx1 (20). Administration of a booster dose was demonstrated to increase and prolong vaccine-induced neutralizing antibody potency against B.1.1.529, thus contributing to sustain control of the evolving pandemic (13, 14, 17, 18, 20).

In the present study, we performed a direct comparative analysis of vaccine-induced total immunoglobulin G (IgG) levels and antibody neutralization titers against different SARS-CoV-2 variants: wildtype (wt), B.1.1.7, B.1.351, B.1.617.2, and B.1.1.529, following primary and booster vaccination with four COVID-19 vaccines: BNT162b2, mRNA-1273, ChAdOx1 and Ad26.COV2.S, in a healthy sub-population of the Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 vaccines (ENFORCE). Furthermore, considering the marked resistance against antibody-mediated neutralization demonstrated by B.1.1.529, the high-throughput, cell- and virus-free ACE2 competition assay was assessed as a surrogate for the pseudovirus neutralization assay. This assay has the potential of measuring lower antibody neutralization capacity with a multiplex readout of different SARS-CoV-2 variants from several samples in 1 day and without the requirement of biosafety level 2 facilities.

Materials and methods

The Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 vaccines (ENFORCE) was designed as an open-label, non-randomized, parallel group, phase IV study. The study enrolled adults in Denmark prior to their first COVID-19 vaccination offered through the Danish vaccination program (clinicaltrails.gov, identifier: NCT04760132). The enrollment took place at seven study sites, covering all five Danish regions, from February to August 2021. The study protocol was approved by the Danish Medicines Agency (#2020-006003-42) and the National Committee on Health Research Ethics (#1-10-72-337-20). All participants provided written informed consent. The ENFORCE cohort has previously been described by Søgaard et al. (21) and Stærke et al. (22).

The present study was a part of the ENFORCE sub-studies, with the primary objective to quantify and compare the neutralizing capacity of vaccine-induced antibodies following primary and booster doses of different COVID-19 vaccines among a healthy sub-population of the ENFORCE participants.

Study design and data collection

This study included study participants from the ENFORCE cohort vaccinated with BNT162b2, mRNA-1273, ChAdOx1 and Ad26.COV2.S. Approximately 25 individuals from each COVID-19 vaccine group, that met the following criteria were randomly selected for inclusion in the sub-study: (1) aged from 18 to 65 years, (2) a Charlson Comorbidity Index score of zero, and (3) data collected at the third study visit (90 days ± 14 days after first vaccination). Information on age, sex, medical history, vaccine priority group, vaccination dates and vaccine type were collected and confirmed by the Danish National Patient Registry and the Danish Vaccination Registry. Serum and plasma samples drawn at the third study visit and the Xc study visit (28 days ± 8 days after booster vaccination) were used to quantify the COVID-19 vaccine-induced antibody response.

Study participants vaccinated with an mRNA vaccine, BNT162b2 or mRNA-1273, received a booster vaccine homologous to the primary vaccine, while participants vaccinated with an adenoviral vector vaccine, ChAdOx1 or Ad26.COV2.S, received an mRNA booster vaccine.

Blood samples from SARS-CoV-2 recovered individuals, infected with SARS-CoV-2 wt at the start of the pandemic (March/April 2020), were collected as part of the CoroNAT study and were used herein as convalescent comparators. The CoroNAT study protocol was approved by the National Committee on Health Research Ethics (#1-10-72-76-20). All participants provided written informed consent (23). Individuals with verified SARS-CoV-2 infection, defined as Spike IgG positive at enrollment (data from Statens Serum Institut) or any previous positive SARS-CoV-2 PCR (data extracted from the Key Infectious Diseases System database and the Danish National Microbiology database) were excluded from the vaccine comparison.

Quantification of SARS-CoV-2-spike IgG

To detect and quantify IgG responses against multiple SARS-CoV-2 VOCs, we utilized a highly sensitive, electro chemiluminescent immunoassay from Meso Scale Discovery (MSD) (Meso Scale Diagnostics LLC, Maryland, USA). Multi-spot, 96-well, V-PLEX plates coated with purified antigens were used for the detection of IgG antibodies against SARS-CoV-2-Spike (SARS-CoV-2-S) wt (Wuhan-Hu-1), B.1.1.7, B.1.351, B.1.617.2, and B.1.1.529; BA.1, BA.2, and BA.3 [V-PLEX SARS-CoV-2 Panel 13 (IgG) kit (K15463U-2) and Panel 25 (IgG) kit (K15583U-2)]. The assays were performed according to the manufacturer's protocol.

Serum or plasma samples were diluted 1:5,000 in diluent buffer, along with a fourfold seven-point dilution of the reference standard and a blank. Plates were read on a

MESO SECTOR S600 Reader. Raw data was processed by MSD Discovery Workbench Software (Version 4.0). Total IgG concentrations were calculated by fitting the electro chemiluminescence signals to the corresponding calibration curves. Quantifications were reported in units per mL (U/mL).

Production of SARS-CoV-2 pseudoviruses

Pseudoviruses with SARS-CoV-2-S were produced according to methods previously described by Nielsen et al. (23). Sub-confluent HEK-293T cells were transfected by polyethylenimine with the S protein expressing plasmid (pCG1-SARS-CoV-2-S wt (Wuhan-Hu-1 including D614G), B.1.1.7, B.1.351, B.1.617.2, and B.1.1.529; BA.1) for 18 h. Following, the cells were transduced with VSV-ΔG pseudovirus (vesicular stomatitis virus which lacks the VSV glycoprotein gene) expressing a green fluorescent protein (GFP) reporter gene (multiplicity of infection = 3). After 2 h of infection, the cells were washed to remove residual virus and fresh medium added. Exceptionally, for the production of pseudoviral particles incorporating the S protein of B.1.1.529; BA.1, anti-VSV-G from I1 hybridoma cells was added to the medium to neutralize remaining virus. Supernatants were collected after 24 h, centrifuged, aliquoted and stored at −80°C. A VSV-ΔG-mock was produced synchronously to allow subtraction of any background.

Pseudovirus neutralization assay

To determine the neutralizing potency of COVID-19 vaccine-induced antibodies, we performed a neutralization assay with VSV-ΔG-SARS-CoV-2-S pseudovirus. Heat-inactivated plasma samples were fivefold eight-point diluted in medium and mixed with the pseudovirus for 1 h. Sub-confluent Vero76 c-myc cells expressing human TMPRSS2 (Transmembrane Serine Protease 2) were incubated with plasma and pseudovirus for ∼18 h, yielding a final plasma dilution of 1:25-1:1,953,125. The cells were washed, trypsinized and fixed in 2% paraformaldehyde, before GFP expression was determined on a Miltenyi Biotec MACSquant 16 flow cytometer. All samples were run in duplicates and virus-only positive controls and cell-only negative controls were included in each assay. The VSV-ΔG-mock background signal was subtracted from all samples.

The measured GFP expression was analyzed using FlowJo (Version 10.8.0). The half maximal neutralization titers (NT50) were reported as the plasma dilution at which infectivity of the pseudovirus was inhibited by 50% relative to the virus-only positive controls. NT50 values were calculated using an inhibitor vs. dose-response curve fit with non-linear regression with a hill slope of −1.0 by GraphPad Prism Software (Version 9.3.1). NT50 was non-quantifiable in cases of less than 95% inhibition of infection in the wells of the least diluted plasma, 1:25. All samples with non-quantifiable NT50 values or calculated values < 25 were adjusted to the lowest plasma dilution factor, NT50 = 25.

Quantification of ACE2 receptor blocking

A multiplexed MSD immunoassay was used to measure the ability of vaccine-induced antibodies in serum or plasma to block ACE2 binding to SARS-CoV-2-S. Thereby evaluating the functional potential of neutralizing antibodies to compete with the ACE2 receptor for binding to SARS-CoV-2-S. Multi-spot, 96-well, V-PLEX plates coated with SARS-CoV-2-S wt (Wuhan-Hu-1), B.1.1.7, B.1.351, B.1.617.2, and B.1.1.529; BA.1, BA.2, and BA.3, were used for the quantification of ACE2 receptor blocking [V-PLEX SARS-CoV-2 Panel 13 (ACE2) kit (K15466U-2) and Panel 25 (ACE2) kit (K15586U-2)]. The assays were performed according to the manufacturer's protocol.

Serum or plasma samples were diluted 1:10 and 1:100 in diluent buffer. For panel 13 assays, an ACE2 calibration reagent provided by the manufacturer was added, but no calibration reagent was provided for panel 25. Plates were read on a MESO SECTOR S600 Reader. Raw data was processed by MSD Discovery Workbench Software (Version 4.0). Quantifications were reported in U/mL and percentage of ACE2 receptor blocking for panel 13 and in percentage of ACE2 receptor blocking for panel 25.

Data and statistical analysis

Demographic characteristics at enrollment of the included participants in this study were analyzed by Chi-squared tests (categorical variables) and one-way ANOVA tests (continuous variables).

Boxplots, showing the median along with the lower and upper quartiles, as well as error bars indicating 95% CI, were used to present all data. Data obtained from MSD immunoassays and pseudovirus neutralization assays were compared using Mann-Whitney tests (two groups) and Kruskal-Wallis tests ($\geq$ three groups). Wilcoxon tests were used to compare the effect of a booster dose. All statistical tests were followed by a *post hoc* Dunn's multiple comparisons test adjusted using Bonferroni correction. *P*-values ≤ 0.05 were considered statistically significant. *P*-values were denoted as follows: $^{*} = p \leq 0.05$, $^{**} = p < 0.01$, $^{***} = p < 0.001$, and $^{****} = p < 0.0001$.

Spearman's rank correlation test was used to assess the correlation between NT50 values measured by the pseudovirus

neutralization assay and ACE2 receptor blocking measured by the ACE2 competition assay.

Data analysis and visualization was conducted in R (Version 4.0.4) and RStudio Desktop (Version 1.4.1106).

Results

In a direct comparison, we assessed the capacity of four COVID-19 vaccines to produce Spike-specific (S-specific) antibodies and induce antibody-mediated neutralization of SARS-CoV-2-S. A total of 96 healthy individuals from the ENFORCE cohort were included in this sub-study. However, to focus on the vaccine-induced antibody response, we excluded previously infected individuals ($n = 11$), eliminating the impact of antibodies generated by previous infection. The vaccine-type comparative analysis included 85 individuals (61.2% females): 22 vaccinated with two doses of BNT162b2 (median age of 54 years), 24 vaccinated with two doses of mRNA-1273 (median age of 54.5 years), 20 heterologous vaccinated with one dose of ChAdOx1 and a second dose of an mRNA vaccine (median age of 45 years), and 19 vaccinated with one dose of Ad26.COV2.S (median age of 33 years). A total of 25 SARS-CoV-2 recovered individuals infected with the original SARS-CoV-2 variant (median age of 47 years) were used in this study as convalescent comparators. The demographic characteristics of the study participants at enrolment are shown in **Table 1**.

Levels of SARS-CoV-2-S IgG after COVID-19 vaccination

Serum samples were collected at the third study visit (90 days ± 14 days after first vaccination) and SARS-CoV-2-S IgG antibodies were quantified.

The median IgG antibody levels specific for SARS-CoV-2-S wt were highest for ChAdOx1/mRNA: 503,992 U/mL [IQR: 359,170-709,457] followed by mRNA-2173: 471,670 U/mL [364,131–692,740] and BNT162b2: 251,511 U/mL [199,365–376,470]. In contrast, significantly lower IgG antibody levels were detected for Ad26.COV2.S: 16,241 U/mL [12,664–29,986], which were comparable with the convalescent individuals: 27,497 U/mL [8,875–55,419] (**Figure 1**).

The quantification of SARS-CoV-2-S IgG specific for B.1.1.7, B.1.617.2, and B.1.351 demonstrated similar vaccine-induced antibody responses as observed for SARS-CoV-2-S wt. Generally, all vaccine recipients had slightly lower levels of B.1.1.529; BA.1 S-specific IgG compared with the other variants. As observed for SARS-CoV-2-S wt, the quantifications displayed significantly higher median antibody titers for BNT162b2, mRNA-1273 and ChAdOx1/mRNA compared with Ad26.COV2.S recipients for all included variants (**Figure 1**).

Neutralizing antibody responses to pseudoviral SARS-CoV-2-S after COVID-19 vaccination

Plasma samples collected at the third study visit were used to analyze the neutralizing capacity of vaccine-induced antibodies by a pseudovirus neutralization assay employing VSV-ΔG-SARS-CoV-2-S pseudovirus and NT50 values were determined.

Correspondingly, as demonstrated for SARS-CoV-2-S IgG levels, the highest NT50 values for SARS-CoV-2-S wt were determined for recipients of ChAdOx1/mRNA: median: 4,292 [IQR: 1,639–11,377] followed by mRNA-1273: 1,285 [466–3,078] and BNT162b2: 643 [300–1,278]. Significantly lower NT50 values were determined for recipients of Ad26.COV2.S: 79 [25–182], which were on par with the convalescent comparators: 182 [60–324]. Additionally, significantly higher NT50 values were observed in the ChAdOx1/mRNA vaccine group compared with the BNT162b2 vaccine group (P-value = 0.012) (**Figure 2A**).

The assessment of SARS-CoV-2-S neutralizing antibodies specific for B.1.1.7, B.1.617.2, and B.1.351 showed a similar order of vaccine-induced neutralizing antibody responses as observed for SARS-CoV-2-S wt. The analysis of SARS-CoV-2-S B.1.1.529; BA.1 showed lower NT50 values and did not display a similar ranking of neutralizing antibody responses (**Figure 2A**).

All data was merged irrespectively of vaccine type and antibody neutralization capacity was assessed with focus on the different SARS-CoV-2 variants. The highest NT50 values were observed for SARS-CoV-2-S wt: 577 [180–1,906], while NT50 values decreased progressively with an increasing number of S protein mutations [B.1.1.7: 348 (121–1,296), B.1.617.2: 225 (25–610), B.1.351: 84 (25–300) and B.1.1.529; BA.1: 25 (25–86)] (**Figure 2B**).

The pseudovirus neutralization assay used a lowest plasma dilution factor of 1:25. The assay was therefore unable to determine antibody neutralization capacity for samples with poor neutralizing activity. In compliance with a decrease in antibody neutralization capacity, a higher frequency of non-quantifiable NT50 values was observed with an increasing number of S protein mutations. Consequently, 69% of samples analyzed for B.1.1.529; BA.1 were below the assay cut-off of 25-fold dilution (**Figure 2C**).

ACE2 competition assay as a surrogate for quantifying antibody neutralization capacity

The pseudovirus neutralization assay facilitated examination of the neutralizing potency of antibodies. However, the assay was unable to estimate the low antibody neutralization titers observed for B.1.1.529. In consequence,

TABLE 1 Demographic characteristics.

	BNT162b2 ($N = 22$)	mRNA-1273 ($N = 24$)	ChAdOx1/mRNA ($N = 20$)	Ad26.COV2.S ($N = 19$)	Convalescent ($N = 25$)	Overall ($N = 110$)
Sex						
Male	8 (36.4%)	12 (50.0%)	5 (25.0%)	9 (47.4%)	20 (80.0%)	54 (49.1%)
Female	14 (63.6%)	12 (50.0%)	15 (75.0%)	10 (52.6%)	5 (20.0%)	56 (50.9%)
Age (years)						
Median	54.0	54.5	45.0	33.0	47.1	48.0
[min, max]	[19.0, 64.0]	[40.0, 63.0]	[19.0, 60.0]	[23.0, 47.0]	[26.0, 67.8]	[19.0, 67.8]
Priority group						
Health care professionals	9 (40.9%)	0 (0%)	20 (100%)	0 (0%)	4 (16%)	33 (30%)
General population	13 (59.1%)	24 (100%)	0 (0%)	19 (100%)	21 (84%)	77 (70%)
Days from 1st vaccine to 3rd study visit						
Median	92.5	91.0	98.5	92.0	108*	95.5
[min, max]	[83.0, 102]	[79.0, 99.0]	[84.0, 114]	[42.0, 101]	[75.0, 119]	[42.0, 119]
Days from 2nd Vaccine to 3rd study visit						
Median	62.0	56.0	19.0	NA	NA	54.0
[min, max]	[45.0, 79.0]	[44.0, 64.0]	[7.00, 35.0]	[NA, NA]	[NA, NA]	[7.00, 79.0]

* Days from confirmed positive SARS-CoV-2 PCR to study visit.

Booster dose evaluation	BNT162b2 ($N = 11$)	mRNA-1273 ($N = 12$)	ChAdOx1/mRNA ($N = 5$)	Ad26.COV2.S/mRNA ($N = 6$)	($N = 34$)
Sex					
Male	5 (45.5%)	5 (41.7%)	1 (20.0%)	2 (33.3%)	13 (38.2%)
Female	6 (54.5%)	7 (58.3%)	4 (80.0%)	4 (66.7%)	21 (61.8%)
Age (years)					
Median	55.0	54.5	55.0	27.0	54.0
[min, max]	[22.0, 64.0]	[48.0, 62.0]	[31.0, 60.0]	[23.0, 34.0]	[22.0, 64.0]
Days from 2nd vaccine to booster vaccine					
Median	190	169	163	161**	170
[min, max]	[146, 273]	[161, 204]	[160, 189]	[128,169]	[128, 273]
Days from booster vaccine to Xc study visit					
Median	28.0	29.5	26.0	25.0	27.5
[min, max]	[21.0, 75.0]	[22.0, 45.0]	[21.0, 30.0]	[15.0, 41.0]	[15.0, 75.0]

**Days from 1st vaccine to booster vaccine.

an ACE2 competition assay was assessed as a surrogate for the pseudovirus neutralization assay with the potential of measuring vaccine-induced antibody neutralization capacity at lower levels. Serum samples were therefore used to quantify the neutralizing capacity of vaccine-induced antibodies reported as SARS-CoV-2-S ACE2 receptor-blocking antibodies in U/mL and as percentage of ACE2 receptor blocking.

In comparison to the findings of SARS-CoV-2-S wt IgG levels and NT50 values, a similar ranking of vaccine-induced responses against SARS-CoV-2-S wt was detected utilizing the ACE2 competition assay for both ACE2 receptor-blocking antibodies in U/mL and percentage of ACE2 receptor blocking (**Figures 3A,B**, respectively). A very strong positive correlation was observed for SARS-CoV-2 wt between NT50 values quantified by the pseudovirus neutralization assay and the ACE2 competition assay for both the calculated concentration of ACE2 receptor-blocking antibodies ($\rho = 0.88$, P-value < 0.0001, **Figure 3C**) and for the percentage of ACE2 receptor blocking ($\rho = 0.87$, P-value < 0.0001, **Figure 3D**).

This strong positive correlation between pseudovirus neutralization and the calculated concentration of ACE2 receptor-blocking antibodies both in U/mL or the percentage of ACE2 receptor blocking was also observed for SARS-CoV-2 S-specific for B.1.1.7, B.1.617.2, and B.1.351 (**Supplementary Figures 1A,B**, respectively).

The ACE2 competition assay was utilized to measure antibody neutralization capacity for SARS-CoV-2-S B.1.1.529; BA.1, BA.2, and BA.3. The quantification of ACE2 receptor blocking demonstrated the same ranking of vaccine-induced responses as shown previously. As demonstrated for the levels of SARS-CoV-2-S IgG and NT50 values, the ACE2 competition data showed significantly higher percentages of ACE2 receptor blocking in individuals vaccinated

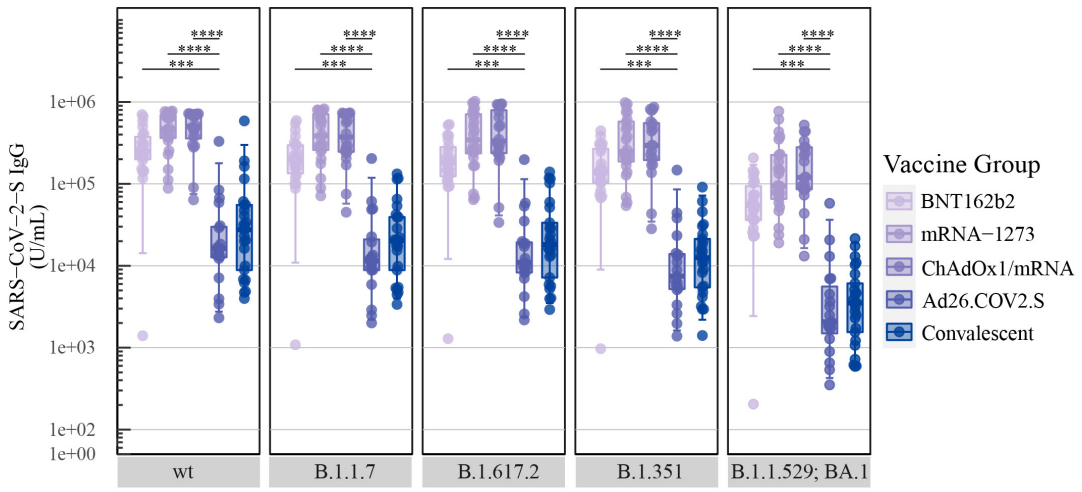

FIGURE 1
Levels of SARS-CoV-2-S IgG after COVID-19 vaccination: Levels of total SARS-CoV-2-S IgG in U/mL induced by primary COVID-19 vaccination with BNT162b2 ($n = 22$), mRNA-1273 ($n = 24$), ChAdOx1/mRNA ($n = 20$) or Ad26.COV2.S ($n = 19$) quantified by the MSD platform (serum 1:5,000). Data from convalescent comparators ($n = 25$) are also displayed, but are not included in the statistical analysis. From left to right: SARS-CoV-2-S wt (Wuhan-Hu-1) and the following SARS-CoV-2-S VOCs: B.1.1.7 (Alpha), B.1.617.2 (Delta), B.1.351 (Beta) and B.1.1.529; BA.1 (Omicron). The boxplots present the lower quartile, median and upper quartile, and the error bars indicate 95% CI. *P*-values were indicated as follows: ***$p < 0.001$ and ****$p < 0.0001$.

with either one of the two mRNA vaccines or with ChAdOx1/mRNA compared with Ad26.COV2.S recipients (Figure 4A).

Further, when merging all data irrespectively of vaccine type, a significant reduction in the percentage of ACE2 receptor blocking was observed for B.1.1.529 [BA.1: 25.7% (IQR: 3–74), BA.2: 34.2% (0–84) and BA.3: 31.7% (0–77)] compared with previous SARS-CoV-2 VOCs [wt: 98.9% (67–100), B.1.1.7: 96.2% (60–99), B.1.617.2: 95.7% (53–99) and B.1.351: 84.8% (32–98)] (*P*-value < 0.0001) (Figure 4B). Again, these findings demonstrate that accumulation of S protein mutations was accompanied by a gradual decline of vaccine-induced neutralizing antibody capacity.

Levels of SARS-CoV-2-S IgG and ACE2 receptor blocking after COVID-19 booster vaccination

Serum samples were collected at the Xc study visit (28 days ± 8 days after booster vaccination) and SARS-CoV-2-S IgG levels and the percentage of ACE2 receptor blocking was quantified.

The booster vaccination caused a small increment in SARS-CoV-2-S IgG levels of B.1.1.529 sub-variants (B.1.1.529; BA.1: primary vaccination: 78,014 vs. booster vaccination: 111,694 U/mL, BA.2: 77,074 vs. 109,595 U/mL and BA.3: 53,315 vs. 79,917 U/mL) (Figure 5A). In concordance with the increase of S-specific IgG following administration of a booster vaccine, a significant increase was observed in ACE2 receptor blocking of B.1.1.529 sub-variants (B.1.1.529; BA.1: 56.5 vs. 89.6%, BA.2: 73.9 vs. 93.2% and BA.3: 62.8 vs. 91.6%) (Figure 5B).

Additionally, when assessing the vaccine-induced antibody-mediated immune response after administration of a booster dose, all previously displayed vaccine-induced differences were no longer present. SARS-CoV-2-S IgG levels and antibody neutralization titers in the form of percentage of ACE2 receptor blocking were equalized, in such manner that no significant differences were observed between the four COVID-19 vaccine-induced antibody responses for both SARS-CoV-2-S wt and B.1.1.529 sub-variants (Supplementary Figure 2 and Figure 5C, respectively).

Discussion

In this study, we presented a direct comparative analysis of four COVID-19 vaccines: BNT162b2, mRNA-1273, ChAdOx1 and Ad.26COV2.S, following primary and booster vaccination, focusing on the vaccine-induced antibody-mediated immune response against diverse SARS-CoV-2 variants: wt, B.1.1.7, B.1.617.2, B.1.351, and B.1.1.529; BA.1, BA.2, and BA.3.

This study demonstrated significantly higher SARS-CoV-2-S IgG levels and antibody neutralization titers in individuals vaccinated with BNT162b2, mRNA-1273 and ChAdOx1/mRNA compared with recipients of Ad26.COV2.S for all SARS-CoV-2

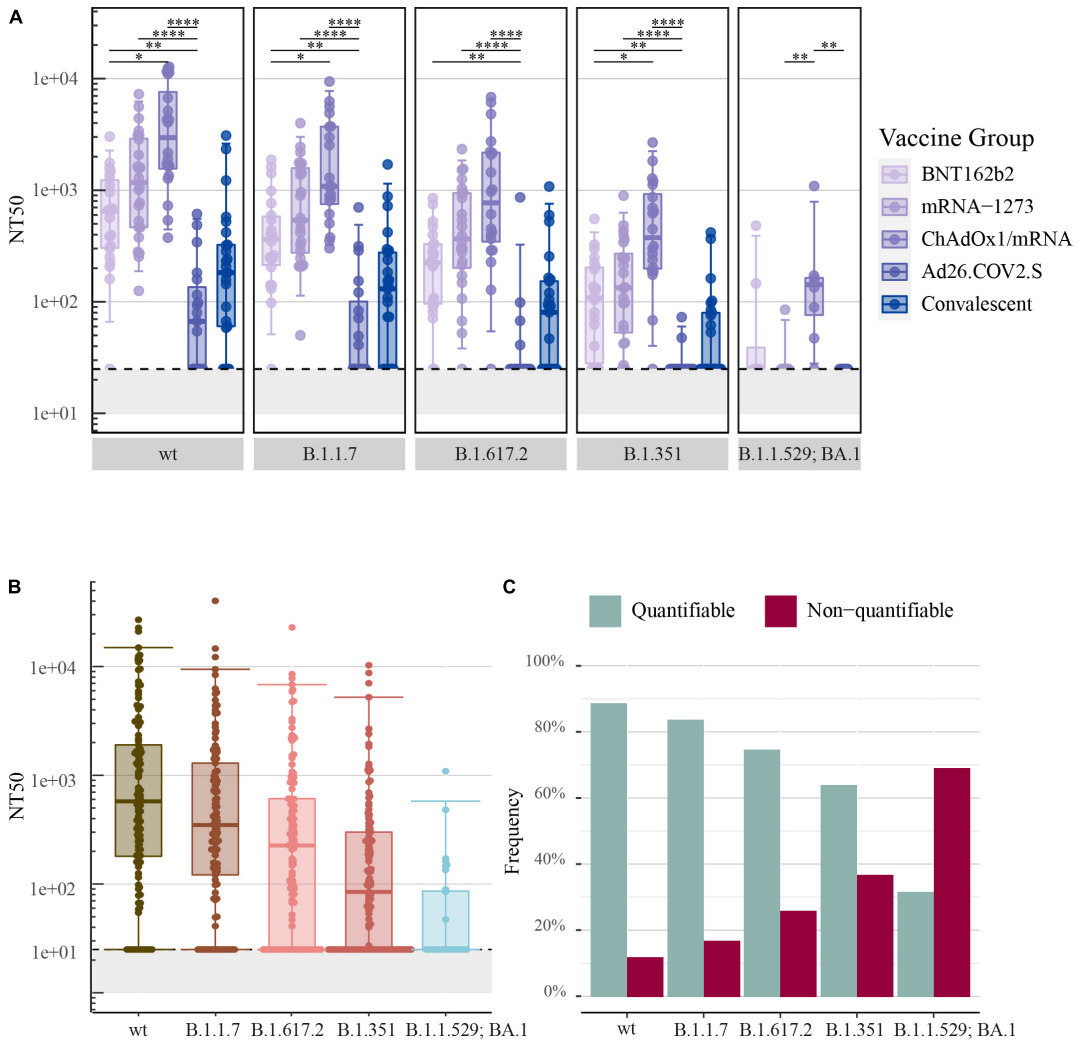

FIGURE 2
Neutralizing antibody responses to pseudoviral SARS-CoV-2-S after COVID-19 vaccination: **(A)** The 50% neutralization titers (NT50) induced by primary COVID-19 vaccination with BNT162b2 ($n = 22$), mRNA-1273 ($n = 24$), ChAdOx1/mRNA ($n = 20$) or Ad26.COV2.S ($n = 19$) quantified by the pseudovirus neutralization assay. Data from convalescent comparators ($n = 25$) are also displayed, but are not included in the statistical analysis. **(B)** NT50 values merged for all vaccine types. **(C)** The frequency of quantifiable (> 25) and non-quantifiable (≤ 25) NT50 values merged for all vaccine types. From left to right: SARS-CoV-2-S wt (Wuhan-Hu-1 including D614G) and the following SARS-CoV-2-S VOCs: B.1.1.7 (Alpha), B.1.617.2 (Delta), B.1.351 (Beta) and B.1.1.529; BA.1 (Omicron) (B.1.1.529; BA.1, $n = 32$: eight individuals per vaccine group). All boxplots present the lower quartile, median and upper quartile, and the error bars indicate 95% CI. *P*-values were indicated as follows: $^{*}p \leq 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$.

variants. We also showed that accumulation of S protein mutations of SARS-CoV-2 was accompanied by a gradual decline in antibody neutralization capacity, particularly demonstrating a marked decline against B.1.1.529. In addition, administration of a booster vaccine was shown to induce increasing levels of SARS-CoV-2-S IgG and a higher percentage of ACE2 receptor blocking against B.1.1.529 sub-variants. The vaccine-type comparative analysis after administration of a booster dose showed that the vaccine-induced SARS-CoV-2-S IgG levels and antibody neutralization titers reached similar levels, to the point were no significant differences between the four COVID-19 vaccines were detected.

All four COVID-19 vaccines evaluated in this study have been administered to reduce the incidence of COVID-19 infections and have been invaluable in reducing and preventing severe disease, hospitalization and death. Phase three trials have demonstrated that all four vaccines have high clinical efficacy against the original SARS-CoV-2 variant with mRNA-based vaccines demonstrating greater efficacy than adenoviral vector-based vaccines (3–5, 24).

The vaccine efficacy of BNT162b2 and mRNA-1273 was nearly equivalent in phase three trials, though subsequent real-world vaccine studies, including our study, have shown

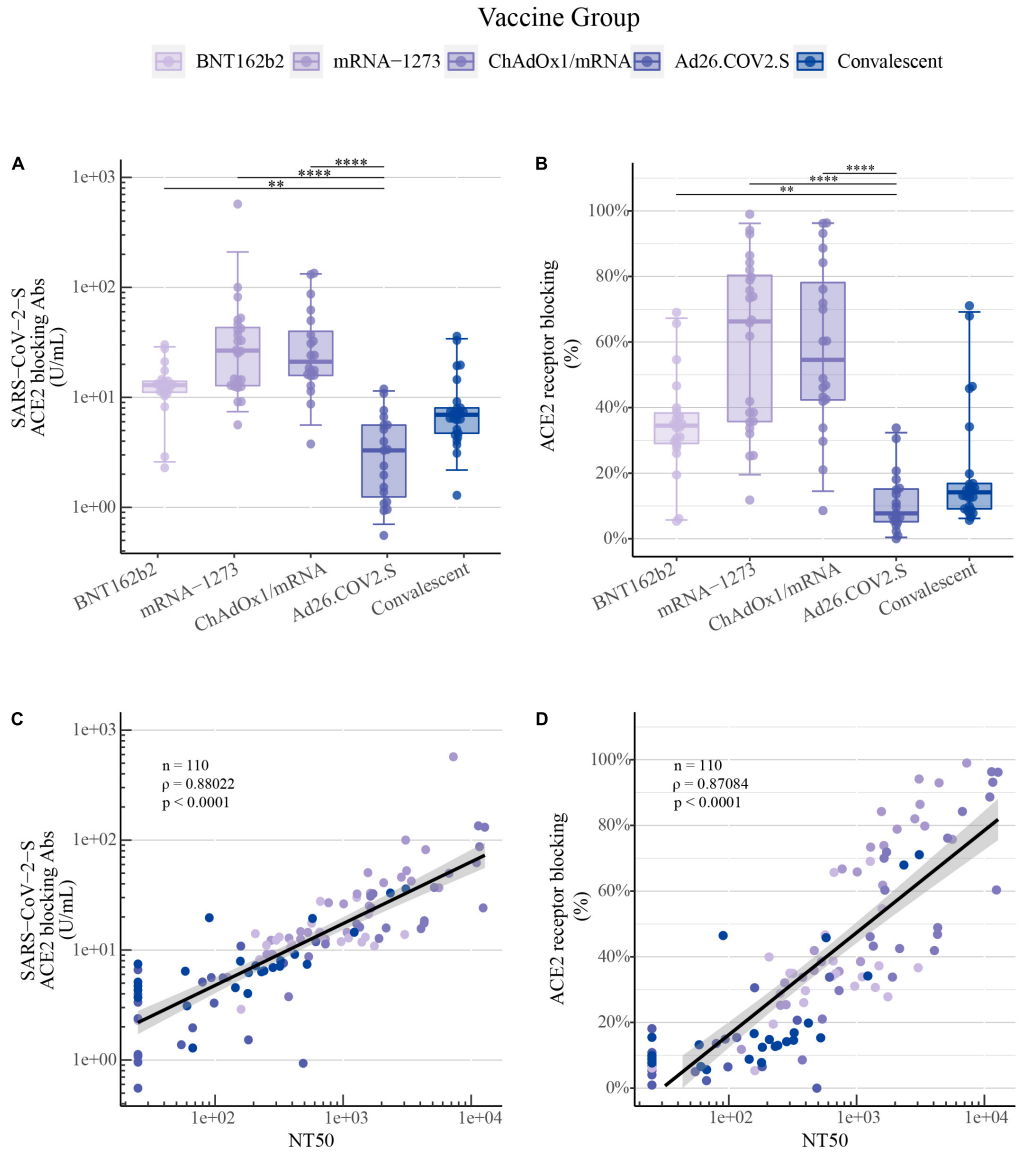

FIGURE 3
ACE2 competition assay as a surrogate for quantifying COVID-19 vaccine-induced antibody neutralization capacity: **(A)** SARS-CoV-2-S ACE2 receptor-blocking antibodies in U/mL and **(B)** ACE2 receptor blocking in percentage for SARS-CoV-2-S wt induced by primary COVID-19 vaccination with BNT162b2 ($n = 22$), mRNA-1273 ($n = 24$), ChAdOx1/mRNA ($n = 20$) or Ad26.COV2.S ($n = 19$) quantified by the MSD platform (serum 1:100). Data from convalescent comparators ($n = 25$) are also displayed, but are not included in the statistical analysis. The boxplots present the lower quartile, median and upper quartile, and the error bars indicate 95% CI. *P*-values were indicated as follows: $^{**}p < 0.01$ and $^{****}p < 0.0001$. **(C)** Spearman's correlation between SARS-CoV-2-S wt NT50 values quantified by the pseudovirus neutralization assay and ACE2 receptor-blocking antibodies in U/mL and **(D)** ACE2 receptor blocking in percentage quantified by the MSD ACE2 competition assay.

higher S-specific IgG levels and more pronounced antibody neutralization potency after two doses of mRNA-1273 compared with BNT162b2 (21, 25–27). This difference may be explained by several factors, including variation in the composition of the lipid nanoparticles for packaging and delivery, the mRNA dose content (30 μg for BNT162b2 and 100 μg for mRNA-1273) and/or the recommended time interval between the two primary vaccine doses (21 days for BNT162b2 and 28 days for mRNA-1273) (3, 24, 28).

Adenoviral vector-based vaccines have demonstrated lower vaccine efficacy compared with mRNA-based vaccines. However, in this study, heterologous vaccination with one dose of ChAdOx1 and a second dose of an mRNA vaccine was shown to induce high levels of SARS-CoV-2-S IgG and high antibody neutralizing titers. This observation can support other studies, including a Swedish study, that showed heterologous ChAdOx1/mRNA vaccine efficacy against symptomatic infection of 68%, which was significantly

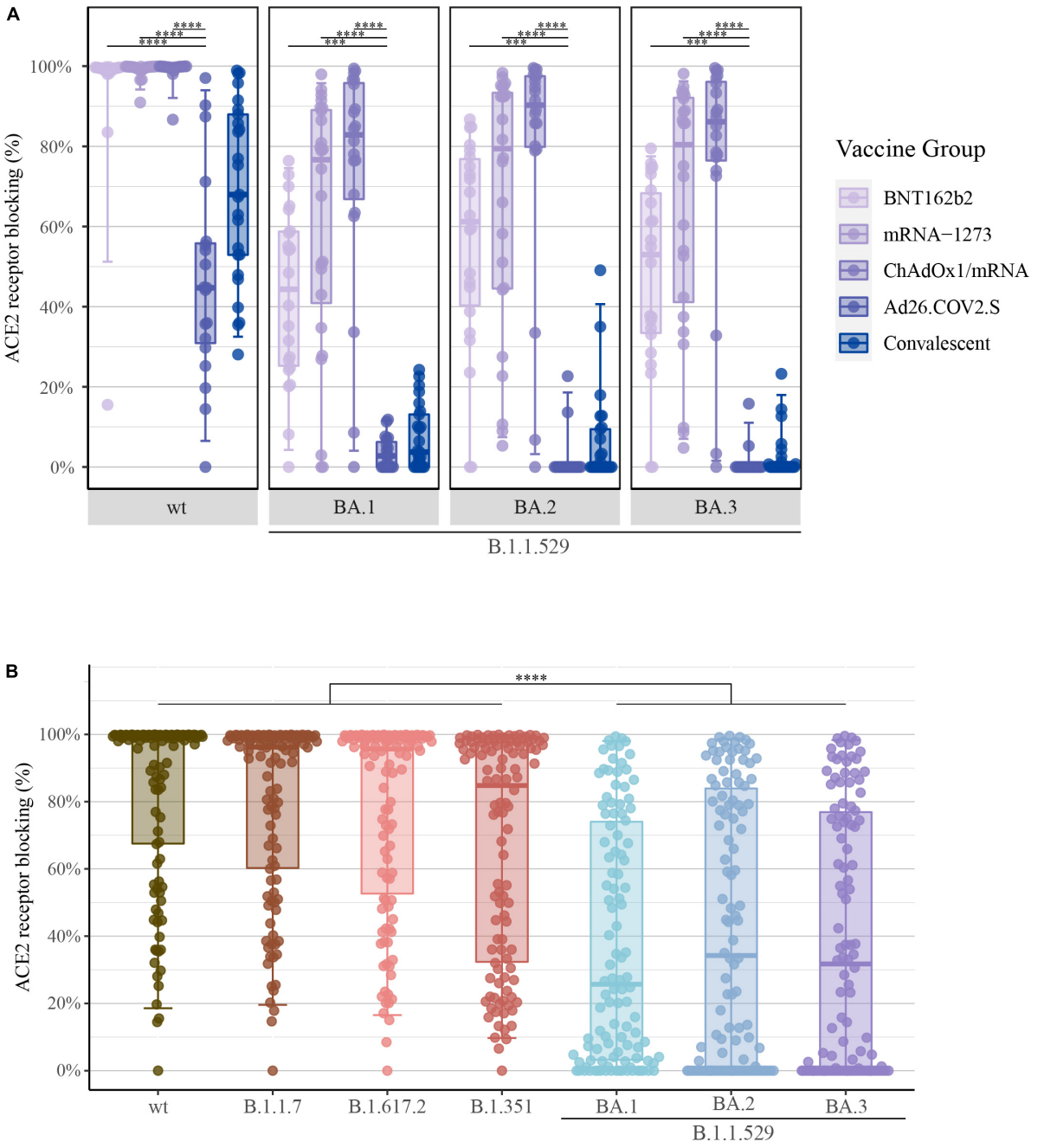

FIGURE 4
Percentage of ACE2 receptor blocking after COVID-19 vaccination: **(A)** Percentage of ACE2 receptor blocking induced by primary COVID-19 vaccination with BNT162b2 ($n = 22$), mRNA-1273 ($n = 24$), ChAdOx1/mRNA ($n = 20$) or Ad26.COV2.S ($n = 19$) quantified by the MSD platform (serum 1:10). Data from convalescent comparators ($n = 25$) are also displayed, but are not included in the statistical analysis. From left to right: SARS-CoV-2-S wt (Wuhan-Hu-1) and B.1.1.529; BA.1, BA.2 and BA.3 (Omicron). **(B)** Percentage of ACE2 receptor blocking merged for all vaccine types. From left to right: SARS-CoV-2-S wt, B.1.1.7 (Alpha), B.1.617.2 (Delta), B.1.351 (Beta), and B.1.1.529; BA.1, BA.2 and BA.3 (Omicron). All boxplots present the lower quartile, median and upper quartile, and the error bars indicate 95% CI. *P*-values were indicated as follows: ***$p < 0.001$ and ****$p < 0.0001$.

greater than the 50% efficacy of homologous ChAdOx1 vaccination (29). Furthermore, additional studies have reported superior immune responses with higher levels of S-specific IgG, neutralizing antibodies and T cell reactivity, inducing a significantly broader and highly potent immune response following heterologous relative to homologous vaccination

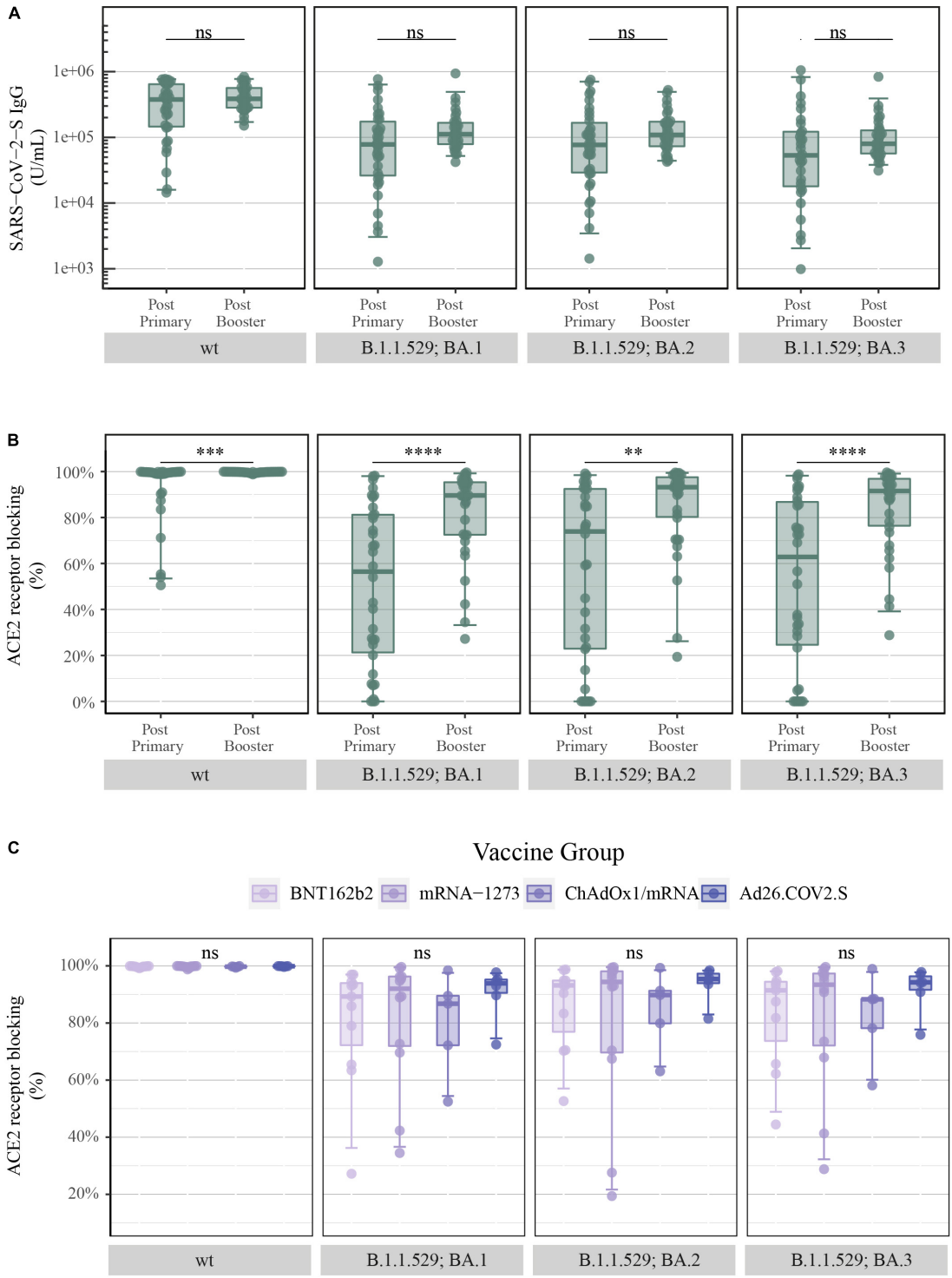

FIGURE 5
Levels of SARS-CoV-2-S IgG and percentage of ACE2 receptor blocking after COVID-19 booster vaccination: **(A)** Levels of total SARS-CoV-2-S IgG in U/mL and **(B)** ACE2 receptor blocking in percentage at the third study visit (after primary vaccination) and at the Xc study visit (after booster vaccination) merged for all vaccine types quantified by the MSD platform (after primary = serum and after booster = plasma, IgG = 1:5,000 and ACE2 = 1:10). From left to right: SARS-CoV-2-S wt (Wuhan-Hu-1) and B.1.1.529; BA.1, BA.2 and BA.3 (Omicron). **(C)** ACE2 receptor blocking in percentage after booster vaccination with BNT162b2 ($n = 11$), mRNA-1273 ($n = 12$), ChAdOx1/mRNA ($n = 5$) and Ad26.COV2.S/mRNA ($n = 6$) quantified by the MSD platform (plasma 1:10). From left to right: SARS-CoV-2-S wt and B.1.1.529; BA.1, BA.2, and BA.3. All boxplots present the lower quartile, median and upper quartile, and the error bars indicate 95% CI. *P*-values were indicated as follows: ns = $p > 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

(30–32). Some of these studies reported that the subsequent mRNA vaccine efficiently stimulated SARS-CoV-2-specific B-cell memory that had been generated by the first dose of ChAdOx1 (33, 34).

The weakest vaccine-induced antibody-mediated immunity discovered in this study was observed in individuals vaccinated with a single dose of Ad26.COV2.S. Several other studies have demonstrated considerably lower antibody levels and neutralizing antibody titers in individuals vaccinated with Ad26.COV2.S (27, 35–37). A priming dose of Ad26.COV2.S followed by an mRNA-based booster vaccination has been demonstrated, including this study, to boost S-specific IgG levels, antibody neutralizing capacity, T cell reactivity and improve vaccine efficacy compared with homologous vaccination with Ad26.COV2.S (38, 39). Heterologous COVID-19 vaccination might provide a favorable alternative for better protection against current and emerging SARS-CoV-2 VOCs by inducing a broader and more robust antibody-mediated and cell-mediated immune profile.

The emergence of novel SARS-CoV-2 variants has repeatedly received global attention. Especially, the current VOC, B.1.1.529, has been proven to have a substantial ability to avoid vaccine-induced and convalescent immune responses, thus affecting COVID-19 protection. Levels of S-specific IgG and antibody neutralization titers have shown to correlate and be highly predictive of clinical protection against symptomatic COVID-19 (40–43). However, the minimum required titers of neutralizing antibodies to provide protection against B.1.1.529 are yet to be determined.

The importance of COVID-19 vaccines was confirmed in a study examining the antibody-mediated immune response following B.1.1.529 infection. Data demonstrated that B.1.1.529 infections in unvaccinated individuals induced a limited immune response that lacked broader effective cross-neutralizing antibodies and displayed limited neutralization of non-B.1.1.529 variants. However, B.1.1.529 breakthrough infections were demonstrated to induce high neutralization titers against all SARS-CoV-2 VOCs. Thus, B.1.1.529 infections are capable of boosting pre-existing immunity induced by vaccination that is effective against B.1.1.529 and other SARS-CoV-2 variants (44).

SARS-CoV-2 B.1.1.529 has been shown to be highly resistant to neutralizing antibodies induced by vaccination and previous infections (11–14). Consequently, an additional dose of the COVID-19 vaccine was offered to boost the immune response and sustain protection against SARS-CoV-2. Our data displayed increased levels of SARS-CoV-2-S IgG and higher antibody neutralization capacity following a booster dose, which is comparable to other studies (17, 18, 45–47). Data on vaccine efficacy likewise demonstrated that a booster vaccination provided increased protection against symptomatic infection with B.1.1.529 (20). Thus, administration of a booster dose provides great potential for improving neutralizing antibody capacity against B.1.1.529 and possible future SARS-CoV-2 VOCs.

Due to the fact that many individuals had non-quantifiable antibody neutralization titers for SARS-CoV-2 B.1.1.529 by the pseudovirus neutralization assay, an additional assay was assessed to measure the potency of B.1.1.529 S-specific neutralizing antibodies with detection sensitivity at lower levels. The pseudovirus neutralization assay is a strong tool to study functional antibody responses against a virus. However, this assay is labor intensive, requires access to biosafety level 2 facilities and the use of living cells, making the assay more difficult to standardize. In addition, the assay has a detection limit at NT50 values of 25, prohibiting the quantification of low neutralizing antibody titers. The most concentrated plasma dilution examined in the pseudovirus assay is 1:25, as cell death has been shown to confound the readout at higher plasma concentrations. The ACE2 competition assay can serve as a high-throughput alternative to the traditional pseudovirus neutralization assay. The ACE2 competition assay is provided as a 96-well microtiter plate with multi-spot panels facilitating the quantification of up to 10 different SARS-CoV-2 variants from a single, small-volume of sample. However, it should be noted that the ACE2 competition assay has a narrow dynamic range and performing a dilution series is favored to ensure that all data points fall in the quantifiable range. As demonstrated in this study, and shown by Nielsen et al. (23), a very strong positive correlation was found between the readouts of the two assays, which was true for all variants tested. Thus, the data support the ACE2 competition assay as a reliable, powerful and large-scale screening tool to measure antibody neutralization titers.

There are some limitations to consider in our study. The ChAdOx1/mRNA group mainly consisted of female healthcare workers and the timing of their second vaccination was significantly closer to the third study visit compared with the BNT162b2 and mRNA-1273 vaccine groups. As immune responses tend to peak shortly after vaccination and wane over time, this is a relevant factor when considering the higher neutralizing antibody responses detected in the ChAdOx1/mRNA group. The age distribution in the four vaccine groups is also not identical. In particular, the Ad26.COV2.S group is considerably younger as a consequence of the restrictive use of Ad26.COV2.S in Denmark. However, increasing age has been shown to correlate with lower IgG levels and antibody neutralization titers (21). Consequently, the differences in age distribution did not appear to have an impact on the vaccine-induced immune responses detected in this study. Another limitation is the relatively small and varying number of participants in each vaccine group included in the comparison of vaccine-induced antibody neutralization following booster vaccination.

This study also had some limitations in regards to the assays that were performed. We measured total levels of SARS-CoV-2-S IgG by utilizing a serum dilution of 1:5,000 as

suggested by the manufacturer. However, after administration of the COVID-19 booster dose, the serum samples appeared to be insufficiently diluted and reached the upper limits of the assay. Due to this, we may only detect small increments in S-specific IgG levels after administration of the booster vaccine.

Conclusion

In conclusion, the direct comparative analysis of vaccine-induced antibody-mediated immune responses, to a range of SARS-CoV-2 variants, demonstrated marked differences in the antibody-mediated immune responses generated by each COVID-19 vaccine. Comparing vaccine types, the study showed lower levels of total S-specific IgG and antibody neutralization titers induced by one dose of the Ad26.COV2.S vaccine, intermediate levels by two doses of the BNT162b2 vaccine, and the highest levels by two doses of the mRNA-1273 vaccine or heterologous vaccination of one dose of the ChAdOx1 vaccine and a subsequent mRNA vaccine. The accumulation of SARS-CoV-2 S protein mutations was accompanied by a marked decline in antibody neutralization capacity, especially against the current VOC, B.1.1.529. However, administration of a booster dose elevated antibody responses significantly for all vaccinated individuals against B.1.1.529. The previously detected differences in antibody-mediated immunity, between the four COVID-19 vaccines after primary vaccination, were no longer detected post-booster vaccination. These findings highlight the importance of the roll-out of booster vaccines and the potential inclusion of future heterologous vaccination strategies for broad protection against current and emerging SARS-CoV-2 VOCs to remain in control of the pandemic.

Data availability statement

All data may be made available to researchers upon request. All data will be provided as de-identified data to comply with GDPR regulations. Requests to access the datasets should be directed to MT, marttols@rm.dk.

Ethics statement

The studies involving human participants were reviewed and approved by the Danish National Committee on Health Research Ethics, Copenhagen, Denmark (#1-10-72-337-20) and The Danish Medicines Agency, Copenhagen, Denmark (#2020-006003-42). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL, LØ, OS, NS, JR, DR, and MT conceptualized the work. HN, IJ, LW, TB, NS, KI, AM, MJ, KP, SO, SL, and LR performed the clinical visits and collected the samples. AH, EB, MS, SA, SRA, LD, and AJ performed the laboratory analyses. AH and EB performed the data analysis and visualization. MT supervised and led the study. AH, EB, and MT drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

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Acknowledgments

The ENFORCE study group members all contributed substantially to the study. A full list of members of the ENFORCE study group is provided as **Supplementary material**.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared parent affiliation with several of the authors AH, EB, OS, NS, MS, SA, AJ, LD, and SRA at the time of the review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.994160/full#supplementary-material

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Validation of a simple risk stratification tool for COVID-19 mortality

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Risk prediction is an essential part of clinical care, in order to allocate resources and provide care appropriately. During the COVID-19 pandemic risk prediction became a matter of political and public debate as a major clinical need to guide medical and organizational decisions. We previously presented a simplified risk stratification score based on a nomogram developed in Wuhan, China in the early phase of the pandemic. Here we aimed to validate this simplified risk stratification score in a larger patient cohort from one city in Austria. Age, oxygen saturation, C-reactive protein levels and creatinine levels were used to estimate the in-hospital mortality risk for COVID-19 patients in a point based score: 1 point per age decade, 4 points for oxygen saturation <92%, 8 points for CRP > 10 mg/l and 4 points for creatinine > 84 μmol/l. Between June 2020 and March 2021, during the "second wave" of the pandemic, 1,472 patients with SARS-CoV-2 infection were admitted to two hospitals in Graz, Austria. In 961 patients the necessary dataset to calculate the simplified risk stratification score was available. In this cohort, as in the cohort that was used to develop the score, a score above 22 was associated with a significantly higher mortality ($p < 0.001$). Cox regression confirmed that an increase of one point in the risk stratification score increases the 28-day-mortality risk approximately 1.2-fold. Patients who were categorized as high risk (≥22 points) showed a 3–4 fold increased mortality risk. Our simplified risk stratification score performed well in a separate, larger validation cohort. We therefore propose that our risk stratification score, that contains only two routine laboratory parameter, age and oxygen saturation as variables can be a useful and easy to implement tool for COVID-19 risk stratification and beyond. The clinical usefulness of a risk prediction/stratification tool needs to be assessed prospectively (https://www.cbmed.at/covid-19-risk-calculator/).

KEYWORDS

COVID-19, risk score, prediction, validation, mortality

Introduction

During the COVID-19 pandemic hospital and ICU beds were scarce resources and hospital capacities became a matter of political and public debate. Accurate risk stratification for patients with COVID-19 admitted to the hospital therefore is a major clinical need to guide medical and organizational decisions. However, reliable risk stratification tools to address this problem were and are still lacking. A multitude of studies aimed to predict the risk of severe disease and mortality as early as possible in the course of COVID-19 infections. These risk stratification attempts were ranging from complex biomarker studies that warrant resource intensive research settings (1) to relatively easy to obtain scores that require only routine laboratory data from hospital admission (2). Also non-laboratory markers such as arterial stiffness (3), lung sonography (3), primary care data (4) or the "repurposing" of established risk stratification scores in general hospital populations were studied (5). The methods to combine biomarker into risk prediction score can range from single-parameter to multiple-parameter and aggregate weighted systems (6). The methods to create and validate such risk scores range from traditional biostatistical approaches to novel artificial intelligence models (7). However so far, none of these scores for COVID-19 disease severity prediction made its way to clinical routine.

Already early in the course of the pandemic, data from a large dataset of the first wave of the pandemic in Wuhan/China showed that routine laboratory markers available at admission could accurately predict COVID-19 disease outcome (8). We aimed to validate this score in a real-world dataset for a European cohort. The validation was successful, however, we noticed that the score, despite being based on routine laboratory parameters, was rather complex to calculate and outside a clinical study setting many missing data would further impaired clinical applicability. We therefore took this nomogram as a basis and developed a simple and easy to calculate risk stratification score. Our score stratifies the mortality risk of hospitalized patients with COVID-19 based on only four variables: age, oxygen saturation, C-reactive protein and creatinine (9). Ding et al. tested the robustness of our simplified model in their original cohort from Wuhan and found that our simplified predictive model can predict 28-day mortality well, however with a somewhat reduced accuracy (10).

We now set out to test the robustness of our simplified score and the initial nomogram from Ding et al. again during the second wave of the pandemic between June 2020 and March 2021 in Graz, Austria.

Methods

We retrospectively collected demographic and laboratory data as well as in-hospital mortality from all patients (without age limitations) hospitalized at either the University Hospital Graz or the State Hospital Graz II between June 2020 and March 2021. Patients' information was extracted using the ICD10 code U07.1. SARS-CoV-2 infection was manually verified by 2 independent investigators in each case by the documentation of a result of a positive SARS-CoV-2 PCR. The study was approved by the institutional review board (32–431 ex 19/20), informed consent was waived due to the retrospective nature of the study and the study was registered at clinicaltrials.gov (NCT04420637).

Risk stratification

Age, oxygen saturation, C-reactive protein levels and creatinine levels were used to estimate the in-hospital mortality risk for COVID-19 patients, as previously proposed in (9): 1 point per age decade, 4 points for oxygen saturation <92%, 8 points for CRP > 10 mg/l and 4 points for creatinine > 84 μmol/l. A score of 22 or higher indicates a significantly increased mortality risk.

Parameters for the risk score calculation were assessed at the day of admission (+1 day) if the patient was admitted with or because of a SARS-CoV-2 infection, or as the day of diagnosis (+1 day), if patients contracted SARS-CoV-2 during an unrelated hospital stay. In case, a parameter was assessed more than once within the defined time period, the earliest documented value was used.

Statistical analysis

The predictive merit of the risk stratification score was validated using different approaches. First, AUROC analysis was performed to test whether the risk stratification score can accurately predict which patients died within the defined time period of 7, 14, 21 or 28 days after admission/diagnosis. Next, the previously published cutoff of 22 was used to categorize the patients in a high risk and a low risk group. Kaplan Meier curves and log rank tests were performed to test whether patients in the high risk group actually have a significantly higher mortality risk compared to patients in the low risk group. The cutoff of 22 was further validated by comparison to a cutoff optimized to the data set at hand. A Monte-Carlo simulation was run to find the cutoff with the highest accuracy for 28-day mortality. In this simulation, the data set was randomly split into a training set (70% cases) and a test set (30% of cases), every possible cutoff (i.e. every integer between 1 and 25) was applied and the overall accuracy in the training set was compared. The best performing cutoff was then applied to the test set and its accuracy was documented. This sequence was repeated 100.000 times and the modus of the three best performing cutoffs was defined as the optimized cutoff for the data set at hand. Chi-square test was

used to compare the proportion of accurate predictions between the proposed and the optimized cutoff.

Cox regression was used to estimate the hazard ratio for the risk stratification score, for the categorization as high or low risk group, as well as for each parameter of the score individually.

Analysis was performed with R and R-Studio using the packages "tidyverse", "readxl", "ggpubr", "data.table", "lubridate", "caret", "survival", "survminer", "pROC", "ROCR" and "foreign".

Results

During the second wave of the pandemic, 1511 individual patients were hospitalized with the diagnosis code U07.1 for COVID-19 infection. After exclusion of 39 patients with no verifiable SARS-CoV-2 infection, from the remaining 1,472 patients, in 961 patients the necessary dataset for our simplified score was available whereas the full nomogram from Ding et al. (8) could only be calculated for 171 patients because of missing data. Compared to the cohort used to establish the risk stratification score, the patients analyzed in this study were younger, were less likely to have reduced oxygen saturation, had higher creatinine levels and consequently also had a higher risk stratification score. Mortality and C-reactive protein levels was comparable between the study cohorts. See Table 1 for details.

TABLE 1 Patient characteristics of the validation cohort and the patients on which the risk stratification score was based (patients 3–6/2020).

Parameter	Second wave (6/2020–3/2021; $n = 961$)	First wave (3–6/2020; $n = 243$)	Standardized difference
Age (years)	**68.6 ± 18.5**	**74.9 ± 14.5**	**0.38**
Female (%)	437 (45.5)	108 (44.4%)	−0.02
Communally acquired infection (%)	859 (89.4)	na	na
Oxygen saturation (%)	92.8 ± 5.8	92.6 ± 5.2	−0.03
oxygen saturation <92% (%)	**268 (27.9)**	**97 (39.9)**	**−0.26**
C-reactive protein (mg/l)	83.7 ± 80.2	80.0 ± 78.3	−0.05
C-reactive protein over 10 mg/l (%)	818 (85.1)	203 (83.5)	0.04
Creatinine (μmol/l)	**123.2 ± 121.4**	**110.9 ± 107.8**	**1.44**
Creatinine over 84 μmol/l (%)	572 (59.5)	133 (54.7)	0.10
Risk stratification score	**16.7 ± 5.1**	**18.4 ± 5.0**	**0.33**
Risk stratification score ≥ 22 (%)	**134 (13.9)**	**70 (28.8)**	**−0.37**
Mortality			
Died within 7 days of hospitalization (%)	99 (10.3)	20 (8.2)	0.07
Died within 14 days of hospitalization (%)	170 (17.7)	56 (23.0)	−0.13
Died within 21 days of hospitalization (%)	196 (20.4)	62 (25.5)	−0.12
Died within 28 days of hospitalization (%)	221 (22.0)	63 (25.9)	−0.09

Comparability of the patient groups is shown as standardized difference. An absolute value below 0.2 signifies good agreement.
Bold values indicate parameters without good agreement.

TABLE 2 AUROC analysis of risk stratification score for 7, 14, 21 and 28-day mortality.

	C-value	95% confidence interval
7-day mortality	0.75	0.71–0.80
14-day mortality	0.75	0.71–0.79
21-day mortality	0.74	0.70–0.78
28-day mortality	0.73	0.69–0.77

Risk stratification score validation

AUROC analysis confirmed that the proposed risk stratification score is predictive of 7, 14, 21 and 28-day mortality of hospitalized COVID-19 patients in the new cohort (6/2020–3/2021). Details are given in Table 2.

When the risk stratification score was proposed on the cohort from 3 to 6/2020, a score of 22 or above indicated an increased COVID-19-related in-hospital mortality risk. Also in this study, patient with a score of 22 or above showed a significantly higher mortality risk compared to patients with a score below 22 ($p < 0.001$). However, the optimized cutoff with the highest overall accuracy for the present cohort was 23. It showed a slightly better accuracy, but there was no significant difference in the number of accurately classified patients compared to the cutoff of 22 points (85.5 vs. 83.7%, respectively, $p = 0.3$). Figure 1 compares the Kaplan Meier curves for both cutoffs.

Estimation of hazard ratios

Cox regression confirmed that an increase of one point in the risk stratification score increases the 28-day-mortality risk approximately 1.2-fold (details are given in Table 3, information about 7, 14, and 21-day mortality is given in the supplements).

Also patients who were categorized as high risk (≥22 points) showed a 3–4 fold increased mortality risk, depending on the observation period. Accordingly, all parameters of the risk stratification score were associated with increased mortality risk to varying degrees. Interestingly, while C-reactive protein levels

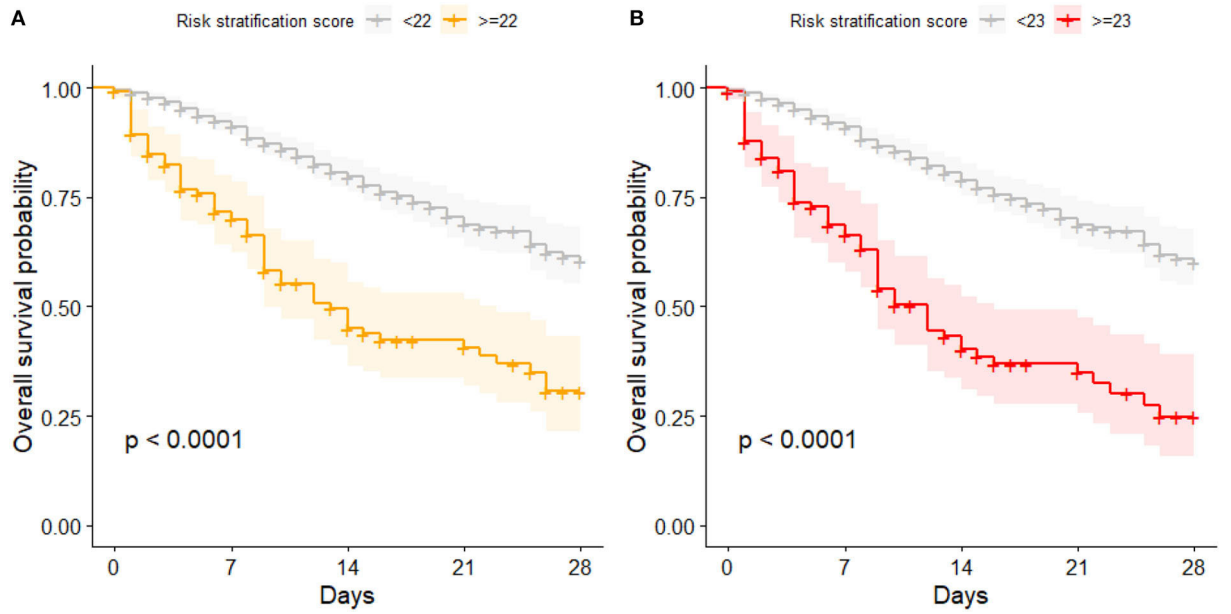

FIGURE 1
Kaplan Meier curves for patients with high and low risk of mortality; **(A)** categorization based on the proposed cutoff of 22 points; **(B)** categorization based on the cutoff optimized for this data set (23 points).

were associated with increased mortality risk, the categorization of high and low levels as initially proposed by the risk stratification score was not a constant significant predictor. Patients showed high levels of C-reactive protein irrespective of the outcome (see Supplementary Figure 1). Although CRP levels were comparable in the initial publication describing the patients from the first wave of the pandemic (3–6/2020), its predictive merit could not be reproduced in patients from the later phase (second wave, 6/202–3/2021). To account for superimposed bacterial infections already at admission, patients with leucocytosis (leucocyte count >11.3 G/l) were temporarily excluded from analysis, however it did not improve the prediction based on increased C-reactive protein levels.

Comparison of the risk stratification score with the nomogram by Ding et al.

In the presented validation cohort, also the nomogram from Ding et al. predicted 28-day in-hospital mortality, whereby an increase of one point is associated with a 1.007-fold (95%CI: 1.003–1.012; $p = 0.002$) increase in mortality risk. Comparing areas under the receiver operated characteristics curve (AUROC) in all available data sets, the full nomogram showed only a slightly but not significantly better prediction when compared to our simplified risk stratification score (AUC-difference: −0.019; $p = 0.7$) (Figure 2). However, full datasets necessary to apply the nomogram from Ding et al. were available for significantly less patients in comparison to our simplified risk stratification score (12 vs. 65%, $p < 0.001$).

TABLE 3 Hazard ratios for 28-day mortality of the risk stratification score, its components and categorizations.

Predictor	Hazard ratio	95% confidence interval	*p*-value
Age	1.056	1.043–1.068	<0.001
Age points	1.682	1.505–1.880	<0.001
Oxygen saturation	0.9501	0.9366–0.9638	<0.001
Oxygen saturation <92%	2.048	1.562–2.685	<0.001
C-reactive protein	1.003	1.002–1.005	<0.001
C-reactive protein >10 mg/l	1.614	0.9952–2.617	0.052
Creatinine	1.106	1.041–1.176	0.001
Creatinine >84 μmol/l	1.702	1.260–2.299	<0.001
Risk stratification score	1.179	1.133–1.226	<0.001
Risk stratification score >22	3.084	2.317–4.105	<0.001
Risk stratification score >23	3.550	2.640–4.775	<0.001

Discussion

Triage management plays important roles in hospitalized patients for disease severity stratification and medical burden analysis. Although risk prediction scores have been extensively researched for many acute and chronic diseases, there was an

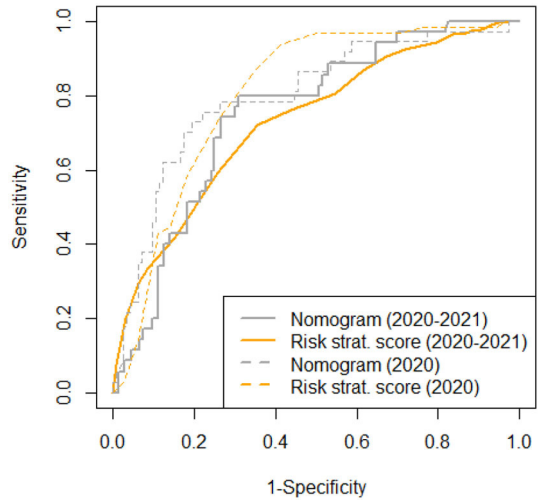

FIGURE 2
AUROC for the risk stratification score and the nomogram (8) for all available data sets ($n = 961$ and $n = 177$, respectively) compared to previously published data (9).

urgent need to adapt and validate risk prediction scores in COVID-19 disease (11).

While the use of complex research biomarkers, such as metabolomic analyses (12) or deep immune phenotyping (13), is of great interest to understand the pathophysiology of this disease better, especially in vulnerable patient groups, the clinical applicability of such complex biomarkers and scores is currently limited due to lack of availability and high costs. A review of 76 different coring systems, ranging from existing scores to newly developed scores, artificial intelligence algorithms and novel biomarker came to the conclusion that all of these scores have limitations but that the combination of single laboratory parameters may have the greatest potential for implementation (14).

Identification of an easily applied and valid evidence-based clinical risk stratification tool is therefore an unmet clinical need that we tried to fulfill. We started from the highly predictive but rather complex nomogram created by Ding et al., that was developed based on the results of a multivariate analysis that contained an extensive routine laboratory parameter workup including full blood count, liver and renal function tests, cardiac troponin I, lactate dehydrogenase, CRP, procalcitonin and cytokines as well as hepatitis B-related antigen or antibodies, and hepatitis C-related antibodies. In addition, age and the findings from a CT scan of the chest were included. From that dataset, 8 laboratory tests (lymphocyte count, platelets, CRP, D-dimer, creatinine, cardiac troponin I, aspartate aminotransferase, direct bilirubin) as well as two clinical parameters (age and severity of pneumonia) were derived and the nomogram was developed. In our initial publication we were able to first of all validate the predictive power of the parameters identified in a Chinese cohort and in a next step we were able to reduce the number of parameters to two clinical and two laboratory parameters without losing diagnostic accuracy (9). In an effort to enhance the accuracy with parameters not included in the nomogram, we also considered comorbidities as potential outcome predictors: First we evaluated 23 comorbidities derived from the Charlson Comorbidity Index separately for their association with COVID19-related outcome. We observed that obesity, cancer, liver disease, arterial hypertension, heart failure and peripheral arterial disease were not associated with outcome. Leukemia, lymphoma, metastatic cancer, AIDS, hemiplegia, connective tissue diseases, gastrointestinal ulcera and inflammatory bowel disease had a low prevalence and therefore did not contribute significantly to outcome prediction in our study population. Dementia, Morbus Parkinson, kidney diseases, diabetes mellitus, coronary artery disease, myocardial infarction, cardiac arrhythmias, cerebrovascular diseases and chronic lung diseases were significantly associated with outcome but highly dependent on age and therefore could not contribute significantly to outcome prediction in a model that strongly featured age as a main predictor. We also used the point score derived from Charlson Comorbidity Index (original, updated and age-adjusted) but age was such a strong factor in both cohorts, that there was no additional benefit in adding comorbidities to the score. Therefore, age, oxygen saturation, C-reactive protein and creatinine were finally implemented in a weighted sums score to predict 28-day mortality. Our validation and the validation performed by Ding et al. (10) shows the robustness of our simplified risk calculation model over different times and across continents. Although the original nomogram from Ding et al. (8) has a slightly better performance, our real-life dataset shows that under routine working conditions outside a study setting, the full dataset necessary to apply the nomogram from Ding et al. was available only from a minority (12%) of patients in the Austrian cohort. In comparison, the simplified risk stratification score was retrospectively calculable in 65% of patients. The cohort characteristics between the first and the second wave of the pandemic differed. In the second wave, patients were younger, had less severe pneumonia as indicated by oxygen saturation <92%, but higher creatinine levels. Despite these differences, the risk stratification score worked equally well with the same cut-off. This indicates the robustness of our model and even allows the hypothesis that this score may be useful outside of COVID-19.

An ideal clinical score requires simplicity of calculation, not too many variables that need to be easily available, independent validation, and should provide clinical detection as early as possible (15). For the field of cardiovascular risk prediction, it is known that factors influencing the successful implementation of risk scoring are related to clinical setting and healthcare system (resources, priorities, practice culture and organization), users (attributes and interactions between users) and the specific

risk tool (characteristics, perceived role and effectiveness) (16). We believe that our COVID-19 risk stratification score fulfills the requirements that would allow successful implementation. Also, from a cost perspective, a score that only requires two laboratory variables instead of eight, also has an advantage, especially in resource limited settings. Our simplified COVID-19 risk stratification score can also be easily calculated without any technical help, however, especially in the younger generation of physicians, online/mobile applications are frequently used and highly accepted in clinical care (17). Therefore we offer our score as an open source online calculator (https://www.cbmed.at/covid-19-risk-calculator/). Ideally this calculator can be implemented in electronic health records, allowing automated calculation of the risk score from data obtained at hospital entry in each patient with COVID-19 infection.

The next step for assessing the clinical usefulness of a risk prediction/stratification tool would be to assess the score prospectively and draw clinical conclusions from the result. This has not been performed yet with our score. Such an undertaking also raises ethical questions: in resource rich settings, a high score, indication a high risk for mortality, would most likely trigger the allocation of resource to this patient (intensive monitoring, early referral to intermediate or intensive care). However, in resource-restricted settings, the opposite may be the case—people with a predicted adverse outcome may be withheld from intensive care treatment in triage situations. Triage here refers to situations where different patient priority groups are established in order to distribute scarce health resources. An in depth review on the literature of triage in the COVID-19 pandemic came to the conclusion that there is consensus to rely on medical prognosis, maximizing lives saved, justice as fairness and non-discrimination (18). Several open points were identified, such as the need for improved outcome predictions, possibly aided by artificial intelligence, the development of participatory approaches to drafting, assessing and revising triaging protocols and the need to learn from experiences with implementation of guidelines with a view to continuously improve decision-making (18).

Our study has some limitations: due to the retrospective nature of our study missing data led to the exclusion of 12% of the datasets. The inclusion of only two centers still warrants further validation of the score in multicenter datasets from different regions to test the robustness across different health care systems. We also did not analyze the impact of different non-specific or specific therapies administered during COVID-19 infection on outcome and on the performance of our score. However, the fact that we could validate the score in the second wave of COVID-19, where treatment with steroids and remdesivir was already well established, as opposed to the first wave, is reassuring that the score is robust.

In conclusion we propose a simple risk stratification score based on age, oxygen saturation (as an indicator for severity of pneumonia), creatinine and C-reactive protein, to differentiate between patients with high and low mortality risk from COVID-19 when admitted to the hospital.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethikkommission Medizinische Universität Graz, Auenbruggerplatz 2, 8036 Graz, IRB00002556. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

TL, NF, and HW collected data. AH and VS analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1016180/full#supplementary-material

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Self-collection of capillary blood and saliva to determine COVID-19 vaccine immunogenicity in patients with immune-mediated inflammatory diseases and health professionals

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Introduction: Being able to independently determine vaccine induced antibody responses by minimal-invasive methods is of great interest to enable a flexible and effective vaccination strategy. This study aimed to evaluate (1) the accuracy, feasibility, usability and acceptability of capillary blood and saliva self-sampling to determine SARS-CoV-2 antibody responses in patients with immune-mediated inflammatory diseases (IMIDs) and health professionals (HP).

Methods: IMID patients and HP having received two doses of SARS-CoV-2 vaccines, self-collected capillary blood (Tasso+) and saliva samples. Capillary samples were considered interchangeable with venous blood if three criteria were met: Spearman's correlation coefficient (r) $>$ 0.8, non-significant Wilcoxon signed-rank test (i.e., $p > 0.05$), and a small bias or 95% of tests within 10% difference through Bland-Altman. Participants completed a survey to investigate self-sampling usability (system usability scale; SUS) and acceptability (net promoter score; NPS). Study personnel monitored correct self-sampling completion and recorded protocol deviations.

Results: 60 participants (30 IMID patients and 30 HP) were analyzed. We observed interchangeability for capillary samples with an accuracy of

98.3/100% for Anti-SARS-CoV-2 IgG/IgA antibodies, respectively. Fifty-eight capillary blood samples and all 60 saliva samples were successfully collected within the first attempt. Usability of both self-sampling procedures was rated as excellent, with significantly higher saliva ratings ($p < 0.001$). Capillary self-sampling was perceived as significantly ($p < 0.001$) less painful compared to traditional venous blood collection. Participants reported a NPS for capillary and saliva self-sampling of +68% and +63%, respectively. The majority of both groups (73%) preferred capillary self-sampling over professional venous blood collection.

Conclusion: Our results indicate that capillary self-sampling is accurate, feasible and preferred over conventional venous blood collection. Implementation could enable easy access, flexible vaccination monitoring, potentially leading to a better protection of vulnerable patient groups. Self-collection of saliva is feasible and safe however more work is needed to determine its application in clinical practice.

KEYWORDS

self-collection, capillary blood, remote care, telehealth, self-sampling, COVID-19

Introduction

Evaluation of an adequate vaccination response and appropriate revaccinations are essential to counteract waning of humoral immune response (1) and to ensure a sustained and adequate level of protection (2, 3). Repeated measurement of anti-SARS-CoV-2 antibody levels is recommended especially for vulnerable patient groups, such as patients with immune-mediated inflammatory diseases (IMIDs) receiving immunsuppressive treatments, likely to have a poor vaccination response and to suffer from a severe COVID-19 infection (4). Due to the already limited number of available health professionals (HP) treating IMID patients (5), HP should try to prevent COVID-related absences, that can be avoided or shortened by maintaining an adequate vaccine immunogenicity.

Ideally, samples to investigate vaccine immunogenicity could be self-collected at home, and having to travel to healthcare facilities including the burden and infection risk, would be obsolete. Self-sampling enables independent, flexible collection of specimen, such as capillary blood (6) and saliva at home. Nwankwo et al. recently demonstrated how remote capillary blood self-sampling provides accurate results for several biomarkers, can improve shared decision making and overall patient experience (7). In a previous randomized controlled trial we showed that patients suffering from rheumatoid arthritis clearly preferred upper arm-based self-sampling with a self-adhesive lancet-based device (Tasso) to traditional finger pricking (8). Furthermore, a recent pilot study demonstrated that this upper-arm device (Tasso+) can be used by healthy and previously infected individuals to reliably collect blood for COVID-19 humoral response evaluation (9). Saliva represents a non-invasive and painless alternative to blood. Recent publications support the accuracy of saliva-based humoral response analysis (10–12). This saliva-based approach enabled a population-based Anti-SARS-CoV-2 antibody study in children, that might otherwise have been reluctant to conventional venous blood collection (11).

To the best of our knowledge, no study has yet directly compared capillary and saliva self-sampling in IMID patients and HP. Therefore, this study aimed to evaluate the accuracy, feasibility, usability and acceptability of capillary blood and saliva self-sampling to determine Anti-SARS-CoV-2 antibody responses in IMID patients and HP.

Materials and methods

Study design

This study was a prospective, single-center, cross-sectional, matched case-control study (WHO International Clinical Trials Registry: DRKS00024787), see Figure 1. Adult IMID patients were consecutively recruited at the outpatient clinic of the Department of Internal Medicine 3 (FAU Erlangen-Nurnberg) between May 2021 and August 2021. Patients were matched with local health professional controls (physicians and nurses), individually matched by same age and sex. The trial was approved by the local ethics authorities (Reg no. 25_21B) and written informed consent was obtained from all study participants. To be included, participants had to have received two doses of SARS-CoV-2 vaccine.

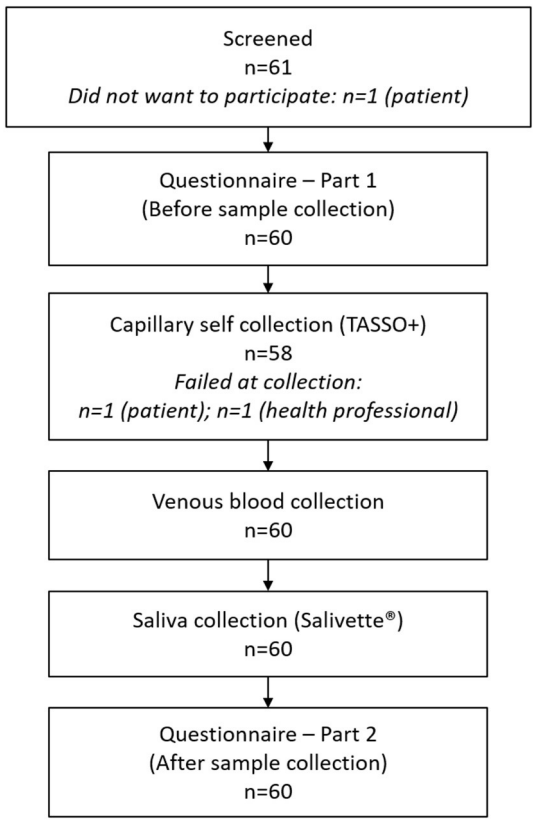

FIGURE 1
Participant flowchart.

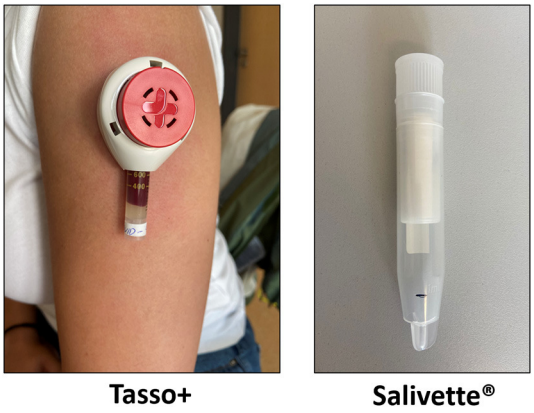

FIGURE 2
Capillary blood upper-arm self-collection device (Tasso+) and saliva collection device (Salivette®).

Participants first completed a questionnaire querying previous self-sampling experience and current attitude. After receiving written instructions, participants independently completed an upper-arm-based capillary and saliva specimen collection under the supervision of local study personnel. Additionally patients were presented a video instruction for the capillary self-sampling device. Deviations from the respective self-sampling protocol were recorded. After a traditional venous blood collection, representing the gold-standard, participants completed a final questionnaire to investigate perceived pain during blood collection and a potentially changed attitude toward self-sampling.

The agreement of anti-SARS-CoV-2 IgG and IgA antibody levels between matched capillary, saliva and venous samples was the primary outcome. Feasibility was assessed by the number of successfully collected samples within the first attempt. Usability of sampling devices was assessed via the ten-item System Usability Scale (SUS) (13). SUS scores range between zero (worst) and 100 (best). A score >68 is considered above average and a score >80 as high (13). Additionally, SUS scores were translated to categories such as "excellent" as previously described by Bangor et al. (14). The Net Promoter Score (NPS) (15, 16) was used to investigate acceptability after sample collection. Participants were queried how likely they are to recommend the self-sampling device to a friend or patient on a 11-point numeric rating scale (zero-not at all likely to 10-extremely likely). Answers between 0– and 6 are categorized as detractors, 7–8 as passives and 9–10 as promoters. The NPS is calculated by subtracting the percentage of detractors from the percentage of promoters. Participants were asked before and after sample collection "I would prefer capillary self-sampling instead of having to see a professional for a traditional venous blood collection" and report their level of agreement (strongly disagree to strongly agree). Pain perception of capillary self-sampling and venipuncture was measured using a 11-point numeric rating-scale (NRS; zero no pain at all, 10 worst imaginable pain) (17) directly after blood collection.

Sample collection and processing

Capillary samples were collected using the upper-arm based Tasso+ device (Tasso Inc., Seattle, WA, USA) and saliva samples were collected using Salivetten Cotton Swab (Sarstedt AG & Co. KG, Nürmbrecht, Germany) by spitting directly into the tube without utilizing the cotton swab (Figure 2).

The Tasso+ device is attached to the upper arm by an adhesive and the lancet is activated by pressing a button. Prior to capillary blood collection, patients were instructed to warm the chosen collection site for 1 min by applying a heat-pad (L x W x H) 135 x 95 x 25 mm, max. heat 55°C, (Conrad Electronics SE, Germany) to increase local blood flow. Blood is then automatically collected using a vacuum. Participants were instructed to remove the device after a maximum collection time of 5 min or as soon as the collection tube was entirely filled with blood. Participants were instructed to collect a target volume of

saliva up to a line mark. Participants should not drink or eat 30 min prior to saliva collection. Matched venous blood samples were collected by trained phlebotomists from all participants within 30 min of capillary blood and saliva collection.

Uncentrifuged capillary samples and centrifuged venous blood reference samples were sent by regular mail using standard postage and UN3373 compliant packaging to Thermo Fisher Scientific research laboratory in Freiburg, Germany. Samples were inspected independently by two lab technicians for quality. Upon arrival in the laboratory the samples were processed, resulting serum was transferred into Sarstedt^{TM} 2 ml Polypropylene Micro Tubes (Sarstedt AG & Co., Nümbrecht, Germany) and stored at −20°C until analysis. Saliva samples were stored at the hospital at −20°C and then sent to Thermo Fisher Scientific research laboratory in Freiburg, Germany on dry ice and stored at −20°C until analysis. Prior to testing saliva samples were transferred to a new salivette tube so that all liquid was absorbed by the cotton pouch, followed by a 5 min, 4°C, 3,000 g centrifugation step. The eluate was collected and stored at −20°C. Saliva samples with ≥100 µl eluate volume were suitable for measurement on a Phadia 250 System.

Serum and saliva samples were tested on the Phadia 250 instrument platform (ThermoFisher Scientific, Phadia AB, Uppsala, Sweden). SARS-CoV-2 Spike 1 (S1) antigen (amino acid 14-681, expressed in mammalian cells) was adsorbed onto irradiated polystyrene EliA^{TM} wells and processed (18, 19). An additional test was developed to detect the IgA isotypes of anti-SARS-CoV-2 Spike 1 antibodies on the EliA^{TM} instrument platform. For both, the EliA^{TM} SARS-CoV-2-Sp1 IgG and the test for IgA isotypes, values above 10 U/ml were considered to be reactive. No measurable correlation of results in the respective immunoglobulin subclass between saliva and corresponding serum samples were observed. Further measurements in saliva were discontinued.

Statistical analysis

We adopted the sample size of previous self-sampling studies (9, 12) and did not perform a power calculation. These studies followed the FDA/EUA recommendation of 30 participants per group (12) and Green's rule of thumb calculation (20) for a linear regression for medium effect size and a minimum of 58 subjects (9).

Study group characteristics were summarized using appropriate descriptive statistics. Agreement between the two blood collection methods was assessed using a combination of three tests: Paired Wilcoxon signed rank test, correlation analysis, and Bland-Altman analysis. Clinical interchangeability between the two methods was a priori defined following the methodology by Nwankwo et al. (7): Non-significant paired Wilcoxon signed rank test, Spearman correlation coefficient >0.8, and small bias or max 10% difference between capillary and venous test results on Bland-Altman analysis. Bland-Altman limits of agreement were plotted and estimated. "Bias" is the average of the differences between the two methods of blood sampling, expressed as a percentage %. Spearman's correlation coefficient was calculated and plotted. Significance level was set as $p < 0.05$ for all statistical tests. The distribution of the pairs of variables, and of the difference between two pairs of variables, was assessed with normality tests (Shapiro-Wilk-Test, quantile-quantile plot). When the distribution of the pairs of variables did not follow a Gaussian distribution, non-parametric statistical tests were applied (Paired Wilcoxon signed rank test, Spearman's correlation). The Wilcoxon signed-rank test was used to compare the System Usability total Score (SUS) between capillary and saliva self-sampling and within the groups (patients and health professionals), when the assumptions for a paired *t*-test were not met. All analyses were completed using the R software environment (R version 4.1.1).

Results

Participants

A total of 61 participants (31 IMID patients, 30 HP) were screened for eligibility (Figure 1). One patient declined to participate, so that a total of 30 sex- and age-matched IMID and HP participants were included, Table 1. About 24/30 (80.0%) of IMID patients were receiving immunosuppressive treatment, most frequently biologic disease-modifying antirheumatic drugs (bDMARDs), 15 (50%), conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), six (10.0%), and targeted synthetic DMARDs (tsDMARDs), three (10.0%). The most common IMIDs investigated were rheumatoid arthritis and psoriatic arthritis. The majoritiy of participants had received mRNA-based vaccines.

Interchangeability of capillary blood and saliva with venous blood

We observed an accuracy of 98.3% (57/58) for anti-SARS-CoV-2 IgG antibodies and 100% (58/58) accuracy for anti-SARS-CoV-2 IgA antibodies, as most of the capillary blood samples fell in the same positive and negative categories as the venous results. Only one variation was observed, where the venous serum value for anti-SARS COV-2 IgG antibodies (6.7 U/ml) was close to the equivocal range of 7 to 10 U/ml and the value measured in the capillary sample (10.5 U/ml) and was just above the cut-off of 10 U/ml. A priori criteria to demonstrate interchangeability to venous blood were also met by capillary blood-based SARS-CoV-2 IgG and IgA. IgG and IgA demonstrated an excellent

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TABLE 1 Participant demographics.

Parameter	Total ($n = 60$)	Patients ($n = 30$)	Health professionals ($n = 30$)
Age, years, mean ± SD	49.4 ± 12.4	49.7 ± 12.2	49.0 ± 12.7
Female, *n* (%)	46 (76.7)	23 (76.7)	23 (76.7)
BMI, kg/m^2, mean ± SD	25.7 ± 5.1	26.2 ± 5.4	25.3 ± 4.8
Previous self-sampling experience, *n* (%)	18 (30.0)	8 (26.7)	10 (33.3)
Previous saliva-sampling experience, *n* (%)	21 (35.0)	10 (33.3)	11 (36.7)
Actively smoking	14 (23.3)	7 (23.3)	7 (23.3)
Diagnosis, *n* (%)			
Rheumatoid arthritis	9 (15.0)	9 (30.0)	–
Psoriatic arthritis	9 (15.0)	9 (30.0)	–
Polymyalgia rheumatica	1 (1.7)	1 (3.3)	–
Systemic lupus erythematosus	1 (1.7)	1 (3.3)	–
Axial spondyloarthritis	3 (5.0)	3 (10.0)	–
Microscopic polyangiitis	1 (1.7)	1 (3.3)	–
Psoriasis	1 (1.7)	1 (3.3)	–
Crohn's disease	2 (3.3)	2 (6.7)	–
Anti-synthetase syndrome	1 (1.7)	1 (3.3)	–
Ulcerative colitis	2 (3.3)	2 (6.7)	–
Education status, *n* (%)			
High School graduate	35 (58.3)	18 (60.0)	17 (56.7)
College graduate	14 (23.3)	7 (23.3)	7 (23.3)
University graduate	11 (18.3)	5 (16.7)	6 (20.0)
Treatment			
No treatment	36 (60.0)	6 (20.0)	30 (100.0)
bDMARDs	15 (25.0)	15 (50.0)	–
csDMARDs	6 (10.0)	6 (20.0)	–
tsDMARDs	3 (5.0)	3 (10.0)	–
Vaccination			
mRNA	58 (96.7)	30 (100.0)	28 (93.3)
mRNA + vector	2 (3.3)	0 (0.0)	2 (6.7)

correlation ($r_s = 0.99$), non-significant Wilcoxon signed-rank test (IgG: 0.12; IgA: 0.29), a small bias (IgG: 1.26%; IgA: −0.44%) and the majority of measurements were within a 10% difference (IgG: 86.3%; IgA: 86.3%), see Figure 3; Supplementary material 2.

The device, with which the saliva measurements were performed was not completely developed at the time of this study, and the values were not directly comparable (see Supplementary material 1).

Usability, acceptability and pain

Usability of both self-sampling procedures was rated as excellent, with significantly higher saliva SUS total scores in both groups, resulting in total SUS scores of 95.9 ± 5.7 vs. 90.4 ± 9.7 ($p < 0.001$), see Table 2.

The percentage of NPS promoters (NRS 9-10), was similar for both devices (Figure 4A), ranging between 67 and 70%, resulting in a slightly higher NPS score for capillary self-sampling: + 68 vs. + 63%. Acceptance of capillary self-sampling was generally high both in patients and HPs and even further increased after having done the procedure (Figure 4B). Furthermore, the majority in both groups preferred capillary self-sampling to professional venous blood collection (IMID: 73%; HP: 73%), see Figure 4B.

Capillary self-sampling was perceived as significantly ($p < 0.001$) less painful compared to traditional venous blood collection (IMID: 1.1 ± 0.3 vs. 2.5 ± 1.9; HP: 1.5 ± 1.2 vs. 1.9 ± 1.1). Sixty-three point three percentage and 36.7% of IMID patients perceived capillary self-sampling as less or equally painful compared to venous blood collection. In the HP group 53.3, 36.7 and 10.0% perceived capillary self-sampling as less, equally or more painful compared to venous blood collection.

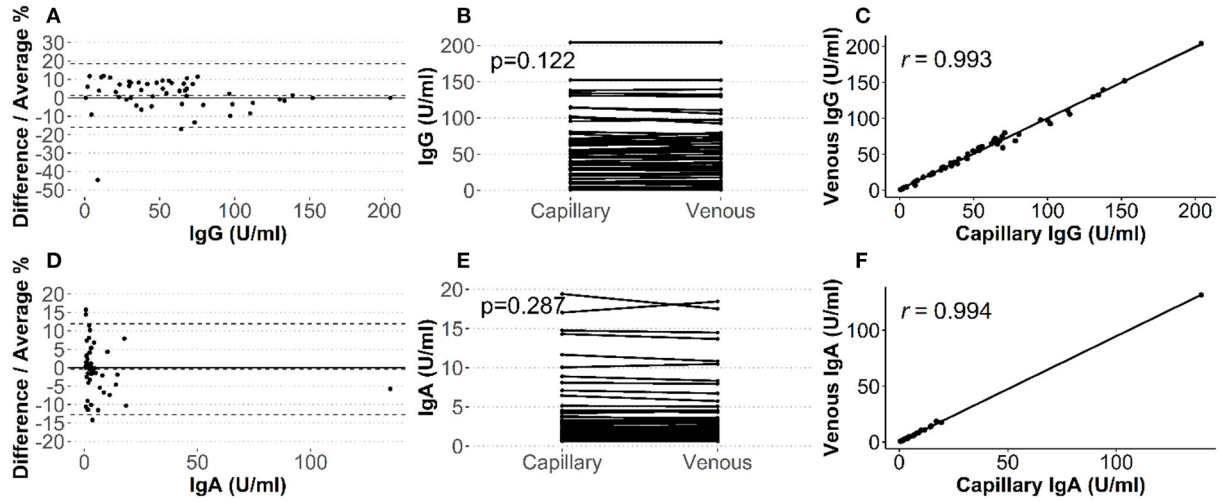

FIGURE 3
Comparison of capillary and venous antibody levels. Single dots represents individual participants. Bland-Altman plot with dashed lines representing upper and lower 95% limits of agreement **(A,D)**, paired Wilcoxon signed rank test, and Spearman's correlation analysis of measurements for IgA **(A–C)** and IgG **(D–F)**, respectively.

TABLE 2 Means and standard deviation scores for the System Usability Scale.

	Total ($n = 60$)		Patients ($n = 30$)		Health professionals ($n = 30$)	
Questions[a] mean ± SD	Saliva	Tasso+	Saliva	Tasso+	Saliva	Tasso+
1. I think I would like to use the system frequently	4.5 ± 1.1	4.4 ± 0.8	4.5 ± 1.1	4.5 ± 0.7	4.5 ± 1.1	4.3 ± 0.8
2. I found the system to be unnecessarily complex	1.1 ± 0.3	1.2 ± 0.4	1.1 ± 0.4	1.1 ± 0.3	1.0 ± 0.2	1.2 ± 0.5
3. I thought the system was easy to use	4.9 ± 0.5	4.8 ± 0.6	5.0 ± 0.2	4.9 ± 0.3	4.8 ± 0.7	4.6 ± 0.7
4. I think that I would need support of a technical person to be able to use the system	1.1 ± 0.4	1.3 ± 0.9	1.1 ± 0.5	1.3 ± 0.7	1.1 ± 0.3	1.4 ± 1.0
5. I found the various functions in the system were well integrated	4.9 ± 0.3	4.6 ± 0.7	5.0 ± 0.0	4.6 ± 0.8	4.9 ± 0.4	4.6 ± 0.6
6. I thought there was too much inconsistency in the system	1.5 ± 0.9	1.7 ± 1.0	1.3 ± 0.7	1.6 ± 1.2	1.6 ± 1.0	1.7 ± 0.9
7. I would imagine that most people would learn to use the system very quickly	5.0 ± 0.2	4.3 ± 1.0	5.0 ± 0.0	4.5 ± 0.7	4.9 ± 0.3	4.1 ± 1.1
8. I found the system very cumbersome to use	1.1 ± 0.3	1.2 ± 0.6	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	1.3 ± 0.7
9. I felt very confident using the system	4.8 ± 0.7	4.6 ± 0.7	4.8 ± 0.8	4.6 ± 0.8	4.8 ± 0.6	4.6 ± 0.7
10. I needed to learn a lot of things before I could get going with the system	1.1 ± 0.2	1.2 ± 0.6	1.1 ± 0.3	1.2 ± 0.6	1.0 ± 0.2	1.2 ± 0.7
System Usability Scale total score (out of 100)	95.9 ± 5.7	90.4 ± 9.7	96.8 ± 5.0	92.1 ± 9.1	95.1 ± 6.2	88.7 ± 10.1

[a]Responses were scored on a five-point Likert scale: 1=strongly disagree, 5=strongly agree.

Self-sampling success rate and supervision

58/60 capillary blood samples and all 60 saliva samples were successfully collected within the first attempt.

Saliva self-sampling supervision

All participants except one (59/60, 98.3%) stated to have adhered to not eating or drinking 30 min prior to saliva collection. 5/60 (8.3%) participants had to be reminded to remove the cotton from the test tube and 3/60 (5.0%) needed assistance to do that. There was uncertainty among 7/60 (11.67%) participants if the small test tube could be thrown in the trash or not. 7/60 (11.67%) participants were unsure when assessing if enough saliva was collected, especially since saliva was often foamy.

Capillary self-sampling supervision

One patient (1/60, 1.7%) and one HP (1/60, 1.7%) failed to collect capillary blood. Both participants stated to be in a hurry, did not pay adequate attention to the instructions and failed

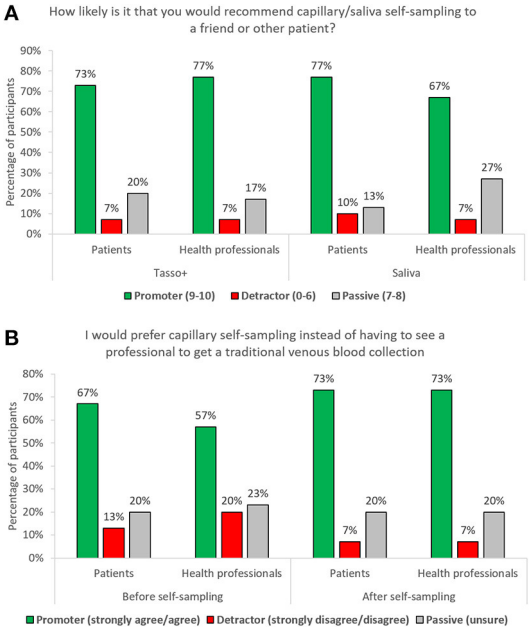

FIGURE 4
(A) Percentage of participants per group according to respective net promoter category and self-sampling device;
(B) participants preference of capillary self-sampling over traditional venous blood collection.

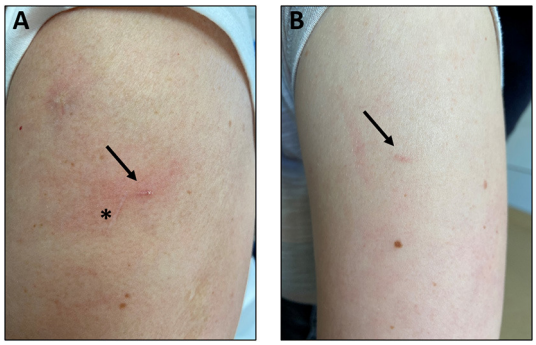

FIGURE 5
Scars (arrows) of two individual participants **(A)** 91 days and **(B)** 89 days after self-sampling. Asterisk to clarify diagonal previous unrelated scar.

to adequately attach the self-sampling device. 17/60 (28.3%) participants did not follow the protocol steps (e.g. wanted to self-sample before attaching the collection tube). 11/60 (18.3%) participants had to be reminded to start the timer while applying the heat pad to the selected spot on the upper arm. Most problems occurred using the heat pad, where 2/60 (3.3%) pointed out that the heat was getting uncomfortable, and one participant stopped the application prematurely. Additionally, 4/60 (6.7%) participants did not understand how to apply the heat pad, 9/60 (15.0%) participants needed assistance with the activation of the heat pad and in 12/60 (20.0%) cases the heat pad was malfunctioning and had to be replaced. About 7/60 (11.7%) did not carry out the disinfection correctly (e.g., had to be reminded, performed too early). 5/60 (8.3%) had difficulties with removing the protective foil. Two participants accidentally teared the adhesive foil off. The device wasn't applicated properly on the selected spot on the upper arm in 4/60 (6.7%) cases. 10/60 (16.7%) participants expressed concern about the device falling off and held on to it during blood collection. After pushing the button, 7/60 (11.7%) participants would have forgotten to start the timer. Assistance for checking the filling state of the test tube was needed in 10/60 (16.7%) cases. Many participants pointed out that they would have used a mirror if they had done the self-sampling at home. The study personnel had to intervene three times when devices (still connected with collection tube) were put on a flat surface with the risk of blood spilling out. Three participants needed assistance to remove and close the test tube. One of them pointed out the lack of strength and fine motor skills in her fingers due to rheumatoid arthritis. The test tube was shaken instead of slowly turned 5/60 (8.3%) times. Three participants had to be reminded of this step. 6/60 (10.0%) participants reported problems with the healing process. Five of them developed a scar, see Figure 5. Tasso has been working on improvements to that effect.

Discussion

In this study comparing capillary- and saliva-based self-sampling in IMID patients and HP we demonstrate that self-collection of capillary blood and saliva is feasible. Importantly, we also demonstrated that capillary blood produces interchangeable results to conventional venous blood. Participants reported high acceptance for self-sampling with a slight preference for capillary self-sampling. The majority in both groups preferred capillary self-sampling over traditional venous blood collection. Supervision of self-collection allowed the identification of pitfalls to improve the self-sampling approach.

Importantly, we were able to demonstrate the interchangeability of capillary-based anti-SARS-CoV-2 antibodies, allowing precise home-based monitoring. These results are in line with a previous study that reported high correlation despite exposing samples to extreme shipping conditions (9) using a previous upper-arm device. Brown et al. also demonstrated the feasibility of capillary self-sampling and that storage of capillary blood at room temperature for up to 7 days post sampling did not affect concordance (21). Similarly, a dried blood spot (DBS) study demonstrated accuracy using only 10 µl of blood and demonstrate the scalability of this home-based approach by conducting a population-based study with a success rate of 82% (22).

SARS-CoV-2 antibody saliva-based analysis has been validated in various populations, including children (11) and COVID-19 patients (10). Contradicting observations of agreement between saliva and serum IgG or IgA levels were reported. Isho et al. (23) described only moderate correlations while others (10, 24) observed good correlation of IgG titers against spike and nucleocapsid antigens. In this study, the values of SARS-CoV-2 spike antigen IgG and IgA antibodies in the saliva were based on a not fully developed device and showed no significant correlation with venous or capillary serum samples. While individual samples showed reasonable concordance it can be speculated that there are multiple contributors to the heterogeneity of saliva samples. Ortega et al. (25) discuss the different sources of saliva IgA (produced locally in salivary gland plasma cells) and IgG (passive diffusion from serum) as a reason for differences in the observed titers. Additionally, saliva sampling shows generally more variations compared to capillary blood because it is more dependent on instruction compliance (no eating/drinking) prior to sampling (24), varying amounts of remaining mucines and individual degrees of viscosity. Recently Campbell et al. (24) reported that salivary antibodies are stable without refrigeration or preservatives for at least 5 days and piloted a saliva collection kit that can be used *via* regular mail, yet in contrast to HIV (26), no saliva-based serology tests are currently commercially available. While many laboratory test kits for the determination of anti-SARS-CoV-2 antibodies are designed for the use with serum or plasma only, it can be speculated that assay technology specifically developed for use with saliva samples may also contribute to higher agreements in antibody titers.

Due to the greater availability of serum-based analysis devices, capillary blood will likely be easier to implement for the time being.

We observed excellent usability (SUS) of both devices and a statistically significant higher saliva SUS score. Compared to the previous RA study (8) with a mean SUS of 83.1 for the upper-arm device and 80.7 for the finger prick, we observed meaningfully higher ratings in this study for the new Tasso device 90.4 and saliva-based sampling, 95.9. Similarly we observed higher NPS ratings in this study (+68%) compared to the previous RA study (+28%). We can only speculate on the reasons for this difference. We believe that the idea of remote COVID-testing (this study) was easier to grasp as participants were already used to COVID self-sampling (antigen) compared to a more novel idea of CRP and RA-related antibody testing (RA study). We could support previous findings, that upper-arm devices are perceived as significantly less painful compared to venous blood collection (8, 27, 28). The number of patients with less pain using the capillary device compared to venous blood collection was very similar to the previous RA study (8) (63 vs. 60%). Interestingly, we were able to show that actual usage of the devices does change the level of acceptance in at least some participants. After usage the majority of participants would prefer capillary self-sampling over traditional venous collection.

58/60 (96.7%) were able to successfully collect capillary blood within first attempt. Medical education (HP) did not seem to have significant effect on success rate or correct completion of self-sampling steps. In a previous study evaluating a former version of the upper-arm device in patients with rheumatoid arthritis (RA), 16% of the patients needed a second attempt and 4% of patients failed to carry out the procedure (8). In a similar study investigating participants with a prior SARS-CoV-2 infection 7% needed a second attempt and no patients failed to perform self-sampling (9). In the same study 32% requested help. Interestingly, in the previous study the most frequent reason for assistance with the device was help to activate it by pressing the button. In contrast to the previous study we tried to standardize the procedure to increase local blood flow and chose heat-pads instead of skin rubbing. The chosen heat-pads devices failed to work multiple times and as we only gave oral instructions to participants, using the heat-pad was the greatest challenge. Additionally, participants needed help to remove the protective film from the self-adhesive patch and accidentally removed the patch itself.

This study has several limitaitons, including the small sample size. A main limitation is that we did not explore the ultimate goal of a home-based remote study. This risk-adverse study setting was chosen, so that correct usage could closely be monitored and study personnel could physically intervene in case of danger. In a next study we want to explore the at-home scenario and provide on-demand help with videoconsultations, as we did not see any major dangers in this study. A home-based study could also involve caring personnel, in case patients cannot use the devices alone. We could gain valuable user feedback regarding usability and acceptance of capillary and saliva sampling. The matched cohorts, including different age groups and diseases are a strength of this study allowing to assess the benefit of having medical training (HP). Usage of a validated composite approach (7) to investigate interchangeability and detailed observation of correct self-sampling execution represent strengths of this study.

Conclusion

Self-collection of capillary blood and saliva is feasible and safe and could facilitate access to antibody testing of the general public. The interchangeability and high acceptance of capillary blood self-sampling enable flexible and convenient vaccine immunogenicity monitoring.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Erlangen-Nürnberg, Germany. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CS and JK wrote the draft manuscript. CS, JK, and E-TG performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors EV and LS were employed by Thermo Fisher Scientific Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.994770/full#supplementary-material

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Clinical characteristics and short-term recovery of hyposmia in hospitalized non-severe COVID-19 patients with Omicron variant in Shanghai, China

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Background: Olfactory dysfunction is a common neurological symptom of Corona Virus Disease 2019(COVID-19). Little is known about hyposmia after COVID-19 infection with Omicron variant in Chinese population.

Objective: To investigate the incidence, clinical characteristics and recovery of hyposmia in hospitalized non-severe COVID-19 patients with Omicron variant in Shanghai, China.

Methods: Three hundred and forty-nine Chinese non-severe COVID-19 patients with Omicron variant were consecutively enrolled in a designated hospital to investigate the incidence of hyposmia in hospitalization and the recovery rate 1 month later. The visual assessment scale (VAS) was used to evaluate the severity of hyposmia. We compared the demographic, clinical features and treatment outcomes, as well as laboratory parameters between patients with and without hyposmia.

Results: The cross-sectional survey showed that 22 (6.3%) hospitalized patients with non-severe COVID-19 had hyposmia. Patients with hyposmia were younger (61.5 vs. 72.0, $p = 0.002$), had more related clinical symptoms (sore throat, cough, poor appetite, diarrhea, myalgia and taste impairment, etc.), a higher proportion of moderate clinical type (31.8 vs. 13.5%, $p = 0.028$) and longer duration of hospitalization (11 vs. 8 days, $p = 0.027$) than those without hyposmia. Whereas, there were no significant differences regarding gender, comorbidity and nucleic acid conversion time between the two groups. Laboratory subgroup analyses demonstrated that patients with hyposmia had slightly low serum IL-6 and TNF-α levels. However, both of the levels were not associated with hyposmia occurrence in multivariate regression analyses. Further follow-up study disclosed that 16 of 22 (72.7%) hyposmia patients had recovered olfaction 1 month later. Serum IL-6 and TNF-α levels were similar between hyposmia recovered patients and those with persistent hyposmia.

Conclusion: Although the incidence of hyposmia after Omicron variant infection is relatively low and the short-term recovery rate is quite high, patients with hyposmia are prone to have a higher proportion of both upper and lower respiratory tract involvements, gastrointestinal and neurological symptoms, contributing to a longer duration of hospitalization.

KEYWORDS

COVID-19, Omicron, hyposmia, IL-6, recovery

Introduction

Novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a pandemic for more than 2 years since December 2019 in Wuhan, China (1). The ongoing COVID-19 pandemic is still a matter of global concern in terms of public health. With the evolution of the virus, Omicron variant, first discovered in southern Africa in November 2021 (2), has replaced the delta variant to become the dominate strain and triggered the fourth wave of COVID-19 worldwide. It also appeared and spread rapidly in Shanghai, China in late February 2022. According to the Shanghai Municipal Health Commission, as of May 4, 2022, more than 600,000 people have been infected, most of them with the fast-spreading Omicron BA.2 variant (3). Clinically, patients infected with COVID-19 Omicron variant had much higher transmissibility, less disease severity and mortality than the previous variants as reported from other countries (4–7).

As one of the neurological manifestations, olfactory dysfunction is a common complaint among COVID-19 patients (8). Hyposmia can be the initial and only symptom during the onset of the disease, and usually shows much improvement within a few weeks in majority of cases (9, 10). Its incidence varies by different virus strains, disease severity and genetic background, ranging from 5.1 to 98.3% (11). It was reported that subjects with older age, Omicron variant, severe clinical classification and East Asian population were associated with low incidence of hyposmia after COVID-19 infection (12, 13). However, infection with Omicron has been rarely examined in East Asia, and only with very small cohorts (14, 15).

The exact pathogenesis of olfactory dysfunction after COVID-19 infection is not fully elucidated. Inflammation of the olfactory system has been reported in COVID-19 related anosmia. Regarding levels of inflammatory markers, Torabi et al. (16) in Iran reported that the pro-inflammatory cytokine, TNF-α level in olfactory epithelium was increased in patients with COVID-19 relative to uninfected controls (16). Experiments have confirmed that virus-infected microglial cells and astrocytes secrete IL-6 and primary glial cells cultured *in vitro* secrete a large number of inflammatory factors, such as IL-6, TNF-α after being infected with coronaviruses (17). In peripheral blood laboratory studies, the results were not consistent. Increased IL-6 levels have been found in serum of patients with hyposmia (18); whereas researchers in Turkey found that serum IL-6 level was lower in patients with COVID-19 related anosmia than those without anosmia (19). Blood tests are easier to obtain than nasal mucosa biopsy. Whether pro-inflammatory cytokines in serum are associated with hyposmia occurrence, severity and recovery of patients with COVID-19 Omicron variant merits investigation.

Therefore, the present study aimed to investigate the incidence, associated clinical characteristics and serum inflammatory parameters associated with olfactory dysfunction in hospitalized non-severe COVID-19 patients with Omicron variant from a Chinese population in Shanghai, China. In addition, the short-term recovery of hyposmia was explored 1 month later *via* telephone interviews.

Materials and methods

Subjects

Between May and June 2022, subjects with non-severe COVID-19 Omicron variant infection admitted in designated hospital of Shanghai Ninth People's Hospital were consecutively screened in this study. All participants were diagnosed with COVID-19 infection according to positive reverse-transcription polymerase chain reaction (RT-PCR) for SARS-CoV2. SARS-CoV-2 viral genomes' phylogenetic characteristics showed that all of the new viral genomes in Shanghai were clustered into the SARS-CoV-2 BA.2.2 sublineage (3). We excluded patients with age under 18 years, pre-existing olfactory dysfunction 1 month before the infection, and obvious cognitive and behavior disorders interfering with further neuropsychological evaluation. We totally screened 362 patients, 13 cases were excluded (1 patient had a history of nasopharyngeal carcinoma, 12 patients could not cooperate to complete the questionnaire). 349 subjects with non-severe COVID-19 Omicron variant infection were enrolled for final analyses (Figure 1). This study was approved by the Medical Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine.

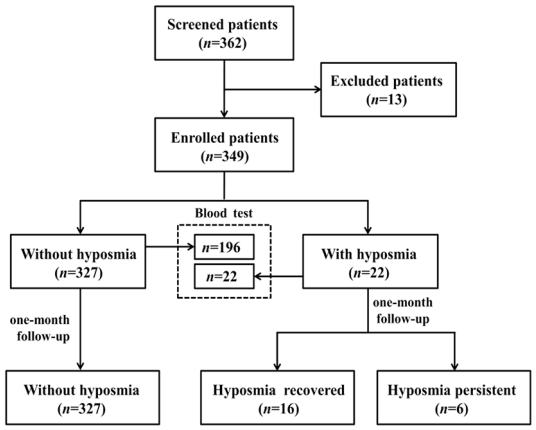

FIGURE 1
Flow chart of the study.

Clinical evaluation

A self-designed structural questionnaire was used to obtain related information of the enrolled subjects. Questionnaires were cross-sectionally administered by the doctors working in the general ward of the designated (20) hospital. We collected demographics (age, sex, smoking, etc.), comorbidities (hypertension, diabetes, chronic obstructive pulmonary disease, coronary heart disease, chronic renal disease, etc.), vaccination status and contact history. Clinical symptoms comprising typical (fever, cough, expectoration, sore throat, etc.), gastrointestinal (poor appetite, diarrhea, nausea, vomiting, etc.) and neurological symptoms (fatigue, myalgia, headache, dizziness, taste impairment, etc.) were carefully recorded (Table 1). Olfactory condition was documented for each participant in hospitalization by a face-to-face interview and 1 month later by a telephone interview. According to a research, hyposmia severity was evaluated by visual assessment scale (VAS) ranging from 0 to 100 score (20). The higher the VAS score, the more severe hyposmia the patients had.

Chest CT scan, clinical treatment (oxygen therapy, corticosteroids, anticoagulation, antibiotic, nutritional support, etc.) and outcomes (duration of hospitalization, time period until the nucleic acid amplification test turned negative, transfer to Intensive Care Unit, death, etc.) were also recorded. Disease classification was determined as asymptomatic, mild, moderate, severe and critical condition, according to the ninth version of Chinese COVID-19 diagnosis and treatment protocol for COVID-19 patients (21). Patients with typical pneumonia changes on CT such as patchy ground-glass opacities were classified into moderate subtype.

Biochemical analyses

To explore the biochemical parameters associated with COVID-19 related hyposmia, two hundred and eighteen patients with detailed biochemical information were enrolled as a subgroup.

Routine blood biochemistry including total white blood cell (WBC), neutrophil, lymphocyte and monocyte count, percentages of neutrophil and lymphocyte hemoglobin, platelet count, C-reactive protein (CRP), coagulation function including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and D-dimer were analyzed during hospitalization. In addition, two pro-inflammatory cytokines in serum, IL-6 and TNF-α were measured in this subgroup of 218 cases.

Statistical analyses

SPSS version 23.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Continuous variables are expressed as the means ± SD or medians [interquartile ranges (IQR), Q1–Q3]; categorical variables are expressed as frequencies and percentages. Comparisons of means between the two groups were performed using the independent *t*-test or the Mann-Whitney U test as appropriate. To compare categorical data among groups, we applied the chi-square test or Fisher's exact test. Two linear regression analyses were used to explore the independent associated factors of serum IL-6 and TNF-α levels, respectively. B value and 95% confidence intervals (CIs) were reported accordingly. The test level (α) was set at 0.05.

Results

Incidence, demographic and clinical characteristics of COVID-19 patients with hyposmia

Among the enrolled 349 cases infected with COVID-19 Omicron variant, 22 patients had hyposmia during hospitalization. So, the prevalence of hyposmia in this cohort was 6.3%. The mean VAS score of these patients with hyposmia was 54.8 ± 25.3 points.

Demographically, COVID-19 patients with hyposmia were younger than those without hyposmia (61.5 vs. 72.0, $p = 0.002$, Table 1). There was no statistically significant difference in gender between the two groups. There was a trend that patients with hyposmia had a marginal increase of vaccination rate (59.1 vs. 39.1%, $p = 0.075$, Table 1) relative to those without hyposmia. Although none of the patients with hyposmia had diabetes, the number of comorbidities was similar between the two groups. Regarding clinical symptoms, patients in the hyposmia group

TABLE 1 Demographic and clinical characteristics of COVID-19 patients with hyposmia.

Items	Total	Without hyposmia	With hyposmia	*p*-Value
n	**349**	**327**	**22**	
Age, years	72.0 (63.0, 82.5)	72.0 (64.0, 84.0)	61.5 (50.7, 71.2)	0.002**
Sex				0.120
Male, *n* (%)	158 (45.4)	152 (46.5)	6 (27.3)	
Female, *n* (%)	191 (54.6)	175 (53.5)	16 (72.7)	
Current smoker, *n* (%)	7 (2.0)	6 (1.8)	1 (4.5)	0.369
COVID-19 vaccination status				0.075
Unvaccinated, *n* (%)	208 (59.6)	199 (60.9)	9 (40.9)	
Vaccinated, *n* (%)	141 (40.4)	128 (39.1)	13 (59.1)	
Comorbidities				
Any, *n* (%)	214 (61.3)	202 (61.8)	12 (54.5)	0.484
Hypertension, *n* (%)	185 (53.0)	177 (54.1)	8 (36.4)	0.125
Diabetes, *n* (%)	67 (19.2)	67 (20.5)	0 (0)	0.011*
COPD, *n* (%)	9 (2.6)	8 (2.4)	1 (4.5)	0.447
Coronary heart disease, *n* (%)	108 (30.9)	103 (31.5)	5 (22.7)	0.480
Chronic renal disease, *n* (%)	57 (16.3)	53 (16.2)	4 (18.2)	0.768
Neurological disease, *n* (%)	92 (26.4)	86 (26.3)	6 (27.3)	1.000
Symptoms				
Typical symptoms				
Fever, *n* (%)	156 (44.8)	143 (43.9)	13 (59.1)	0.188
Cough, *n* (%)	252 (72.2)	232 (70.9)	20 (90.9)	0.049*
Expectoration, *n* (%)	195 (55.9)	179 (54.7)	16 (72.7)	0.122
Sore throat, *n* (%)	131 (37.5)	117 (35.8)	14 (63.6)	0.012*
Runny nose, *n* (%)	106 (30.4)	95 (29.1)	11 (50)	0.053
Nasal obstruction, *n* (%)	57 (16.3)	51 (15.6)	6 (27.3)	0.227
Gastrointestinal symptoms				
Poor appetite, *n* (%)	65 (18.6)	54 (16.5)	11 (50.0)	<0.001***
Diarrhea, *n* (%)	46 (12.3)	35 (10.7)	8 (36.4)	0.002**
Nausea, *n* (%)	21 (6.0)	15 (4.6)	6 (27.3)	<0.001***
Vomiting, *n* (%)	13 (3.7)	10 (3.1)	3 (13.6)	0.041*
Abdominal pain, *n* (%)	12 (3.4)	11 (3.4)	1 (4.5)	0.548
Neurological symptoms				
Fatigue, *n* (%)	87 (25.0)	78 (23.9)	9 (40.9)	0.123
Myalgia, *n* (%)	72 (20.6)	62 (19)	10 (45.5)	0.006**
Headache, *n* (%)	47 (13.5)	41 (12.5)	6 (27.3)	0.097
Dizziness, *n* (%)	46 (13.2)	38 (11.6)	8 (36.4)	0.004**
Taste impairment, *n* (%)	19 (5.4)	11 (3.4)	8 (36.4)	<0.001**
Vision impairment, *n* (%)	19 (5.4)	16 (4.9)	3 (13.6)	0.109
Emotional disorder, *n* (%)	17 (4.9)	16 (4.9)	1 (4.5)	1.000
Acute cerebrovascular disease, *n* (%)	3 (0.9)	3 (0.9)	0 (0)	1.000
Impaired consciousness, *n* (%)	2 (0.6)	2 (0.6)	0 (0)	1.000
Seizure, *n* (%)	2 (0.6)	2 (0.6)	0 (0)	1.000
COVID-19 disease classification (admission)				0.430
Asymptomatic or mild, *n* (%)	319 (91.4)	299 (91.4)	20 (90.9)	
Moderate, *n* (%)	30 (8.6)	28 (8.6)	3 (13.6)	
COVID-19 disease classification (discharge)				0.028*
Asymptomatic or mild, *n* (%)	298 (85.4)	283 (86.5)	15 (68.2)	
Moderate, *n* (%)	51 (14.6)	44 (13.5)	7 (31.8)	

(Continued)

TABLE 1 (Continued)

Items	Total	Without hyposmia	With hyposmia	*p*-Value
Clinical treatments				
Oxygen therapy, *n* (%)	40 (11.5)	39 (11.9)	1 (1.5)	0.491
Antiviral-paxlovid, *n* (%)	222 (63.6)	209 (63.9)	13 (59.1)	0.653
Corticosteroids, *n* (%)	58 (16.6)	55 (16.8)	3 (13.6)	1.000
Anticoagulation, *n* (%)	58 (16.6)	55 (16.8)	3 (13.6)	1.000
Antibiotic, *n* (%)	59 (16.9)	53 (16.2)	6 (27.3)	0.234
Intravenous immunoglobulin, *n* (%)	8 (2.3)	7 (2.1)	1 (4.5)	0.409
Thymosin, *n* (%)	72 (20.6)	69 (21.1)	3 (13.6)	0.587
Nutritional support, *n* (%)	106 (30.4)	100 (30.6)	6 (27.3)	0.477
Clinical outcomes				
Duration of Hospitalization	8 (5, 11)	8 (5, 11)	11 (7, 13)	0.027*
Turning to nucleic acid negative duration	10 (7, 13)	10 (7, 13)	10 (8, 12)	0.901

*p < 0.05; **p < 0.01; ***p < 0.001.

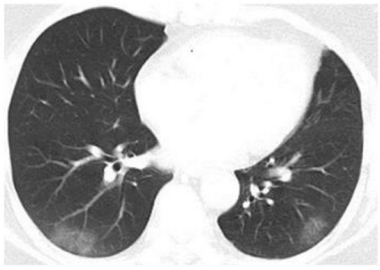

FIGURE 2
Chest CT image of a COVID-19 patient with hyposmia. Axis chest CT scan showed bilateral patchy ground-glass opacities consistent with typical moderate COVID-19.

had more typical [cough (90.9 vs. 70.9%, $p = 0.049$) and sore throat (63.6. vs. 35.8%, $p = 0.012$)], gastrointestinal [(poor appetite (50.0 vs. 16.5%, $p < 0.001$), diarrhea (36.4 vs. 10.7%, $p = 0.002$), nausea (27.3 vs. 4.6%, $p < 0.001$) and vomiting (13.6 vs. 3.1%, $p = 0.041$)] and neurological [myalgia (45.5 vs. 19.0%, $p = 0.006$), dizziness (36.4 vs. 11.6%, $p = 0.004$) and taste impairment (36.4 vs. 3.4%, $p < 0.001$)] symptoms, in comparison with those without hyposmia (Table 1).

Concerning COVID-19 severity, patients in the hyposmia group had a higher proportion of moderate COVID-19 (31.8% vs. 13.5%, $p = 0.028$) at discharge relative to those without hyposmia (Table 1), indicating a higher proportion of lung involvement in this subgroup (Figure 2). Although the clinical treatment and time period of conversion of the nucleic acid amplification test from positive to negative were similar, COVID-19 patients with hyposmia had longer duration of hospitalization (11 vs. 8 days, $p = 0.027$, Table 1). None of the enrolled subjects were transferred to intensive care unit (ICU) or died.

Laboratory analysis of COVID-19 patients with hyposmia

Subgroup analysis based on 218 patients (Figure 1) demonstrated that subjects with hyposmia had slightly lower serum IL-6 (3.71 vs. 6.11, $p < 0.001$) and TNF-α (5.72 vs. 9.34, $p = 0.010$) levels than those without hyposmia (Table 2). There was no statistical difference in terms of blood routine, coagulation function, C-reactive protein and other inflammatory indicators between the two groups. However, linear regression analyses demonstrated that older age was independently associated with IL-6 levels; also, older age and diabetes were independently associated with TNF-a levels in serum (Table 3).

We also did a correlation analysis between those two cytokines and hyposmia VAS score. It revealed that neither IL-6 ($r = -0.022$, $p = 0.929$) nor TNF-a ($r = -0.008$, $p = 0.974$) levels in serum were related to hyposmia severity.

Olfactory recovery of COVID-19 patients with hyposmia at one-month follow-up

COVID-19 patients with hyposmia ($n = 22$) were followed up by telephone interviews 1 month after discharge (Figure 1). Olfactory function still did not return to normal in 6 of 22 patients (27.3%). Subsequently, we compared the differences in baseline VAS scores of hyposmia and laboratory indicators between the hyposmia recovered ($n = 16$) and persistent group ($n = 6$). It demonstrated that there were no significant differences in terms of initial VAS score (52.2 ± 25.7 vs. 61.7 ± 24.8, $p = 0.528$), serum IL-6 (3.56 vs. 4.46, $p = 0.803$) or TNF-α (5.07 vs. 7.83, $p = 0.184$) levels between hyposmia recovered and persistent groups.

TABLE 2 Laboratory findings of COVID-19 patients with hyposmia ($n = 218$).

Laboratory finding	Without hyposmia	With hyposmia	*p*-Value
n	**196**	**22**	
White blood cell count, *10^9/L	5.29 ± 1.78	5.25 ± 2.36	0.235
Neutrophil cell count, *10^9/L	2.97 (2.09, 4.14)	2.31 (1.65, 3.64)	0.197
Lymphocyte count, *10^9/L	1.30 (1.00, 1.70)	1.60 (1.00, 2.10)	0.107
Monocyte count, *10^9/L	0.51 (0.39, 0.67)	0.50 (0.42, 0.72)	0.940
Hemoglobin, g/L	130.25 ± 17.53	128.23 ± 16.11	0.223
Platelet count, *10^9/L	187.95 ± 66.36	208.59 ± 78.05	0.268
Neutrophil/ Lymphocyte	1.95 (1.44, 3.51)	1.48 (1.25, 2.20)	0.052
Monocyte / Lymphocyte	0.39 (0.27, 0.57)	0.38 (0.25, 0.46)	0.178
C-reactive protein, mg/L	3.81 (1.58,10.30)	2.29 (0.68, 6.60)	0.121
PT, s	10.90 (10.40, 11.40)	10.60 (10.40, 11.10)	0.097
APTT, s	28.50 (26.50, 30.50)	28.60 (26.88, 30.12)	0.808
Fibrinogen, g/L	3.04 (2.58, 3.60)	2.90 (2.55, 3.29)	0.427
D-dimer, mg/L	0.42 (0.22, 0.79)	0.26 (0.13, 1.29)	0.398
IL-6, pg/mL	6.11 (4.01, 9.11)	3.71 (0.00, 4.81)	<0.001***
TNF-α, pg/mL	9.34 (6.73, 12.13)	5.72 (0.00, 8.01)	0.010**

*p < 0.05; **p < 0.01; ***p < 0.001.

TABLE 3 Independent associated factors of serum IL-6 and TNF-α levels in COVID-19 patients ($n = 218$).

Items	Univariate regression		Multivariate regression	
	***B* (95%CI)**	***p*-value**	***B* (95%CI)**	***p*-value**
Model 1: Dependent factor: serum IL-6 level				
Age: > 72 years	5.96 (3.34, 8.58)	<0.001***	5.40 (2.47. 8.32)	<0.001***
COVID-19 disease severity: Moderate	2.03 (−1.73, 5.79)	0.288	0.36 (−3.38, 4.01)	0.849
Vaccination status: Unvaccinated	3.00 (0.26, 5.74)	0.032*	1.26 (−1.58, 4.10)	0.383
With diabetes	2.68 (−0.68, 6.04)	0.117	1.76 (−1.57, 5.09)	0.299
With hyposmia	−1.22 (−6.18, 3.74)	0.630	1.29 (−3.68, 6.26)	0.609
Model 2: Dependent factor: serum TNF-α level				
Age: > 72 years	3.81 (2.28, 5.35)	<0.001***	2.79 (1.12, 4.46)	0.001**
COVID-19 disease severity: Moderate	1.20 (−1.01, 3.42)	0.286	0.80 (−1.34, 2.93)	0.462
Vaccination status: Unvaccinated	2.03 (0.42, 3.65)	0.014*	0.80 (−0.82, 2.43)	0.331
With diabetes	3.57 (1.62,5.52)	<0.001***	2.70 (0.78, 4.61)	0.006**
With hyposmia	−4.10 (−6.91, −1.30)	0.004**	−2.44 (−5.22, 0.35)	0.087

CI, confidence interval; *p < 0.05; **p < 0.01; ***p < 0.001.

Altogether, our results suggested that although there was a slight reduction of serum IL-6 and TNF-α levels in the hyposmia group, both of the two pro-inflammatory cytokine levels in serum were not associated with hyposmia occurrence, severity or recovery in COVID-19 patients. Aging and diabetes may influence the expression of the two cytokines in serum.

Discussion

To our knowledge, this is the first study in China reporting the epidemiological data of olfactory dysfunction after Omicron variant infection. Our results based on 349 patients with non-severe COVID-19 Omicron variant enrolled in designated hospital revealed that (1) the incidence of hyposmia after Omicron infection was relatively low and the short-term recovery rate was quite high; (2) patients with hyposmia had more associated clinical symptoms and increased proportions of both upper and lower respiratory tract involvements, contributing to a longer duration of hospitalization;(3) serum IL-6 and TNF-α levels were not related to hyposmia occurrence, severity or recovery.

We validated that Chinese COVID-19 patients with Omicron variant also had relatively low hyposmia incidence.

This finding was consistent with the reports from other countries. Loss of smell was less likely among people infected during Omicron prevalence than during delta prevalence, according to a ZOE COVID study conducted in the UK (16·7 vs 52·7%) (4). A systematic review based on the first 12 reports revealed that approximately 13% of patients with Omicron infection had involvement of Smell (22), which was 3–4-fold lower than the prevalence in times and regions when the alpha and delta variants prevailed. All these findings indicate that Omicron variant largely spares the olfactory function. In comparison to earlier strains, the new mutations make Omicron more hydrophobic and alkaline, which may lessen mucus layer penetration. Omicron very slightly alters receptor binding affinity, however, entry efficiency into host cells is reduced in cells expressing the TMPRSS2 protease. The sustentacular cells in the olfactory epithelium, which are the novel Omicron variant's primary target cells, may be less likely to become infected because they abundantly express TMPRSS2. In addition, genetic background may also contribute to the low incidence of hyposmia in Chinese population (12). Shelton et al. reported that the UGT2A1/UGT2A2 locus was associated with COVID-19-related loss of smell or taste, which differed significantly between ethnicities (23). All these factors may explain the low incidence and high recovery of hyposmia in our cohort. Compared with those in Western Countries, patients in East Asia had less olfactory impairment. During the battle against COVID-19 in the past 2 years, more and more residents received COVID-19 vaccination in China. The usefulness of vaccination in reducing the severity of COVID-19 has been adequately proven (24); however, there is not enough evidence to establish a link between vaccination and the low occurrence of chemosensory disorders (25).

A novel finding is that patients with hyposmia had more upper respiratory (sore throat), lower respiratory (cough), gastrointestinal (poor appetite, diarrhea, nausea and vomiting) and neurological (myalgia and taste impairment) symptoms as demonstrated by our detailed symptomatic descriptions. Also, such kind of patients were more likely to have lung infiltration as revealed by COVID-19 clinical classification. All these factors could result in a possible longer hospital stay. Burges Watson et al. in Italy reported that COVID-19 patients with hyposmia also had a higher proportion of altered eating, appetite loss and weight changes (26). Smell and taste impairments are typical chemosensory dysfunctions, and usually correlated to each other after COVID-19 infection (27). It was also reported that COVID-19 related myalgia was a risk factor for persistent hyposmia (28). Although higher proportions of lung infiltration at discharge in patients with hyposmia were found, none of them had converted to severe/critical stage, indicating the pulmonary infiltration in such kind of patient is not severe. The underlying mechanism is still unknown. Some previous studies found that hyposmia appeared less in severe COVID-19 patients and may represent a favorable prognosis (29). Our study suggested that hyposmia could be a marker indicating high proportions of both upper and lower respiratory involvements. Hyposmia in COVID-19 patients may not be as benign as reported. This has important clinical implications. For these patients, more attention should be paid to their pulmonary conditions. Close monitoring and active treatment are required.

The pathogenesis of hyposmia related to COVID-19 is still not fully elucidated. Accumulating evidence suggested that pro-inflammatory cytokines, IL-6 and TNF-α may be associated with hyposmia secondary to COVID-9 infection. We found the serum IL6 and TNF-α levels were not correlated with hyposmia occurrence, severity or recovery, which was consistent with Sanli's report in Turkey (19) and Vaira's report in Italy (30). Regarding nasal biopsies, Torabi et al. (16) reported that the pro-inflammatory cytokine, TNF-α level in olfactory epithelium was increased in patients with COVID-19 relative to uninfected controls (16). One autopsy study in two patients found that there was inflammatory olfactory neuropathy, mainly axonal damage in olfactory epithelium in two patients with COVID-19 (31), whereas, the olfactory tracts were largely unremarkable. Significant pathology in central nervous system structures, including those related to olfaction, appears to be relatively rare (32). Based on these results, we infer that local inflammation in nasal mucosa rather than the systemic inflammation may contribute to COVID-19 related hyposmia in the acute stage (33). More mechanism research of COVID-19 related hyposmia is warranted in future.

In our study, 6 of 22 patients (27.3%) still had olfactory deficits at one-month follow-up. Whether their persistent hyposmia will develop into long-term sequelae merits investigation. Possibly, COVID-19 patients with persistent hyposmia had affections of a larger area of the sensory epithelium, presumably with more extensive epithelium damage that resulted in the loss of more olfactory receptor neurons (12). The notion of the SARS-CoV-2 virus being neurotropic in humans and invading the brain through the olfactory nerve is highly controversial (34). A more extensive viral propagation into other brain regions would have been expected if systemic hematogenous involvement occurred. However, other studies have suggested that olfactory transmucosal virus invasion is a port of central nervous system entry in individuals with COVID-19 (35). Neurodegenerative diseases may be accelerated by an inflammatory signal from the nasal olfactory epithelium to the olfactory bulbs and associated brain areas. Long-term longitudinal follow-up is needed to explore the association between persistent olfactory dysfunction and phenotypic conversion of neurodegenerative diseases (36), such as Parkinson's disease.

This study has a few limitations. First, severe/critical patients were not included in the study, since the proportion of such

kind of patients was relatively low, and they were usually transferred to ICU, not treated in general ward. Second, we used subjective VAS score to evaluate the occurrence and severity of hyposmia, which may underestimate the real hyposmia incidence compared with objective evaluation method such as sniffin' sticks. Third, the sample size of patients with hyposmia is relatively small. The findings are exploratory. Multi-center registry studies for patients with hyposmia are needed in future.

Conclusion

Our study based on Chinese population broadens the epidemiological data and phenotypic characteristics of Omicron related hyposmia. Although the incidence of hyposmia after Omicron infection is relatively low and the short-term recovery is quite high, patients with hyposmia are prone to have higher proportions of both upper and lower respiratory tract involvements, gastrointestinal and neurological symptoms, contributing to longer hospitalized duration. COVID-19 with hyposmia may not be as benign as reported. Close monitoring and active treatment are needed for such kind of patients. Systematic inflammation in serum may not contribute to COVID-19 related hyposmia in the acute stage. More mechanism research and long-term follow-up of hyposmia in COVID-19 are warranted in future.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China (2022-T130-2). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JS, LW, WC, and JL had a role in the design of the study. JS, LW, XS, PW, YJ, WC, and JL had a role in the acquisition and interpretation of data. LW and WC analyzed the data and interpreted it. JS, LW, and WC drafted the manuscript. WC revised it. The final version of the manuscript was amended with input from all authors, who also gave their approval.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Sex difference in circulating soluble form of ACE2 protein in moderate and severe COVID-19 and healthy controls

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Introduction: Membrane-bound angiotensin-converting enzyme-2 (ACE2) in epithelial cells is the main receptor for SARS-CoV-2. The extracellular portion of ACE2 may be shedded to plasma in which process ADAM17 (a disintegrin and metalloproteinase 17) is important. Results on the relationship between circulating levels of the soluble form of ACE2 (sACE2) and disease severity are inconclusive. This study investigates if sACE2 concentration correlates with COVID-19 severity, and whether this is affected by sex.

Materials and methods: Soluble form of ACE2 was analyzed in three groups: 104 patients (23 women and 81 men) with severe COVID-19 admitted to an intensive care unit (ICU), patients with moderate COVID-19 who required hospital care (n = 19, 4 women and 15 men), and age and sex matched healthy controls (n = 20, 4 women and 16 men). Blood samples were collected at hospital admission between 18 March 2020, and 3 May 2021, and at follow-up between 27 October 2020, and 19 October 2021. Circulating sACE2 (μg/L) was measured in EDTA plasma with a sensitive enzyme-linked immunosorbent assay. Additionally, CRP, ferritin, and lymphocyte count were analyzed during hospital stay.

Results: In total, 23 patients (22%) died in the ICU. When comparing healthy controls [mean age 58.1 (SD 11.4) years] and patients with moderate COVID-19 [mean age 61.0 (SD 13.2) years] with patients in the ICU [mean age

63.6 (SD 11.6) years], we found that sACE2 concentration decreased (70% reduction) with disease severity in men (p = 0.002) but increased 3.7-fold with severity in women (p = 0.043), suggesting a sex-related difference in how COVID-19 severity is related to sACE2 concentration. Moreover, we identified a relationship between inflammatory biomarkers and sACE2 concentration during the intensive care treatment, such that higher CRP and higher ferritin concentration correlated with lower sACE2 concentration in men.

Conclusion: The decrease in sACE2 concentration, selectively in men, in severe COVID-19 is of pathophysiological interest since men are affected more severely by the disease compared to women. Additionally, the inflammatory biomarkers, CRP and ferritin, correlated inversely with sACE2 concentration, suggesting a role in severe disease. Our findings imply that sACE2 is a possible biomarker of disease severity in a sex-specific manner.

KEYWORDS

COVID-19, disease severity, sex, intensive care unit, sex difference, angiotensin-converting enzyme 2 (ACE2)

Introduction

Membrane-bound angiotensin-converting enzyme 2 (ACE2) in epithelial cells is the main receptor for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The Transmembrane Serine Protease 2 (TMPRSS2) also plays an important role (1). Another enzyme, a disintegrin and metalloproteinase 17 (ADAM17), may cause shedding of ACE2 from cells in the epithelia and from exosomes. This is supposed to have an important regulatory function in the immune system (2, 3).

It is known since long that angiotensin II differently affects the regional blood circulation in various tissues (4). The enzymatic activity of ACE2 is to convert angiotensin II to angiotensin 1–7, attenuating the effects of angiotensin II including vasoconstriction and inflammation (5, 6). Circulating soluble ACE2 (sACE2) may depend on the density of membrane-bound ACE2 in epithelial cells but also on the local ADAM17 activity. Thus, sACE2 may not entirely reflect the expression of ACE2 on epithelial cells. ACE2 expression varies in different tissues and the density in the small intestine seems to be high, thereby representing a major source of the enzyme entering the circulation (6). The balance between sACE2 and the tissue levels is not determined so far. It has been suggested that circulating sACE2 may protect from tissue infection by trapping SARS-CoV-2, and therapeutic attempts are even made to engineer human sACE2 to optimize binding to the spike protein in the virus (7). sACE2 may be analyzed with methods to evaluate the specific protein content as proteomics like OLINK (8–10), mass spectrometry (11), and enzyme-linked immunosorbent assay (ELISA) (12, 13), as well as, with enzymatic methods (14–18).

Early in the coronavirus disease 2019 (COVID-19) pandemic it was suggested in observational studies that the renin-angiotensin-aldosterone system (RAAS)-blockade by ACE inhibitors or angiotensin II type-I receptor blockers (ARBs) would increase the risk of severe SARS-CoV-2 outcomes by upregulating the expression of membrane-bound ACE2. However, many of these studies included a critical risk of confounding or selection bias, and the initial finding that RAAS inhibitor use increases the risk of severe COVID-19 has not been confirmed in later high-quality studies (19).

There is a strong support for a role of ACE2 and TMPRSS2 in severe COVID-19, and sACE2 has been proposed as a potential predictor of disease severity (20–22). Several reports have also tried to investigate the relationship between sACE2 and disease severity. However, studies of circulating sACE2 in severe COVID-19 have shown confusing results, such that plasma levels of sACE2 may be raised or reduced. Differences in enzymatic, ELISA, and immunoprecipitation methods make it difficult to compare the results, which may explain the divergence of sACE2 in different COVID-19 studies. Further, the population samples studied are often heterogenous with regard to age and gender. It is well-known that sACE2 in healthy men are higher than in healthy women (23). Despite this, the sACE2 response to moderate and severe COVID-19 has not been studied separately in men and women although being of possible importance in view of the higher probability of severe COVID-19 progression in men. Moreover, most previous studies have been cross-sectional without longitudinal follow-up of changes. In summary, the results on the relationship between

levels of sACE2 and severity of COVID-19 are controversial and not entirely conclusive, and analyzes regarding sex differences are lacking.

The aim of the present study was to investigate sACE2 concentration in relation to COVID-19 severity, and potential associations with sex.

Materials and methods

Participants

In total, 104 patients with COVID-19, who were admitted to an intensive care unit (ICU) at the Sahlgrenska University Hospital, Gothenburg, Sweden, were included in the study (severe/critical COVID-19). All of them received mechanical ventilation. For comparison analyses, we included 19 patients with moderate COVID-19 who required hospital care but were not high flow nasal oxygen (HFNO)-dependent, at the Department of Infectious Diseases at the Sahlgrenska University Hospital, Gothenburg, Sweden (24). All cases were confirmed with reverse transcriptase polymerase chain reaction (RT-PCR) from nasopharyngeal and throat aspirates. Additionally, twenty healthy age and sex matched volunteers, mostly health care workers, were included as controls. The study was a sub-study of an ongoing prospective COVID-19 cohort study (25, 26), and was conducted in accordance with the ethical principles set out in the declaration of Helsinki. It was approved by the Swedish Ethical Review Authority (Dnr: 2020-01771). Written informed consent was obtained from all participants.

Blood sampling and laboratory analyses

Blood samples at hospital admission were collected between 18 March 2020, and 3 May 2021. Blood samples at follow-up were collected between 27 October 2020, and 19 October 2021. Concentration of sACE2 (μg/L) was measured in EDTA plasma with an enzyme-linked immunosorbent assay (ELISA) using the High Sensitivity Human Soluble Angiotensin-Converting Enzyme 2 (ACE2) immunoassay (Catalog No. SK00707-06, Aviscera Bioscience Inc., Santa Clara, CA) according to the manufacturer's instructions. The inter-assay coefficient of variation was <10%. Samples with sACE2 concentrations below the lower limit of detection (<0.3 μg/L, n = 8) were adjusted to 0.15 μg/L, and samples with sACE2 concentrations above the upper limit of detection (>631 μg/L, n = 2) were excluded due to uncertain values related to the possible impact of heterophilic antibodies. Concentration of C-reactive protein (CRP) and ferritin were analyzed using standard laboratory techniques and automated Alinity Instruments (Abbott Laboratories, Chicago, IL, USA). Lymphocyte count was measured in whole blood with the auto-hematology analyzer ADVIA® 2120i System (Siemens Healthcare GmbH, Erlangen, Germany). All analyses were performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden.

Statistics

Descriptive statistics are shown for all variables involved in the analyses, presented as means with standard deviations and medians with interquartile ranges. For statistical analyses, continuous variables were $\log_{10}$ transformed. Student's t-test was used for group comparisons and stratified by sex or severity. Associations between numeric variables were analyzed with linear regression and measured with Pearson correlation. Some associations were stratified by sex, and slope differences were investigated by an interaction term.

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) software version 25 (SPSS, Chicago, Illinois, USA) or Prism (GraphPad software version 8.0, La Jolla, California, USA). A significance level of 0.05 was used.

Results

Of the 104 patients aged 23–85 years [mean age 63.6 (SD 11.6) years] with COVID-19 requiring intensive care treatment, including mechanical ventilation, 23 (22%) were women. A total of 23 patients (22%) died in the ICU. Blood samples were collected at hospital admission [mean 11.5 (SD 6.4) days since symptom onset] and at follow-up [mean 227.5 (SD 39.8] days since symptom onset) (**Table 1**). Moreover, we identified 19 patients aged 37–79 years [mean age 61.0 (SD 13.2) years] with moderate COVID-19, not requiring ICU treatment but who were admitted to hospital. Four of these patients (21%) were women. None of the patients in this group died during follow-up. Blood sampling at hospital admission was performed in average 13.5 (SD 7.7) days since symptom onset, and at follow-up, in average, 241.5 (SD 48.3) days since symptom onset. The healthy controls were aged 42–81 years [mean age 58.1 (SD 11.4)], and four of them (20%) were women (**Table 1**).

When comparing sACE2 concentrations between men and women in each severity group, we found that women displayed significantly lower levels among healthy controls, as expected, but also among patients with moderate COVID-19. However, there was no difference between men and women among patients with severe COVID-19 treated in the ICU (**Figure 1**). sACE2 concentrations did not differ between patients who died in the ICU and survivors (data not shown), and sACE2 levels did not correlate to age or days since symptom onset in any of the COVID-19 severity groups (**Supplementary Figures 1**, **2**).

Healthy controls and patients with moderate disease displayed similar sACE2 concentrations without any significant

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TABLE 1 Descriptive statistics of included COVID-19 patients and healthy controls.

	COVID-19 in ICU* $n = 104$	Moderate COVID-19 $n = 19$	Healthy controls $n = 20$
Age, mean (SD)	63.6 (11.6)	61.0 (13.2)	58.1 (11.4)
Women, n (%)	23 (22)	4 (21)	4 (20)
Died, n (%)	23 (22)	–	–
Hypertension, n (%)	46 (44)	9 (47)	1 (5)
Diabetes mellitus, n (%)	24 (23)	5 (26)	1 (5)
Obesity, n (%)	26 (25)	6 (32)	**
Chronic heart disease***, n (%)	14 (13)	1 (5)	–
Corticosteroid treatment, n (%)	67 (64)	6 (32)	–
Tocilizumab (RoActemra) treatment, n (%)	2 (2)	–	–
Blood sampling at hospital admission, n (%)	77 (74)	19 (100)	19 (95)
Days since symptom onset, mean (SD)	11.5 (6.4)	13.5 (7.7)	–
sACE2 (μg/L), mean (SD)	30.5 (55.1)	58.9 (109.3)	42.7 (52.8)
sACE2 (μg/L), median (IQR)	9.4 (3.4–28.5)	12.0 (6.7–54.0)	21.0 (6.3–50.3)
Blood sampling at follow-up, n (%)	80 (77)	19 (100)	–
Days since symptom onset, mean (SD)	227.5 (39.8)	241.5 (48.3)	–
sACE2 (μg/L), mean (SD)	44.0 (84.2)	65.1 (122.7)	–
sACE2 (μg/L), median (IQR)	14.0 (5.1–38.5)	15.0 (4.7–37.0)	–

*Critical disease. **There was no information regarding BMI in healthy controls. ***Includes coronary heart disease, heart failure, cardiomyopathy. ICU, intensive care unit; SD, standard deviation; sACE2, soluble angiotensin-converting enzyme 2; IQR, interquartile range.

difference, regardless of sex (Figure 2). Therefore, we merged these groups and compared them as one group to patients with severe disease. In these analyses, severely ill men treated at an ICU displayed lower levels of sACE2 (70% reduction, $p = 0.002$) than men with moderate COVID-19 and healthy controls (Figure 2). Conversely, women with severe disease had significantly higher sACE2 values (3.7-fold increase, $p = 0.043$) than the group of women with moderate disease and healthy controls (Figure 2). Thus, our data suggest that sACE2 concentrations decrease with the severity of COVID-19 among men whereas contrariwise sACE2 concentrations increase with disease severity among women.

In a longitudinal analysis of sACE2 levels at hospital admission and at follow-up, we found no change in sACE2 over time in moderately ill or severely ill COVID-19 patients (Figure 3). Moreover, six patients with moderate COVID-19 and 67 patients with severe COVID-19 received corticosteroid treatment. The sACE2 levels, however, did not differ at hospital admission or follow-up between patients with or without corticosteroid treatment (Supplementary Figure 3). Only two COVID-19 patients in the study were treated with the IL-6 receptor blocking antibody tocilizumab (RoActemra), and its effect on sACE2 levels could therefore not be studied. When analyzing preexisting treatment with ACE inhibitors or ARBs among patients with moderate COVID-19 ($n = 9$) and COVID-19 in the ICU ($n = 34$), there was no significant difference in sACE2 levels in either group (Supplementary Figure 4). Among patients treated in the ICU, sACE2 concentration did not correlate with days with mechanical ventilation (Figure 4). Nor was there any correlation if only including men in this analysis ($r = -0.03$, $p = 0.83$).

To investigate if cardiovascular comorbidities affected the sACE2 concentration among patients in the ICU, we compared patients with and without hypertension ($n = 44$), diabetes mellitus ($n = 20$) and chronic heart disease (coronary heart disease, heart failure, cardiomyopathy; $n = 14$). There were no significant differences in sACE2 levels for any of these comorbidities (Supplementary Figure 5). Further, sACE2 concentration did not correlate with BMI ($r = -0.07$, $p = 0.58$).

The relationship between inflammatory biomarkers and sACE2 levels during the intensive care was studied in correlation analyses including maximum levels of C-reactive protein (CRP, mg/L), maximum concentrations of ferritin (μg/L), and minimum lymphocyte counts ($\times 10^9$/L), recorded during hospitalization. When including all patients, significant correlations were observed for CRP and ferritin, in such manner that higher CRP and higher ferritin levels correlated with lower sACE2 concentrations (Figure 5). Thus, the inflammatory state in severe COVID-19 may be associated with lower sACE2 levels. When stratifying for sex, we found no significant sex differences in how CRP and lymphocyte count were correlated with sACE2 (interaction terms: $p = 0.26$, $p = 0.46$). However, there was a statistically significant sex difference in how ferritin concentration correlated with sACE2 level (interaction term: $p = 0.02$) (Supplementary Figure 6).

Discussion

The main finding of the present study is that sACE2 concentration decreases with the severity of COVID-19 among men, while sACE2 concentration increases with disease severity among women. We also found that the inflammatory biomarkers CRP and ferritin correlated inversely with sACE2 concentration in men, suggesting a role in severe disease. Our results indicate that sACE2 has potential to be a valuable biomarker of disease severity in patients with SARS-CoV-2

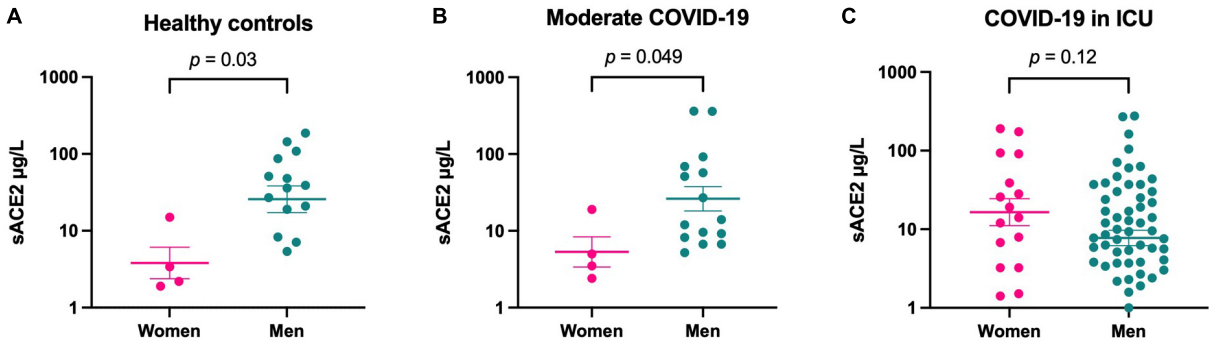

FIGURE 1
Soluble form of ACE2 (sACE2) concentrations (µg/L) in healthy controls **(A)**, at hospital admission for patients with moderate COVID-19 **(B)**, and at hospital admission for patients with COVID-19 in the intensive care unit (ICU) **(C)**, divided by sex. Individual values and mean value with SEM are shown for each group. Student's *t*-test was used for group comparisons.

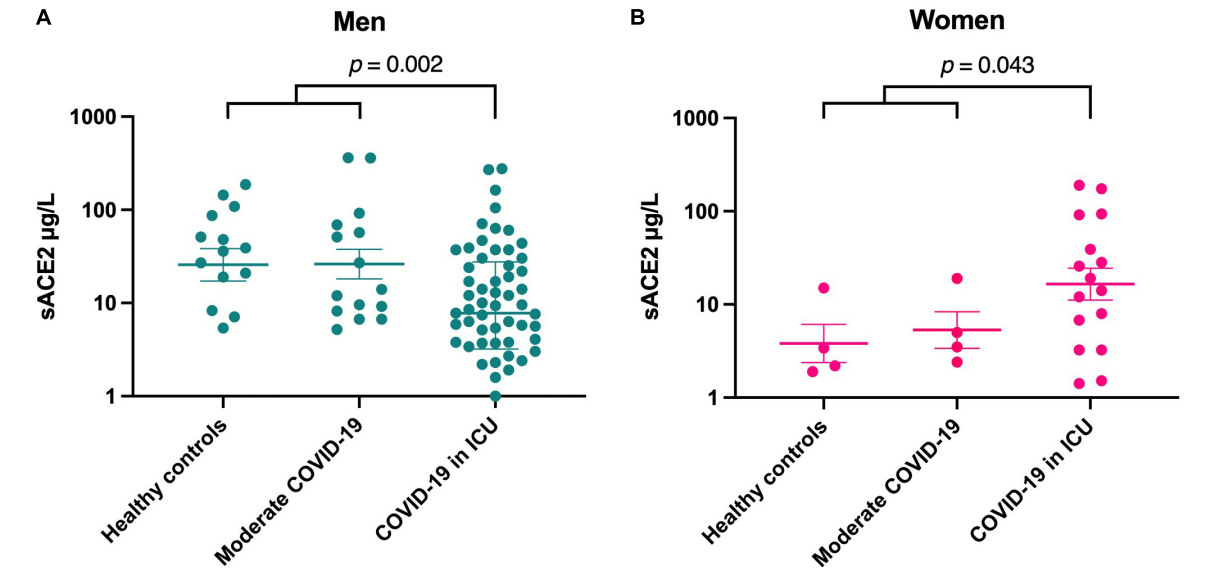

FIGURE 2
Soluble form of ACE2 (sACE2) concentrations (µg/L) in men **(A)** and women **(B)**. Individual values and mean value with SEM are shown for each group. Student's *t*-test was used for group comparisons (ICU, intensive care unit).

infection. However, sex has to be considered based on the different trends in sACE2.

Due to its role as the primary host cell receptor of SARS-CoV-2, several studies have speculated whether sACE2 levels could explain why some people are prone to develop severe disease. sACE2 may reflect both the level of membrane-bound ACE2 but also ADAM17 activity. As we did not analyze membrane-bound ACE2 and ADAM17, we were not able to decide to what extent they affected sACE2 levels in the present study. On one hand, elevated levels of sACE2 have been suggested to competitively inhibit the binding of SARS-CoV-2 to the membrane-bound ACE2, thereby protecting from disease progression (27). On the other hand, Swärd et al. proposed that high levels of sACE2 indicate increased ACE2 expression and elevated ADAM17 activity, leading to higher susceptibility to SARS-CoV-2 (28). In the present study, we found that sACE2 concentrations in men decreased with the severity of COVID-19. Our results are supported by another study where COVID-19 patients who were admitted to the ICU had lower sACE2 values than patients admitted to the ward or who were discharged (29). These findings are in line with the hypothesis that sACE2 plays a protective role in patients infected with SARS-CoV-2. Apart from competitively inhibiting binding of the virus, sACE2 may also protect from severe disease by

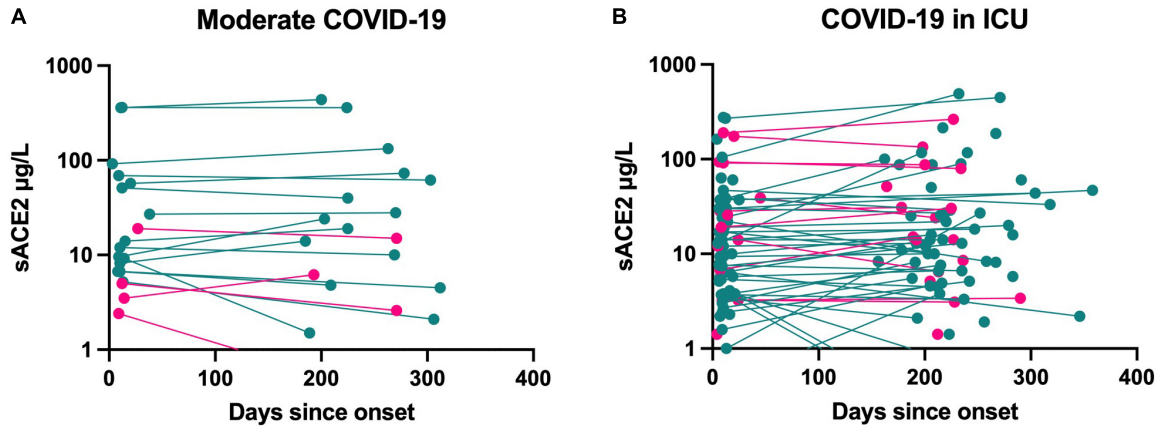

FIGURE 3
Longitudinal measurements of sACE2 (µg/L) in patients with moderate COVID-19 (*n* = 19) **(A)** and with COVID-19 requiring intensive care (*n* = 104) **(B)**. Men = green, women = pink (ICU, intensive care unit).

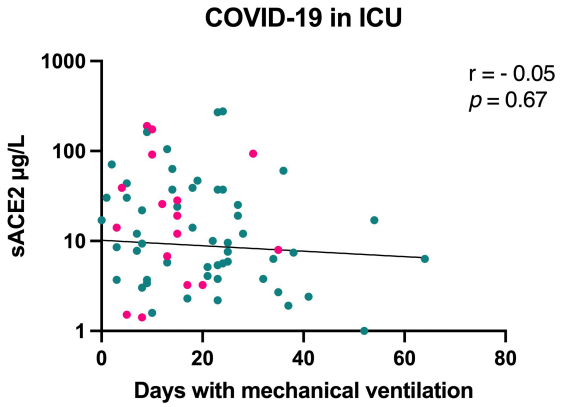

FIGURE 4
Correlation between sACE2 level (µg/L) at hospital admission and days with mechanical ventilation for patients with COVID-19 in the intensive care unit (ICU). Men = green, women = pink.

reducing the activation of the renin-angiotensin system through negative feedback (30). Interestingly, we found an opposite trend in women where sACE2 concentration was increasing with disease severity. To our knowledge, we are the first to report these sex-dependent diverging trends in sACE2 concentration, which may be of particular interest as men are affected more severely by COVID-19 than women.

Our finding that healthy men have higher sACE2 than healthy women confirms previous results where higher sACE2 levels have been found in men from the age of 15 compared to women (28). Among patients with moderate COVID-19, we found the same pattern with higher sACE2 levels in men than in women. This is supported by another study including 114 hospitalized COVID-19 patients of which 22% were treated at an intermediate or intensive care unit (22). The sex difference disappeared when we analyzed sACE2 levels in severely ill patients requiring intensive care, illustrating the opposite trends

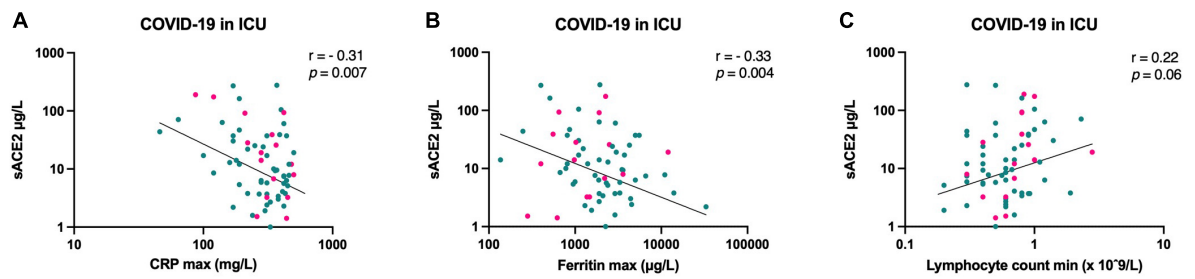

FIGURE 5
Correlation between sACE2 concentrations (µg/L) at hospital admission and maximum C-reactive protein (CRP, mg/L) **(A)**, maximum ferritin (µg/L) **(B)**, and minimum lymphocyte count (x 10^9/L) **(C)**, respectively, for patients with COVID-19 in the intensive care unit (ICU). Men = green, women = pink.

where sACE2 concentration decreased with disease severity in men, but increased with disease severity in women. Since circulating sACE2 is sex-hormone dependent (31), the levels of sex hormones may play a partial role in these sex-related differences. More research is needed to confirm this and explain potential mechanisms. In the longitudinal analysis of sACE2 at hospital admission and at follow-up, we found no change in sACE2 levels. In line with this, Patel et al. (15) showed that the levels persisted at least 114 days post-infection. Thus, potential alterations in sACE2 after COVID-19 may require a longer time period than the present study spans.

Several risk factors for severe COVID-19, such as male sex, older age, hypertension, diabetes mellitus, high BMI, and heart failure are associated with chronically elevated levels of sACE2 (8, 32, 33). However, we found no difference in sACE2 levels in patients with cardiovascular comorbidities or with RAAS-blockade treatment. The latter may support the previous findings that RAAS-blockade is not associated with disease severity (19).

In the current study, the levels of CRP and ferritin inversely correlated with sACE2 concentration, although the correlation was rather weak. An increase in CRP and a decrease in sACE2 with disease severity were previously shown in a study from Spain with 963 patients tested for SARS-CoV-2 (29). Thus, the inflammatory state in severe COVID-19 may be associated with lower sACE2 levels. When stratifying for sex, however, the correlation between sACE2 and ferritin concentration was only seen for men, suggesting a sex-specific divergent trend. On the other hand, sACE2 was negatively correlated to CRP in female ICU patients, similar to men, despite the trend of increasing sACE2 with COVID-19 severity in women. Possibly, the mechanism(s) causing increased sACE2 with disease severity among women is overturned by the severe inflammatory state often seen in ICU patients. Alternatively, CRP may not be perfectly correlated to disease severity in critically ill women.

At present, there are no recognized models for predicting the disease course of COVID-19. Soluble ACE2 is involved in the pathophysiology of COVID-19, but its role as a biomarker of disease severity has been unclear. Our findings indicate that sACE2 has potential to be a valuable marker of disease severity in patients with SARS-CoV-2 infection. However, sex has to be considered based on the different trends in sACE2.

Limitations of this study include the single-center design and the relatively small number of individuals in the groups with healthy controls and patients with moderate COVID-19, especially women. The timing of blood sampling at hospital admission and follow-up differed between patients, which is also a limitation and could have affected the sACE2 levels. Moreover, lack of data regarding smoking habits is a limitation, since there is a hypothesis that ACE2 expression is upregulated in smokers which could increase their sensitivity to infection (34). Strengths of the present study include the comparison of sACE2 levels between three groups including healthy controls, hospitalized patients with moderate COVID-19 and patients with COVID-19 in the ICU. We have also performed a longitudinal follow-up of individual changes in sACE2 levels, which is not often seen in earlier studies.

Conclusion

The decrease in sACE2 concentration, selectively in men, in severe COVID-19 is of pathophysiological interest since men are affected more severely by the disease compared to women. Additionally, the inflammatory biomarkers CRP and ferritin correlated inversely with sACE2 concentration in men, suggesting a role in severe disease. Our findings imply that sACE2 is a possible biomarker of disease severity in a sex-specific manner.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Swedish Ethical Review Authority (Dnr: 2020-01771). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JR, MG, and JS were responsible for the conception and design of the study, as well as for acquisition and analysis of data. BN collected the patients from the ICU. JR and SN performed the statistical analyses. LH and HZ performed the laboratory analyses. All authors took part in drafting the manuscript and approved the final version.

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Conflict of interest

MG has received research grants from Gilead Sciences and Janssen-Cilag and honoraria as speaker, DSMB committee member and/or scientific advisor from Amgen, AstraZeneca, Biogen, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline/ViiV, Janssen-Cilag, MSD, Novocure, Novo Nordic, Pfizer, and Sanofi. HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1058120/full#supplementary-material

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