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RESEARCH ARTICLE

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Autoantibodies against angiotensin-converting enzyme 2 (ACE2) after COVID-19 infection or vaccination

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Abstract

Autoantibodies against angiotensin-converting enzyme 2 (ACE2) are frequently reported in patients during coronavirus disease 2019 (COVID-19) with evidence for a pathogenic role in severe infection. However, little is known of the prevalence or clinical significance of ACE2 autoantibodies in late convalescence or following COVID-19 vaccination. In this study, we measured ACE2 autoantibodies in a cohort of 182 COVID-19 convalescent patients, 186 COVID-19 vaccine recipients, and 43 adolescents with post-mRNA vaccine myopericarditis using two ACE2 enzymatic immunoassays (EIAs). ACE2 IgM autoantibody EIA median optical densities (ODs) were lower in convalescent patients than pre-COVID-19 control samples with only 2/182 (1.1%) convalescents testing positive. Similarly, only 3/182 (1.6%) convalescent patients tested positive for ACE2 IgG, but patients with history of moderate-severe COVID-19 tended to have significantly higher median ODs than controls and mild COVID-19 patients. In contrast, ACE2 IgG antibodies were detected in 10/186 (5.4%) COVID-19 vaccine recipients after two doses of vaccination. Median ACE2 IgG EIA ODs of vaccine recipients were higher than controls irrespective of the vaccine platform used (inactivated or mRNA). ACE2 IgG ODs were not correlated with surrogate neutralizing antibody levels in vaccine recipients. ACE2 IgG levels peaked at day 56 post-first dose and declined within 12 months to baseline levels in vaccine recipients. Presence of ACE2 antibodies was not associated with adverse events following immunization including myopericarditis. One convalescent patient with ACE2 IgG developed Guillain–Barre syndrome, but causality was not established. ACE2 autoantibodies are observed in COVID-19 vaccine recipients and convalescent patients, but are likely innocuous.

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KEYWORDS
ACE2, autoantibody, COVID-19, COVID-19 vaccine, enzymatic immunoassay, SARS-CoV-2

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is one of the largest in recorded history. The pathogenesis of COVID-19 has been extensively investigated with several models being proposed for severe COVID-19 and postacute sequelae of SARS-CoV-2 infection (PASC).[1–3] Autoimmunity is a promising line of inquiry with multiple studies finding autoantibodies targeting immune components (cytokines, chemokines, or their receptors) as well as tissue antigens in COVID-19 patient sera.[4–8] Some types of autoantibodies are clearly mechanistically linked to poor outcomes for example, autoantibodies against type I interferons.[9] However, associations between other autoantibodies and clinical outcomes are less certain. Autoimmunity has also been proposed as a mechanism of rare COVID-19 vaccine induced side effects.[10] Recently, myocarditis following mRNA vaccination has been linked to autoantibodies against endogenous interleukin-1 receptor antagonist.[11]

In this study, we focus on autoantibodies against angiotensin-converting enzyme 2 (ACE2). ACE2 is a vital component of the renin-angiotensin-aldosterone system (RAAS). Its key function is catalytic conversion of the vasoconstrictor angiotensin II into angiotensin (1–7). Angiotensin II is proinflammatory and ACE2 counters this harmful aspect of the RAAS. ACE2 is also the primary receptor engaged by the receptor binding domain (RBD) of the SARS-CoV-2 spike (S) protein enabling cell entry.[12]

Many studies have found elevated levels of ACE2 autoantibodies during severe COVID-19.[13–15] ACE2 autoantibodies have been causally linked to adverse COVID-19 outcomes by causing endothelial dysfunction and complement activation.[15] However, few studies have examined their presence in COVID-19 vaccine recipients and post-recovery COVID-19 patients. It is conceivable that these autoantibodies, which are possibly pathogenic during acute COVID-19, might also be involved in phenomena such as vaccine adverse effects or PASC. Therefore, in this study, we screened for ACE2 autoantibodies and linked clinical sequelae in COVID-19 convalescent and vaccine recipient populations.

2 | MATERIALS AND METHODS

2.1 | Convalescent patient and vaccine recipient samples

Serum samples were obtained from COVID-19 convalescent patients between 1 and 4 months after symptom onset. Patients were classified into mild and moderate-severe COVID-19 based on the WHO clinical progression scale with modifications (Supporting Information S1: Table 1).[16] "Mild" refers to patients not requiring oxygen therapy (scores ≤4 on the WHO clinical progression scale) while moderate-severe COVID-19 patients required oxygen therapy, noninvasive ventilation, or mechanical ventilation (scores 5–9 on the WHO clinical progression scale; Supporting Information S1: Table 1). Individuals who died within 1 month of COVID-19 symptom onset were excluded as they could not provide convalescent sera. None of the patients received COVID-19 vaccines before serum collection.

For the vaccine recipient cohort, COVID-19 vaccine recipients who had completed a primary series (i.e., two doses) provided serum at Day 56 from first dose of vaccine. Vaccine recipients either received CoronaVac (Sinovac Biotech), a whole-virus inactivated vaccine, or Comirnaty (Fosun/BioNTech), an mRNA vaccine. Sera were also obtained from a cohort of adolescents with suspected post-Comirnaty myopericarditis as described previously.[17]

In addition, sera from 60 potential organ donors obtained were used as controls. All donor sera were obtained before 2019 and were stored at −80°C before testing. Clinical and demographic details of patients were retrieved from the electronic patient record. Presence of adverse events following immunization (AEFI) was recorded. This study was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority West Cluster (UW 21-605).

2.2 | ACE2 peptides

Two different ACE2 peptides were used as capture antigens for enzymatic immunoassays (EIAs) in this study. Active recombinant human ACE2 (Gln18-Ser740) expressed in HEK293 cells was sourced commercially (ABclonal; Cat#: RP01277). In addition, we expressed human ACE2 (Ser19-Arg708) in-house using a baculovirus insect cell system as described previously.[18] Both commercial and in-house ACE2 peptides were characterized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis using a monoclonal antibody against ACE2 (R&D Systems; Cat#:-AF933). Detailed protocols for in-house ACE2 expression and western blot analysis are described in the Supporting Information Material.

2.3 | ACE2 EIAs

Microtiter plates were coated overnight at 4°C with 50 µL/well of either 1 µg/mL in-house expressed ACE-2 or commercial recombinant ACE-2 protein diluted in 0.05 M carbonate-bicarbonate coating buffer. After overnight incubation, 150 µL blocking buffer was added to each well and incubated overnight at 4°C. Controls, convalescent patient and vaccine recipient sera were tested with EIAs based on both commercial and in-house ACE2 peptides. Each serum sample

was diluted 1:100 in 1% casein in PBS and added to EIA plates for 1 h at 37°C. The plates were washed with PBS-T (0.3% Tween-20). Each well was incubated with 50 µL of 125 ng/mL HRP conjugated goat anti-human IgG Secondary Antibody (Invitrogen; Cat#: A18811) or 200 ng/mL HRP conjugated goat anti-human IgM Secondary Antibody (Invitrogen; Cat#: A18841) diluted in second Antibody Dilution Buffer for 30 min at 37°C. Plates were washed with PBS-T again and incubated with 50 µL/well of TMB substrate for 10 min. Reactions were stopped by adding 0.3 M sulfuric acid and plates were examined in a microplate reader (Thermo Fisher Scientific) at 450 nm and reference at 620 nm.

Background reactivity was assessed using pre-COVID organ donor sera. Cutoffs were set as mean + 3 standard deviations of these sera. Samples with optical densities (ODs) exceeding cutoffs in both in-house and commercial peptide EIAs were considered positive. Ten control sera with low ODs were selected with a random number generator and used as negative controls for subsequent EIA runs to control for inter-assay variation.

2.4 | SARS-CoV-2 antibody assays

Surrogate SARS-CoV-2 neutralizing antibodies were measured using the iFlash-2019-nCoV NAb kit, a one-step competitive chemiluminescence immunoassay on the iFlash 1800 analyzer (Shenzhen YHLO Biotech) as described previously.[19] SARS-CoV-2 anti-NP IgG antibodies were measured for the CoronaVac cohort using a chemiluminescence immunoassay kit (Shenzhen YHLO Biotech).[20] The cutoff values for the surrogate neutralizing antibody assay and anti-NP antibody assay were 15 and 10 AU/mL respectively.

2.5 | Statistical analysis

All graphs were plotted with GraphPad Prism version 10.0.1. Comparisons of group medians were performed using the Kruskal–Wallis test. If significant, pairwise comparison of group medians was performed using Dunn's multiple comparisons test. Proportions were compared using the Fisher's exact test. Interrater agreement was measured using Cohen's Kappa.

3 | RESULTS

3.1 | Characterization of in-house and commercial ACE2 peptides and application in EIAs

Block diagrams of HEK293 cell-expressed commercial ACE2 and Sf9 cell-expressed in-house ACE2 peptides are depicted in Supporting Information S1: Figure 1A. Both peptides formed smeared bands around 95–130 kDa on SDS-PAGE indicating glycosylation in eukaryotic expression systems (Supporting Information S1: Figure 1B). Both peptides reacted strongly with a specific monoclonal antibody against human ACE2 (Supporting Information S1: Figure 1C).

3.2 | Characteristics of study participants

Baseline demographic and clinical data of convalescent patients and COVID-19 vaccine recipients are presented in Table 1. For the 182 COVID-19 patients, 100 had mild COVID-19 and 82 had moderate-severe COVID-19 as per study definitions. Serum was obtained at a median of 2 months post-symptom onset for COVID-19 patients.

TABLE 1 Clinical and demographic data of study participants.

	Mild COVID-19 (*n* = 100)	Moderate/severe COVID-19 (*n* = 82)	CoronaVac recipients (*n* = 91)	Comirnaty recipients (*n* = 95)
Age (median [IQR])	50 (36.8–60.3)	62 (57–67.8)	56 (46.5–62)	51 (38.5–59.5)
Male (*n* [%])	55 (55.0)	55 (67.1)	36 (39.6)	25 (26.3)
Chronic medical conditions				
Hypertension (*n* [%])	21 (21.0)	35 (42.7)	25 (27.5)	17 (17.9)
Diabetes mellitus (*n* [%])	13 (13.0)	23 (28.0)	17 (18.7)	7 (7.4)
Chronic lung disease (*n* [%])	1 (1.0)	1 (1.2)	2 (2.2)	3 (3.2)
Chronic kidney disease (*n* [%])	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.1)
Immunosuppression (*n* [%])	0 (0.0)	0 (0.0)	3 (3.3)	4 (4.2)
Pregnancy (*n* [%])	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)
Medications				
ACEI (*n* [%])	6 (6.0)	6 (7.3)	9 (9.9)	1 (1.1)
ARB (*n* [%])	1 (1.0)	9 (11.0)	8 (8.8)	8 (8.4)

Abbreviations: ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; COVID-19, coronavirus disease 2019; IQR, interquartile range.

These patients were infected between March 2020 and January 2021, corresponding to three separate small outbreak waves when lineages B.1, B.1.1.63, and B.1.36.27 dominated locally.[21,22] Most moderate-severe COVID-19 patients (69/82; 84.1%) were at WHO score 5 or 6 (requiring oxygen via nasal prongs or high-flow oxygen) whereas 13/82 (15.9%) required intubation and mechanical ventilation at WHO scores of 7–8. 27/82 (32.9%) moderate-severe COVID-19 patients required admission to ICU. No patients required extracorporeal membrane oxygenation.

One hundred and eighty-six vaccine recipients provided sera at Day 56 post-first dose of vaccination. Vaccine recipients received two doses of either CoronaVac ($n = 91$) or Comirnaty ($n = 95$). Individuals received first doses of vaccinations between July 2021 and December 2021. As per manufacturer's recommendations, CoronaVac was administered 28 days apart while Comirnaty was administered 21 days apart. None of the recipients had COVID-19 before vaccine administration.

The post-Comirnaty myopericarditis cohort comprised 43 individuals with median age of 14. Most patients (35/43; 86.8%) were males. Six (14%) developed myopericarditis after the first dose of Comirnaty, 29 (67.4%) developed myopericarditis after the second dose, and eight (18.6%) more developed it after a Comirnaty booster.

3.3 | ACE2 IgM autoantibodies in convalescent patient sera

Median ACE2 IgM ODs of both mild and moderate-severe patient groups were significantly lower than donor controls in both commercial and in-house peptide EIAs (Figure 1A,B). Only one patient each in mild (1/100, 1%) and moderate-severe (1/82, 1.2%) groups tested positive for ACE2 IgM antibodies (defined as OD ≥mean + 3SD of donor sera ODs in both in-house and commercial EIAs) at 2- and 3-months post-symptom onset, respectively. We hypothesized that the low positivity rate was due to the long duration from symptom onset that would lead to decline of T-independent IgM antibodies. Therefore, we decided to focus on ACE2 IgG responses for the rest of the study.

3.4 | ACE2 IgG autoantibodies in convalescent patient and vaccine recipient sera

Convalescent patients with moderate-severe COVID-19 had significantly higher median ODs than donors in both in-house and commercial ACE2 IgG EIAs (Figure 1C,D). Although these patients also had significantly higher median ODs than mild COVID-19 patients in the in-house ACE2 IgG EIA, this was not observed when the commercial peptide was used. Proportions of individuals in each group exceeding assay cutoffs in in-house and commercial IgG EIAs are presented in Table 2. One mild COVID-19 convalescent patient (1/100, 1%) and two moderate-severe COVID-19 convalescent patients (2/82, 2.4%) tested positive for ACE2 IgG antibodies (defined as OD ≥mean + 3SD of donor sera ODs in both in-house and commercial EIAs).

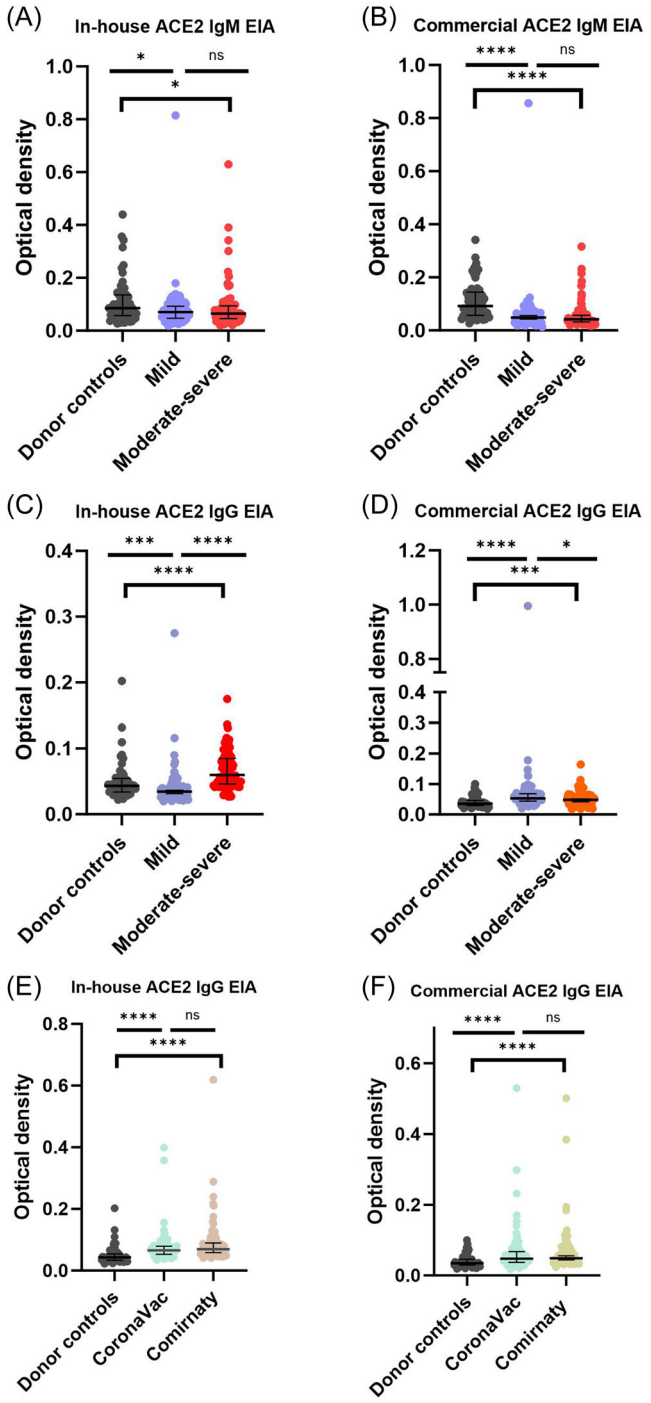

FIGURE 1 In-house and commercial ACE2 enzymatic immunoassay (EIA) results of pre-COVID-19 donor control sera, COVID-19 convalescent patient, and vaccine recipient sera. (A, B) IgM EIA results of COVID-19 convalescent sera classified based on severity. (C, D) IgG EIA results of COVID-19 convalescent sera classified based on severity. (E, F) IgG EIA results of COVID-19 vaccine recipients based on type of vaccine. Bars represent median and interquartile range. Intergroup comparisons of medians were performed using Dunn's multiple comparisons test. Ns: not significant; *$p \le 0.05$; ***$p \le 0.001$; ****$p \le 0.0001$. ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019.

TABLE 2 ACE2 autoantibody IgG enzymatic immunoassays (EIA) results for convalescent COVID-19 patients and vaccine recipients.

Category	In-house ACE2 EIA positive (*n* [%])[a]	Commercial ACE2 EIA positive (*n* [%])[a]	Positive in both EIAs (*n* [%])
Mild COVID-19 (*n* = 100)	1 (1.0)	8 (8.0)	1 (1.0)
Moderate-severe COVID-19 (*n* = 82)	2 (2.4)	4 (4.9)	2 (2.4)
CoronaVac recipients (*n* = 91)	3 (3.3)	13 (14.3)	3 (3.3)
Comirnaty recipients (*n* = 95)	9 (9.5)	12 (12.6)	7 (7.4)

Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019.

[a]Positivity in EIA defined as exceeding mean + 3 SDs of donor sera tested in the same EIA format.

TABLE 3 Characteristics of convalescent patients or vaccine recipients testing positive for ACE2 antibodies in both commercial and in-house peptide EIAs.

Patient	Group	Antibody subtype	Age	Gender	Background medical history	ACEI/ARB	PASC/AEFI
S029	Convalescent mild COVID-19	IgM	44	M	Good past health	No	No
OPD001	Convalescent severe COVID-19	IgM	68	F	Good past health	No	No
S008	Convalescent mild COVID-19	IgG	78	M	Hypertension	Yes	No
S140	Convalescent severe COVID-19	IgG	54	M	Hypertension	Yes	GBS
OPD013	Convalescent severe COVID-19	IgG	94	M	Diabetes, chronic kidney disease	No	No
SNV020	CoronaVac recipient	IgG	52	M	Good past health	No	No
SNV027	CoronaVac recipient	IgG	56	M	Good past health	No	No
SNV058	CoronaVac recipient	IgG	58	F	Hypertension	No	No
BNT007	Comirnaty recipient	IgG	48	F	Good past health	No	No
BNT012	Comirnaty recipient	IgG	29	M	Good past health	No	No
BNT032	Comirnaty recipient	IgG	58	F	Good past health	No	No
BNT081	Comirnaty recipient	IgG	61	F	Hypertension, diabetes, chronic kidney disease	No	No
BNT086	Comirnaty recipient	IgG	39	F	Good past health	No	No
BNT090	Comirnaty recipient	IgG	44	F	Diabetes	No	No
BNT092	Comirnaty recipient	IgG	50	F	Good past health	No	No

Abbreviations: ACE2, angiotensin-converting enzyme 2; ACEI, angiotensin converting enzyme inhibitors; AEFI, adverse events following immunization; ARB, angiotensin receptor blockers; COVID-19, coronavirus disease 2019; EIA, enzymatic immunoassays; GBS, Guillain–Barre syndrome; PASC, post-acute sequelae of SARS-CoV-2 infection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

We then tested ACE2 IgG antibodies in vaccine recipient sera obtained Day 56 from first dose. Median ODs of both CoronaVac and Comirnaty groups were significantly higher ($p < 0.0001$) than the donor sera group in both ACE2 IgG EIAs (Figure 1E,F). There was no significant difference between CoronaVac and Comirnaty groups. CoronaVac and Comirnaty recipients' median ODs were significantly higher than mild convalescent patient sera in in-house IgG EIAs (Supporting Information S1: Figure 2). 3/91 (3.3%) individuals in the CoronaVac group and 7/95 (7.4%) in the Comirnaty group tested positive for ACE2 IgG antibodies in both in-house and commercial EIAs (Table 2). Proportions of COVID-19 convalescents (3/182) and vaccine recipients (10/186) testing positive for ACE2 autoantibodies did not differ significantly despite a higher trend in the latter ($p = 0.087$).

In-house and commercial ACE2 IgG EIA ODs were moderately correlated as shown in Supporting Information S1: Figure 3 (Spearman's r: 0.45, $p < 0.0001$). Cohen's Kappa measuring interobserver agreement of in-house and commercial IgG EIAs was 0.473 (95% CI: 0.299–0.647) indicating moderate agreement between the two assays. The in-house peptide EIA assay was more stringent than the commercial peptide EIA (Supporting Information S1: Table 2).

3.5 | Characteristics of individuals testing positive for ACE2 autoantibodies

Characteristics of the 15 patients testing positive for ACE2 IgG or IgM in both EIAs are presented in Table 3. The median age of seropositive

individuals is 54 with male: female ratio of 7:8. Most individuals (9/15) had unremarkable medical history. Only two were taking RAAS-modulating medications. With the exception of one patient (S140) diagnosed with mild Guillain–Barre syndrome (GBS) after COVID-19, none of the individuals had documented PASC or AEFI. Approximately 2 months after recovery from COVID-19 of moderate severity (WHO score 5), patient S140 developed mild hyporeflexic lower limb weakness. Cerebrospinal fluid showed albuminocytological dissociation and nerve conduction studies were compatible with GBS. Anti-ganglioside antibodies tested negative. The patient recovered spontaneously.

3.6 | Correlation with antibody responses

ACE2 autoantibodies have been proposed to arise due to an anti-idiotypic antibody response. We questioned whether ACE2 IgGs ODs were proportional to the strength of the neutralizing antibody response. We measured surrogate neutralizing antibody levels at day 56 post-first dose for patients receiving the CoronaVac and Comirnaty vaccines using a chemiluminescence immunoassay. We also measured anti-NP antibodies for CoronaVac vaccine recipients as this inactivated whole virus vaccine includes this antigen. As previously reported, surrogate neutralizing antibody levels were higher in the Comirnaty cohort than the CoronaVac cohort. However, ACE2 IgG EIA ODs were not correlated with surrogate neutralizing antibody levels in either vaccine cohort (Figure 2). ACE2 IgG EIA ODs were also not correlated with anti-NP antibodies in the CoronaVac recipient cohort (Supporting Information S1: Figure 4).

3.7 | Kinetics of ACE2 IgG antibodies in vaccine recipients

For nine of 10 vaccine recipients with detectable ACE2 IgG antibodies in both EIAs, we retrieved sera obtained before first dose (Time 0), before second dose (Day 21 post-first dose for Comirnaty recipients and Day 28 post-first dose for CoronaVac recipients), 6 months post-first dose, and 1 year post-first dose. We tested all

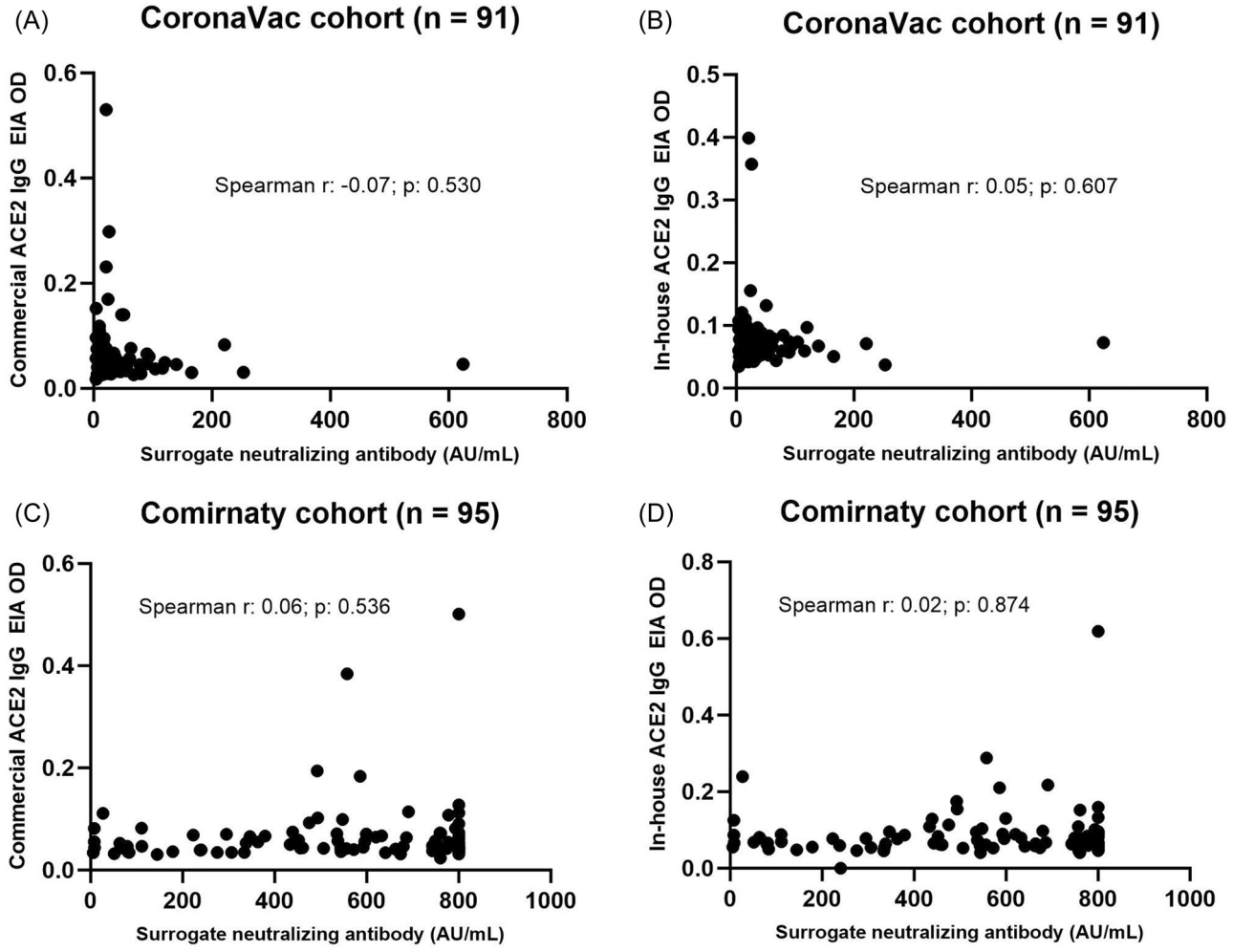

FIGURE 2 Correlations between ACE2 IgG enzymatic immunoassay optical densities (OD) and surrogate neutralizing antibody levels of CoronaVac (A, B) and Comirnaty (C, D) cohorts using commercial and in-house ACE2 peptides. Strength of correlation was assessed using Spearman's rank correlation. ACE2, angiotensin-converting enzyme 2.

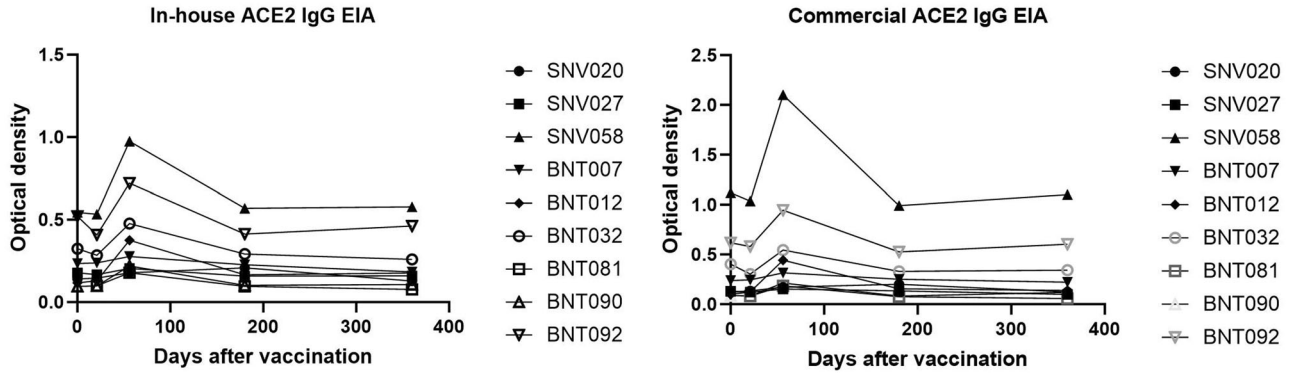

FIGURE 3 Trends of ACE2 IgG optical densities (ODs) using in-house (A) and commercial (B) peptides for vaccine recipients testing positive at Day 56 post-first dose. Each line represents trend for individual recipients. SNV020, SNV027, and SNV058 are CoronaVac recipients. BNT007, BNT012, BNT032, BNT081, BNT090, and BNT092 are Comirnaty recipients. The second timepoint is either Day 21 (for Comirnaty recipients) or Day 28 (for CoronaVac recipients). ACE2, angiotensin-converting enzyme 2.

sera together in in-house and commercial ACE2 IgG EIAs. All patients had highest ODs at day 56 post-first dose (approximately 1 month after the second dose) and levels tended to fall back to baseline levels by 6 months to 1 year post-first dose (Figure 3). Interestingly, two vaccine recipients (SNV058 and BNT092) had elevated ODs at baseline even before receiving COVID-19 vaccines, but levels rose above baseline at Day 56.

3.8 | ACE2 IgG in Comirnaty-induced myopericarditis patients

We then questioned whether ACE2 IgG might be involved in cardiovascular complications of Comirnaty. We tested sera from a cohort of adolescents presenting with myopericarditis after one to three doses of Comirnaty. Each serum was tested in both commercial and in-house ACE2 IgG EIAs (Supporting Information S1: Figure 5). Samples from two patients produced ODs marginally exceeding previously established cutoffs of both IgG EIAs. However, we noted that negative controls in this particular assay run were higher than those of previous runs. If the cutoff was changed to mean + 3 SDs of the 10 control sera used in this assay run, then all myopericarditis patient samples would be classified as negative. Therefore, we concluded that all patients tested negative for ACE2 IgG antibodies.

4 | DISCUSSION

Autoantibodies against ACE2 have previously been implicated in the pathogenesis of constrictive vasculopathies, systemic sclerosis, and connective tissue disease.[23,24] With the advent of the COVID-19 pandemic, there has been a renewed interest in the role of these autoantibodies in severe infectious disease outcomes. Although the first study on this topic found that more than 90% of inpatients carried anti-ACE2 autoantibodies, subsequent investigations have generated more conservative estimates.[13,14,25] However, most studies agree that ACE2 autoantibodies (of either IgG or IgM subtypes) are produced by at least some patients during the course of moderate-severe COVID-19.[14,15,26]

Fewer studies have examined these autoantibodies in later convalescence or in vaccine recipients. A recent study by Lebedin et al. found that ACE2 IgG autoantibodies peaked within the first month of symptom onset and persisted for up to 2 months in some individuals.[26] In this study, we found that a small proportion of convalescent individuals harbored autoantibodies beyond this time irrespective of disease severity. But, for the first time, we additionally show that COVID-19 vaccine recipients may also mount ACE2 IgG autoantibodies irrespective of vaccine platform.

The mechanism whereby these autoantibodies arise is uncertain. Hypotheses include anti-idiotypic responses, humoral responses to circulating spike-ACE2 complexes, cross-reactive antibodies, and nonspecific polyreactivity.[26,27] We initially favored an anti-idiotype response considering that the strongly immunogenic mRNA vaccine tended to elicit these autoantibodies more frequently, but we were unable to find a correlation between ACE2 autoantibodies and day-56 surrogate SARS-CoV-2 neutralizing antibody levels, which might be expected in an anti-idiotypic response. However, we only measured neutralizing antibody responses at a single timepoint, which may not reflect the overall strength of RBD-specific humoral antibody responses. ACE2 IgG ODs in vaccine recipients were highest immediately after the second dose of vaccine, which might represent correlation with frequency of SARS-CoV-2 spike protein exposure. It is unlikely that mRNA vaccines elicit the same kind of nonspecific autoantibody reactivity observed in natural COVID-19 infections; indeed, there is good evidence that mRNA vaccines effectively decouple antiviral immunity from humoral auto-immunity.[28] Soluble ACE2 plays an important role in SARS-CoV-2 cell entry, but previous studies have shown that circulating soluble ACE2 levels are unrelated with ACE2 IgG levels.[13,26,29] Furthermore, circulating spike-ACE2 complexes, which might be considered

essential for a breakdown in peripheral tolerance, are yet to be detected in human tissues. Therefore, it is currently unclear why COVID-19 vaccine recipients mount ACE2 autoantibodies.

The functional significance of ACE2 autoantibodies is also controversial. One early study suggested possible inhibition of ACE2 activity, but a recent study found that ACE2 IgG levels in COVID-19 patients are too low to impact regulatory activity.[13,26] However, another study implicates T-independent ACE2 IgM antibodies in complement activation and functional changes in endothelial cells in microvessels.[15] In our study, these autoantibodies do not appear to have clinical significance in terms of predisposing to AEFI or PASC. One patient with detectable ACE2 IgG following COVID-19 developed GBS. GBS is a rare complication of COVID-19 that is similar in spectrum to GBS after other infections.[30] As ACE2 autoantibodies have not been implicated in other forms of post-infectious GBS, we believe that presence of ACE2 IgG in this patient was coincidental. We have also excluded a role for these autoantibodies in mRNA vaccine induced myopericarditis.

A major strength of our study was inclusion of convalescent and vaccine recipient cohorts that were not widely represented in previous studies. We were also very stringent in defining positive cases by performing two separate EIAs. This is important because validated positive controls and confirmatory functional assays for ACE2 autoantibody detection are not available to evaluate the sensitivity and specificity of our EIA assays. It is possible that these criteria may have led to the exclusion of some genuinely positive cases due to discrepancies in length between the peptides leading to lack of certain epitopes in in-house ACE2, but we accepted this tradeoff for higher specificity. We did not perform soluble ACE2 detection or functional ACE2 assays because these assays are difficult to interpret and of doubtful significance given the lack of sequelae in individuals with detectable ACE2 autoantibodies. Soluble ACE2 is likely derived from tissue-bound ACE2 and is unlikely to be related to development of these autoantibodies.

In conclusion, ACE2 autoantibodies reported by other groups during acute COVID-19 may persist into convalescence. Furthermore, COVID-19 vaccine recipients may also mount these autoantibodies transiently, peaking after the second dose and declining subsequently for those completing a primary series. ACE2 autoantibodies following COVID-19 or vaccines were not linked to postinfection or postvaccination sequelae.

AUTHOR CONTRIBUTIONS

Siddharth Sridhar: Conceptualization, funding acquisition, data curation, supervision, writing—original draft. **James Yiu Hung Tsoi**: Data curation, formal analysis, methodology, investigation, validation, writing—original draft. **Jianpiao Cai**: Methodology, validation, writing—review and editing. **Jianwen Situ**: Methodology. **Winston Jim Lam**: Data curation. **Estie Hon Kiu Shun**: Methodology. **Joy Ka Yi Leung**: Investigation. **Lin Lei Chen**: Methodology. **Brian Pui Chun Chan**: Methodology. **Man Lung Yeung**: Methodology, writing—review and editing. **Xin Li**: Data curation. **Kwok Hung Chan**: Data curation, methodology, writing—review and editing. **Joshua Sung Chih Wong**: Data curation. **Mike Yat Wah Kwan**: Data curation, writing—review and editing. **Kelvin Kai Wang To**: Data curation, writing—review and editing. **Kwok Yung Yuen**: Supervision, writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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