

Hypersensitivity may be involved in severe COVID-19

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Abstract

Background: Deaths attributed to Coronavirus Disease 2019 (COVID-19) are mainly due to severe hypoxemic respiratory failure. Although the inflammatory storm has been considered the main pathogenesis of severe COVID-19, hypersensitivity may be another important mechanism involved in severe cases, which have a perfect response to corticosteroids (CS).

Method: We detected the serum level of anti-SARS-CoV-2-spike S1 protein-specific IgE (SP-IgE) and anti-SARS-CoV-2 nucleocapsid protein-specific IgE (NP-IgE) in COVID-19. Correlation of levels of specific IgE and clinical severity were analysed. Pulmonary function test and bronchial provocation test were conducted in early convalescence of COVID-19. We also obtained histological samples via endoscopy to detect the evidence of mast cell activation.

Result: The levels of serum SP-IgE and NP-IgE were significantly higher in severe cases, and were correlated with the total lung severity scores (TLSS) and the PaO₂/FiO₂ ratio. Nucleocapsid protein could be detected in both airway and intestinal tissues, which was stained positive together with activated mast cells, binded with IgE. Airway hyperresponsiveness (AHR) exists in the early convalescence of COVID-19. After the application of CS in severe COVID-19, SP-IgE and NP-IgE decreased, but maintained at a high level.

Conclusion: Hypersensitivity may be involved in severe COVID-19.

KEYWORDS

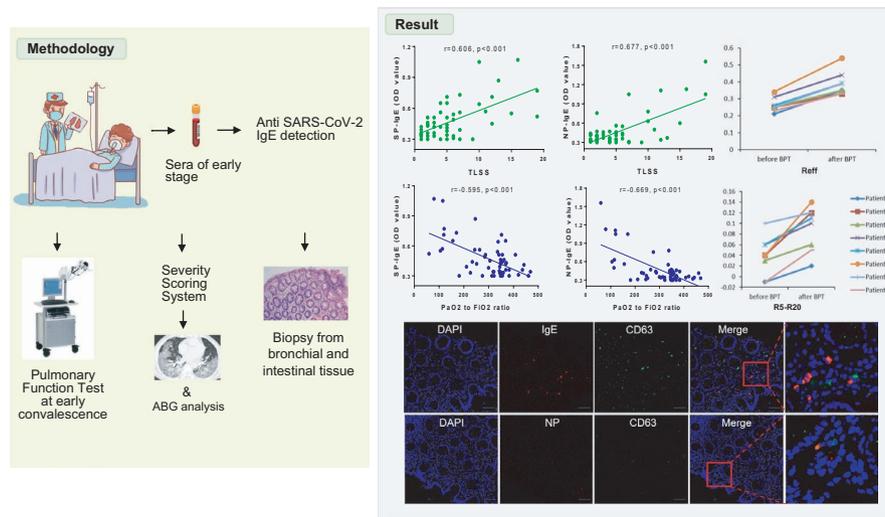
Anti-SARS-CoV-2 specific IgE antibodies, IgE-mediated hypersensitivity reactions, pulse methylprednisolone therapy, severe COVID-19

1 | INTRODUCTION

At present, Coronavirus Disease 2019 (COVID-19) continues to spread worldwide, with more than 3,144,028 deaths reported as of 29 April 2021 and no indications that disease spread will be controlled in the near future.¹ The most common cause of death from COVID-19 has been reported to be severe hypoxemic respiratory failure.² Therefore, it is extremely important to clarify the precise

pathophysiology for the development of severe COVID-19, as well as to develop appropriate treatment strategies. Increasing studies indicate that "cytokine storm" contribute to the mortality of COVID-19.³ Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may cause a hyperactivated innate immune response, elevating serum levels of inflammatory chemokines (interleukin-6, interleukin-1 β , tumour necrosis factor- α , etc.), thus resulting in the exhaustion of lymphocytes.⁴ This might ultimately cause organ failure and fatal

Hypersensitivity may be involved in severe COVID-19.



GRAPHICAL ABSTRACT

Level of IgE to SARS-CoV-2-spike protein and nucleocapsid protein positively correlated with clinical severity in COVID-19 patients. Airway hyperresponsiveness (AHR) exists in early convalescence of COVID-19. Mast cells with concurrently positive staining for IgE and CD63 were observed in lamina propria of the bronchus and duodenum in severe COVID-19. We deduce that hypersensitivity reaction may be involved in severe COVID-19. Note: SP-IgE: anti-spike protein (SP) IgE; NP-IgE: anti-nucleocapsid protein (NP) IgE; TLSS: Total lung severity scores; BPT: bronchial provocation test; Reff: effective airway resistance; R5-R20: difference between the resistance at 5 Hz and 20 Hz.

respiratory distress. On the contrary, coagulopathy in multiple vascular territories may account for severity in COVID-19, either related to a systemic immunopathology.⁵ As some data reported, referred to the clinical manifestation and lung CT performance, it is probably that the hypoxic respiratory failure caused by COVID-19 may be related to hypersensitivity within the lungs.⁶ Mast cells (MCs) are activated by SARS-CoV-2.⁷ Although only recently recognized, MC activation syndrome (MCAS), usually due to acquired MC clonality, is a chronic multisystem disorder with inflammatory and allergic themes, and an estimated prevalence of 17%.⁸ Mast cells may react to viruses in collaboration with other cells and lung autopsy findings from patients that died from the coronavirus disease that emerged in 2019 (COVID-19) showed accumulation of mast cells in the lungs that was thought to be the cause of pulmonary oedema, inflammation and thrombosis.⁹ CD63 is an important surface marker of activated human blood-derived MCs.^{10,11} We herein detected the anti-SARS-CoV-2 specific immunoglobulin E (IgE) and CD63+ cells in COVID-19 patients to figure out the correlations of IgE, MCs and COVID-19.

The humoral immune system plays an important role against viral infection. Investigations in humoral immune responses provide supportive evidence to vaccine development. It is clear that most people infected with SARS-CoV-2 display an antibody response after infection. Seroconversion occurs approximately 10 days after the symptom onset. Specific IgM appears between 10 and 14 days after infection.¹² The IgG antibody titres rise from day 10 onwards to reach a peak whose height is likely to be influenced, on a case-by-case basis, by disease severity and virus load.¹³ IgA serum concentrations peaked 3 weeks after symptom onset but persisted for several more weeks in saliva, and serum IgA was more potent than IgG in

Key messages

- Level of IgE to SARS-CoV-2-spike protein and anti-SARS-CoV-2 nucleocapsid protein positively correlated with clinical severity in COVID-19 patients.
- Mast cells with concurrently positive staining for IgE and CD63 were observed in lamina propria of the bronchus and duodenum in severe COVID-19.
- Based on the pulmonary function test, airway hyperresponsiveness (AHR) exists in the early convalescence of COVID-19.

neutralizing SARS-CoV-2.¹⁴ However, the dynamic feature of IgE after SARS-CoV-2 infection is still unknown. Our team successfully managed 102 COVID-19 cases. Among these patients, 27 developed hypoxemic respiratory failure, but no deaths occurred.¹⁵ We retrospectively reviewed our cases and conducted a study to investigate the pathophysiology of COVID-19. We found that various COVID-19-related clinical features, from as severe as near-fatal hypoxemic respiratory failure to as mild as gastrointestinal discomfort, might be due to hypersensitivity reactions. As the treatment for COVID-19-related hypoxemic respiratory failure, we compared specific IgE, total lung severity scores (TLSS), and the arterial oxygen tension to inspiratory oxygen concentration ratio ($\text{PaO}_2/\text{FiO}_2$) before and after administration of pulse methylprednisolone, and found the fast improved clinical manifestation and above index, which might suppress not only inflammatory storm but also the hypersensitivity reactions. There are few studies about pulmonary function in patients with

COVID-19, which focus on the recovery period, and airway ventilation dysfunction is not common in these studies.^{16,17} However, because the bronchial provocation test (BPT) is not carried out routinely, prolonged clinical symptoms of chest tightness, cough and wheezing in COVID-19 patients were attributed to diffusion dysfunction mostly. In this study, we conducted BPT in COVID-19 patients with prolonged clinical symptoms to find out whether airway hyperresponsiveness (AHR) exists.

2 | METHODS

2.1 | Study population

This was a retrospective case-control study. COVID-19 patients hospitalized in the Fifth Hospital of Sun Yat-sen University from 1 January 2020–25 April 2020 were enrolled. The diagnosis of COVID-19 infection was made based on the criteria of the “Diagnosis and Treatment of New Coronavirus-Infected Pneumonia” (Seventh trial version) drafted by the National Health Commission of China. We used 50 pre-COVID-19 sera from volunteers collected between October 2015 and November 2018 as negative controls. The samples are mainly from routine yearly health screening of staff members, who underwent chest X-ray to rule out pulmonary diseases. According to their medical record, patients with severe systemic disease, congestive heart failure, active malignant tumour, hepatic or renal disease, were also excluded. The other exclusion criteria were: age <18 or >80 years, underlying autoimmune diseases, underlying primarily or acquired immunodeficiencies, and refusal to participate. Our study was approved by the Research Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University (Ethics approval number K30-1). Written informed consent was obtained from all the participants.

2.2 | Chest CT

Chest CT was performed in all subjects using 1-mm slice thickness CT on a UCT 760 scanner (United Imaging; Shanghai, China). CT images were read by two experienced specialists with over five years of experience, and were evaluated for the presence or absence of the following findings: ground-glass opacity, centrilobular nodules and high-density linear or strip shadows. The distribution of lung lobes involved was described as subpleural, central, random or diffuse.¹⁸

2.3 | COVID-19 severity

Total lung severity scores (TLSS) are assessed as previously reported.¹⁸ First, each of the five lung lobes was assessed for degree of involvement and classified as none (0%), minimal (1%–25%), mild (26%–50%), moderate (51%–75%) or severe (76%–100%). Then, no involvement corresponded to a lobe score of 0, minimal to a lobe

score of 1, mild to a lobe score of 2, moderate to a lobe score of 3, and severe to a lobe score of 4. Subsequently, the total lung severity score was calculated by summing the five lobe scores (range of possible scores, 0–20). In addition, the severity of COVID-19 was assessed by PaO₂/FiO₂ and respiratory dysfunction was defined as PaO₂/FiO₂ less than 300 mm Hg.

2.4 | Collection of biological specimens from COVID-19 patients and control participants

All biological specimens used in this study were acquired from the specimen bank of our hospital with the approval of the participants. The sera and tissues from COVID-19 patients were obtained during hospitalization and stored at –80°C and in liquid nitrogen, respectively. Sera of the control group were obtained for routine health screening several months prior to the COVID-19 outbreak.

2.5 | Detection of peripheral blood-specific anti-SARS-CoV-2 IgE antibody levels in patients with COVID-19

We developed an enzyme-linked immunosorbent assay (ELISA) system to measure the levels of anti-SARS-CoV-2 IgE antibodies in the serum of all participants. The ELISA is a common method in the detection of specific IgE in clinical practice.¹⁹ However, there lacks a specific IgE kit of SARS-CoV-2. We developed an indirect ELISA system to detect sera anti-SARS-CoV-2 spike protein antibody and anti-SARS-CoV-2 N protein antibody. Checkerboard titration was used to determine the optimal antigen coating concentration, serum dilution and peroxidase-conjugate secondary antibody working dilution. Good repeatability was showed in the experiment. We tried to determine a cut-off value by the detection of sera from negative control individuals (detailed information in Figure S3). However, because of the small sample of the control group, it may cause some bias of the result. Therefore, instead of using a “positive” or “negative” in the paper when comparing the specific IgE between groups, we would like to compare their exact value.

The procedure of ELISA was as follows:

1. Coating: In this, 96-well microtiter ELISA plates were coated with 2-μg/ml recombinant SARS-CoV-2 spike S1 protein and SARS-CoV-2 nucleocapsid protein antigens (Sino Biological, Beijing, China), which was accepted as SARS-CoV-2-specific proteins, generally, overnight at 4°C.
2. Blocking: Wells were blocked with 20% non-fat dried milk overnight at 4°C and incubated at 37°C for 2 h.
3. Sample application: Plates were washed thrice with phosphate-buffered saline (PBS) containing 0.04% Tween-20 (PBST, Solarbio). For the detection of IgE antibodies, 50-μl undiluted serum was added and incubated at 37°C for 2 h.

4. Antibody detection: Antigen-specific antibodies were detected by adding 100- μ l horseradish peroxidase (HRP)-labelled goat anti-human IgE (1/5000 dilution, Abcam ab3901), followed by incubation at room temperature for 1 h, and after some time the plates were washed thrice.
5. Coloration: The plates were washed thrice with PBST, and the signal was developed by adding 100- μ l TMB (Solarbio) for 15 minutes at room temperature.
6. Stopping: The reaction was stopped with 2-M sulphuric acid; a 50- μ l stop solution was added to each well.
7. Signal detection: Plates were read on a Spectramax Plate Reader at 450 nm using SoftMax Pro, with subtraction of the optical density (OD) values of the background.

2.6 | Detection of SARS-CoV-2 in respiratory specimens

The presence of SARS-CoV-2 in nasal and pharyngeal swab specimens was detected by real-time PCR assays approved by the China Food and Drug Administration.

2.7 | Histologic and immunofluorescent staining: IgE-mediated activated mast cells testing

Bronchial mucous and duodenal mucous tissues were obtained via endoscopy. Samples were embedded in paraffin and processed by hematoxylin and eosin (H&E) and immunofluorescent staining. For immunofluorescent staining, 3- μ m-thick sections were de-waxed in xylene and rehydrated in alcohol, followed by antigen retrieval by citric acid buffer microwave repairing for 20 min. After rinsing with PBST thrice, the sections were blocked with goat serum (ZSGB-BIO, ZLI-9022) for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies anti-CD63 (Abcam ab252919, 1:1000); anti-IgE, (Abcam ab7382, 1:250); anti-NP (Sino Biological, 40143-MM08, 1:500). After rinsing with PBST, slides were incubated with secondary antibodies (Alexa Fluor 647-conjugated goat anti-mouse IgG; Bioss, bs-0295G-AF647, 1:100; Dylight 549-conjugated goat anti-rabbit IgG; Abbkine, A23320, 1:100) at room temperature for 1 h. Nuclei were then counterstained with 4',6-diamidino-2-phenylindole (DAPI) in the VECTASHIELD Hardset Antifade Mounting Medium after washing thrice with PBST. Slides were imaged using a laser scanning confocal microscope (LSM880; Carl Zeiss MicroImaging). At last, the immunofluorescent staining results in specimens were observed by a confocal microscope.

2.8 | Pulmonary function test in early convalescence in COVID-19 patients

Partial subjects underwent a standard pulmonary function test and bronchial provocation test 3 months after discharge (Master

Screen; Jaeger). Body plethysmography, spirometry and impulse oscillation system (IOS) assessment included specific effective airway resistance (sReff), effective airway resistance (Reff), forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), maximal mid-expiratory flow (MMEF), resonant frequency (Fres), airway viscosity resistance at an oscillation frequency of 5Hz(R5), and central airway resistance at an oscillation frequency of 20Hz (R20) and the difference between the resistance at 5 Hz and 20Hz (R5-R20). In the bronchial provocation test (BPT), we used methacholine as a stimulator and observed the changes in airway resistance after medication. The measurements were expressed as a percentage of predicted normal values. To protect the lung function of the laboratory staff, lung function tests were performed in a room with a negative pressure device. Staff wore personal protective equipment, including N95 respirators, protective glasses, gloves and gowns. In addition, each patient used disposable virus and bacterial filters during the test.

2.9 | Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) Version 13.0 (SPSS, Inc). The data of normal distribution were shown as the mean \pm standard deviation (SD). Continuous variables were compared using an independent sample t-test, whereas the rank-sum test was used for nonparametric data. The categorical variables were expressed as count (percentage) and compared using the chi-square test. The associations between the specific IgE and TLSS, or PaO₂/FiO₂ ratio were analysed by Pearson correlation coefficient. All statistical tests were two-tailed. Statistical significance was taken as $p < .05$.

3 | RESULTS

3.1 | Characteristics of study participants

This was a retrospective case-control study. One hundred and two patients with confirmed COVID-19 were included in this study, 27 of whom (26.5%) were severe cases according to the criteria of PaO₂/FiO₂ less than 300 mm Hg. The age of the patients ranged from 15 to 80 years old (median 47.4 years old). Among the 102 patients, 1 patient (male) had a history of asthma,²⁰ 1 had allergic rhinitis, 6 patients had a cured malignancy, 6 had a history of diabetes and 16 had a history of hypertension. The major clinical symptoms were fever, shortness of breath, cough, diarrhoea, loss of appetite, haemoptysis, fatigue, chest tightness and sputum production. The two groups (severe vs. non-severe group) had no significant differences in coexisting disorders (all $p > .05$). Comparison of patients with severe vs. non-severe disease indicated that haemoptysis ($p < .001$), shortness of breath ($p < .001$) and fever ($p = .006$) were more common in the severe

disease group. The two groups had no significant differences in coexisting disorders (all $p > .05$). Among the COVID-19 patients, a peak value of mean TLSS in severe ones was higher than that in non-severe ones ($p < .001$), accompanied by a higher mean $\text{PaO}_2/\text{FiO}_2$ ratio ($p < .001$). At acute stage, the level of SP-IgE and NP-IgE were both higher than that in the control group (both $p < .001$), particularly in severe COVID-19 patients ($p = .002$; $p = .005$; Table 1).

3.2 | Significant associations between SARS-CoV-2-specific IgE levels and severity of COVID-19

Further analysis showed that the significant associations between specific SP-IgE/NP-IgE levels and TLSS or $\text{PaO}_2/\text{FiO}_2$ ratio were observed (Figure 1). The level of specific IgEs were all found to have positive correlations with the TLSS (SP-IgE, $r = 0.606$, $p < .001$; NP-IgE, $r = 0.677$, $p < .001$; Figure 1) and have negative correlations with the $\text{PaO}_2/\text{FiO}_2$ ratio (SP-IgE, $r = -0.595$, $p < .001$; NP-IgE, $r = -0.669$, $p < .001$). However, a negative correlation between the TLSS and $\text{PaO}_2/\text{FiO}_2$ ratio ($r = -0.775$, $p < .001$) was obtained.

3.3 | Histological evidence for SARS-CoV-2-specific IgE-mediated activated mast cells in COVID-19

Bronchoscopy was performed on a patient with severe hypoxemic respiratory failure receiving mechanical ventilation, and bronchial mucosa tissues were obtained. Intestinal endoscopy was performed on selected patients with gastrointestinal symptoms, and intestinal mucosa tissues were obtained. As shown in Figure 2, activated mast cells (CD63+) with concurrently positive staining for IgE and NP were observed in lamina propria of the bronchus, and intestinal tissues, which indicated that IgE-mediated mast cell activation is triggered by SARS-CoV-2 occurred in COVID-19. In the histological aspect, hypersensitivity reactions in COVID-19 can be partially proved.

3.4 | Effect of corticosteroids treatment to SARS-CoV-2-specific-IgE and prognosis in COVID-19

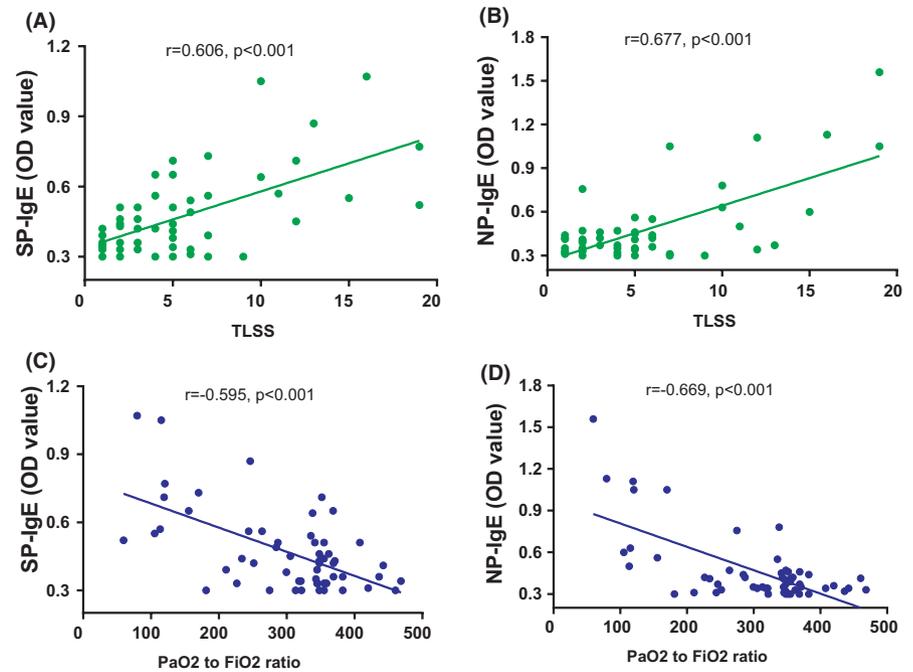
Notably, each of the 16 critically ill patients experienced clinically significant improvements in the lung oxygenation index after each administration of systemic corticosteroids and these 16 patients

TABLE 1 Demographic and clinical characteristics of COVID-19 and healthy controls

Clinical characteristics	All patients	Non-severe group	Severe group	Control group	p -value*	p -value**
	(n = 102)	(n = 75)	(n = 27)	(n = 50)		
Age, median (range), years	47.6 (15–80)	41.0 (15–75)	61.0 (32–80)	49.0 (25–76)	.056	<.001*
Sex						
Male, N (%)	45/102 (44.1)	29/75 (38.7)	16/27 (59.3)	26/50 (52.0)	.121	.083
Coexisting disorders, N (%)						
Hypertension	16/102 (15.7)	10/75 (13.3)	6/27 (22.2)	8/50 (16.0)	.571	.454
Diabetes mellitus	6/102 (5.9)	2/75 (2.7)	4/27 (14.8)	5/50 (10.0)	.069	.072
Cured malignancy	6/102 (5.9)	4/75 (5.3)	2/27 (7.4)	2/50 (4.0)	.82	1.000
Allergic diseases	2/102 (2.0)	1/75 (1.3)	1/27 (3.7)	2/50 (4)	.598	0.461
Chronic pulmonary disease	6/102 (5.9)	3/75 (4)	3/27 (11.1)	3/50 (6)	.730	.188
Respiratory symptoms, N (%)						
Fever	59/102 (57.8)	37/75 (49.3)	22/27 (81.5)			.006
Hemoptysis	7/102 (6.9)	0/75 (0)	7/27 (25.9)			<.001
Shortness of breath	34/102 (33.3)	7/75 (9.3)	27/27 (100.0)			<.001
Asymptomatic on admission	12/102 (11.8)	12/75 (16.0)	0/27 (0)			.062
Gestational intestinal symptoms, N(%)	62/102 (60.8)	48/75 (64)	14/27 (51.8)			.358
Total lung severity scores (TLSS)	5.38 ± 4.14	3.42 ± 2.22	9.37 ± 4.30			<.001
Worst PaO_2 to FiO_2 ratio (mmHg)	295.76 ± 96.92	332.76 ± 61.30	220.38 ± 112.56			<.001
Specific IgE level on admission (ODvalue)						
anti S protein	0.47 ± 0.18 (n = 58)	0.42 ± 0.12 (n = 45)	0.58 ± 0.24 (n = 13)	0.31 ± 0.01	<.001	.002
anti N protein	0.47 ± 0.25 (n = 58)	0.39 ± 0.09 (n = 45)	0.66 ± 0.38 (n = 13)	0.30 ± 0.01	<.001	.005

Note: p values* indicate differences between the COVID-19 patients and control group p values** indicate differences between the non-severe and severe groups.

FIGURE 1 The correlations of anti-SARS-CoV-2-specific IgE with TLSS and the PaO₂/FiO₂ ratio in COVID-19 patients. SP- IgE: anti-SARS-CoV-2-spike S1 protein-specific IgE; NP- IgE: SARS-CoV-2 nucleocapsid protein specific IgE; TLSS: the total lung severity scores



all recovered without any complications.¹⁵ Our experience is that a patient with an oxygenation index of 150 mm Hg or less should receive methylprednisolone (MP) at a dose of 4–8 mg/kg/day to block the hypersensitivity reaction in the lungs. Among these 16 patients treated with corticosteroids, we had tested SP- IgE and NP-IgE in succession. Our results indicated that the application of corticosteroids significantly decreased specific-IgE (Figure 3), at the same time increased the PaO₂/FiO₂ ratio (Figure S1). We found that the level of anti-SARS-CoV-2-specific IgE antibodies decreased significantly after corticosteroids, but were still higher than healthy controls in the recovery period (Figure S2). In addition, we did not find the effect of corticosteroids application on SARS-CoV-2 clearance.

3.5 | Airway hyperresponsiveness (AHR) exists in the early convalescence of COVID-19

During the follow up of COVID-19 patients, we found chest tightness, cough and wheezing existed in 17 cases in early convalescence of COVID-19. Depending on the bronchial provocation test (BPT), we considered that AHR existed in 8 patients with enhanced airway resistance (Figure 4), following the Criteria (1) FEV₁ and FVC were based on the 2005 ATS/ERS criteria, Δ FEV₁ \geq 12% and 200 ml, Δ MMEF \geq 35%, or Δ PEF \geq 12%; (2) Δ Reff \geq 35% (Reff, airway resistance); and (3) Δ R5 \geq 30% or Δ Fres \geq 30%.^{21–24} AHR, enhanced SP-IgE and NP-IgE in patients indicate that hypersensitivity might occur in the pathogenesis of COVID-19 and go on until convalescence.

4 | DISCUSSION

The ongoing COVID-19 outbreak has caused global disruption, with great concern for the mortality rate. There is an urgent need to

determine the causal mechanisms of COVID-19-induced hypoxemic respiratory failure and to develop an effective treatment strategy. To date, quite a few studies presenting autopsy findings from COVID-19 patients have been published, but there is still little evidence revealing the exact mechanism by which SARS-CoV-2 infection directly causes death.^{25–27} Though vasculitis and microvascular thrombosis have been linked to COVID-19 as a suspected pathological pattern by some authors, the evidence is still far from being conclusive.^{28–30} Recent studies showed that SARS-CoV-2 may result in cytokine storm syndrome (CSS).³ CSS is a cascade of escalated immune responses that can cause the immune system to become exhausted, which might ultimately result in organ failure and fatal respiratory distress.³¹ CSS can lead to apoptosis of epithelial and endothelial cells in the lungs, vascular leakage and acute respiratory distress syndrome (ARDS). Evidence had shown that the responses of MCs to SARS-CoV-2 may promote cytokine storms.⁹ Autopsy findings from the lungs of the patients that died from COVID-19 showed an accumulation of MCs that was speculated to be the cause of pulmonary oedema, inflammation and thrombosis in the pathophysiology of COVID-19.^{32,33} The classic trigger for MCs activation is through the IgE-mediated cross-linking the IgE receptor, FcεRI, thus resulting in the degranulation of MCs. Immediate hypersensitivity reactions, historically called type I hypersensitivity reactions, are mediated by IgE antibodies produced by the immune system in response to environmental antigens. IgE antibodies secreted by plasma cells bind to the mast cells. When individuals encounter an antigen, it binds to the IgE antibody and triggers the release of histamine granules by mast cells, resulting in the dilation and increased permeability of capillaries. Although IgE is typically the least abundant isotype of immunoglobulin, it is capable of triggering the most powerful, life-threatening inflammatory reactions.³⁴

The present study revealed the presence of anti-SARS-CoV-2-specific IgE antibodies in COVID-19 patients by comparing the serum levels of these antibodies between COVID-19 patients and

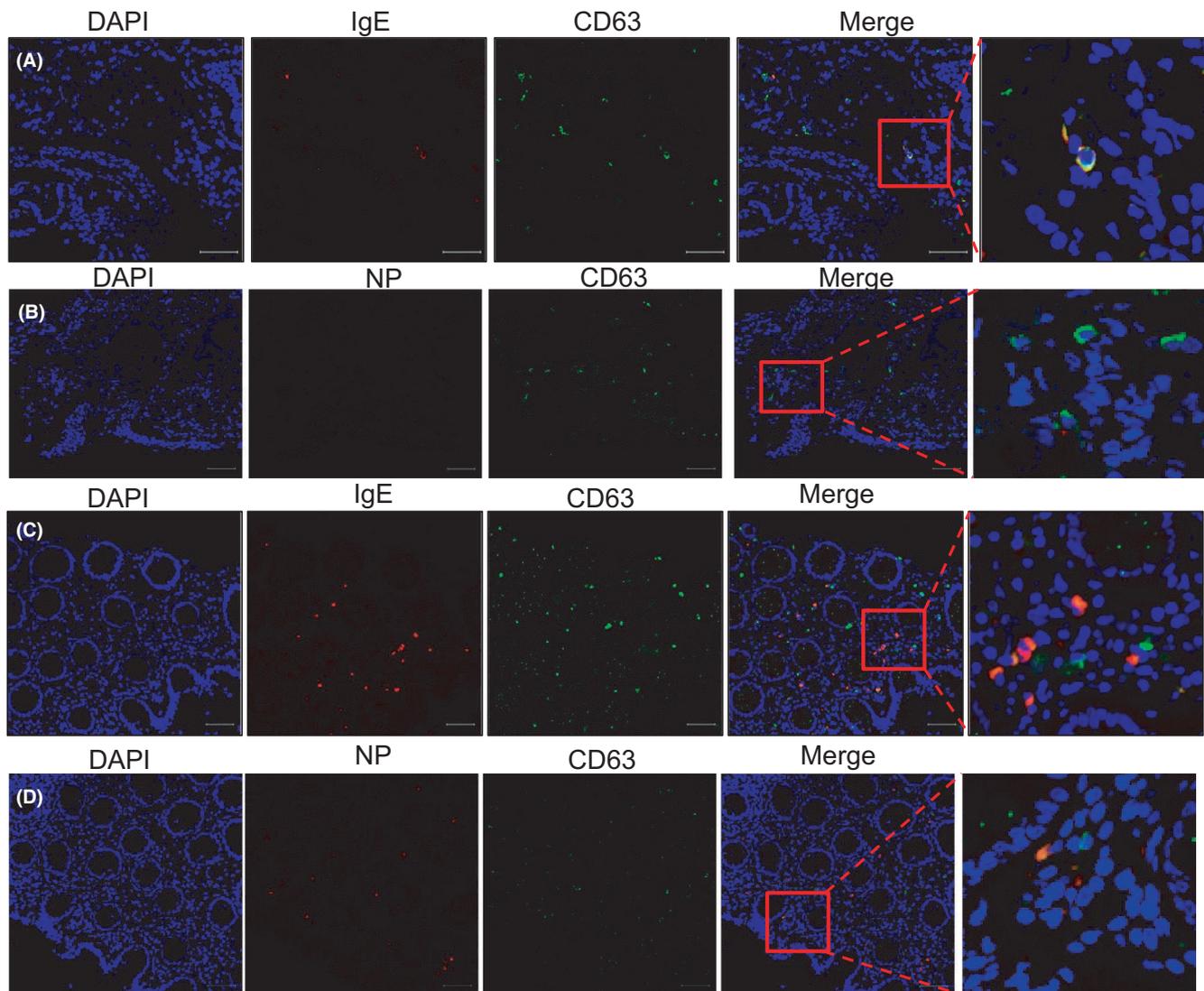


FIGURE 2 IgE-mediated NP-specific CD63⁺ mast cells in the bronchus and intestinal mucosa in COVID-19 patients. Histologic and immunofluorescent staining had been done in specimens in bronchus (A–B) and intestinal mucosa (C–D) from COVID-19 patients. DAPI: 40,6-diamidino-2-phenylindole. NP: SARS-CoV-2 nucleocapsid protein

healthy controls. Moreover, we discovered that the appearance of anti-SARS-CoV-2-specific IgE antibodies is an important clinical feature of COVID-19, as the levels were significantly higher in patients who developed hypoxemic respiratory failure than in those without respiratory failure. In a recent study, it was found that the recruitment of CD45RA⁺ T cells in COVID-19 is consistent with IgE-mediated allergic response.³⁵ However, interestingly, the specific IgE levels are low in patients with mild COVID-19. This means that the levels of specific IgE are associated with the progression of COVID-19 and the severity of COVID-19 at admission. This is explained as follows: The immune responses to viral infection are characterized by initial activation of innate immunity and production of type I and III interferons (IFN- α/β and - λ , respectively), a negative link exists between IgE and IFN- α and then antiviral responses may be suppressed in the process.^{36–39} In addition, the serum level of a specific IgE against N protein appears to have a higher correlation coefficient with TLSS and PaO₂/FiO₂ ratio, compared with that of

specific IgE against S protein. In terms of the association between specific IgE and PaO₂/FiO₂ ratio, it may be explained that during the progression of the disease, pulmonary exudation increased with increasing levels of specific IgE against SARS-CoV-2 antigens, and then the oxygen saturation drops gradually; however, as we all know, correlation does not always mean causality. To demonstrate causality, more rigorous empirical evidence is needed.

The risk of asthma from a severe viral infection is well documented.⁴⁰ The two major pathogens linked to asthma pathogenesis, i.e. respiratory syncytial virus and rhinovirus, are both known to potentially induce the production of IgE in humans.^{41,42} It appears that IgE may act as a signalling molecule for lung conventional dendritic cells (DCs). Once an effective adaptive immune response has been generated, virus-specific IgE will be produced, bind to the conventional DCs, and be cross-linked by viral antigen.⁴³ Anti-IgE treatment (omalizumab) can increase virus-induced IFN- α responses in the presence of IgE cross-linking and reduced DCs surface high-affinity IgE receptor

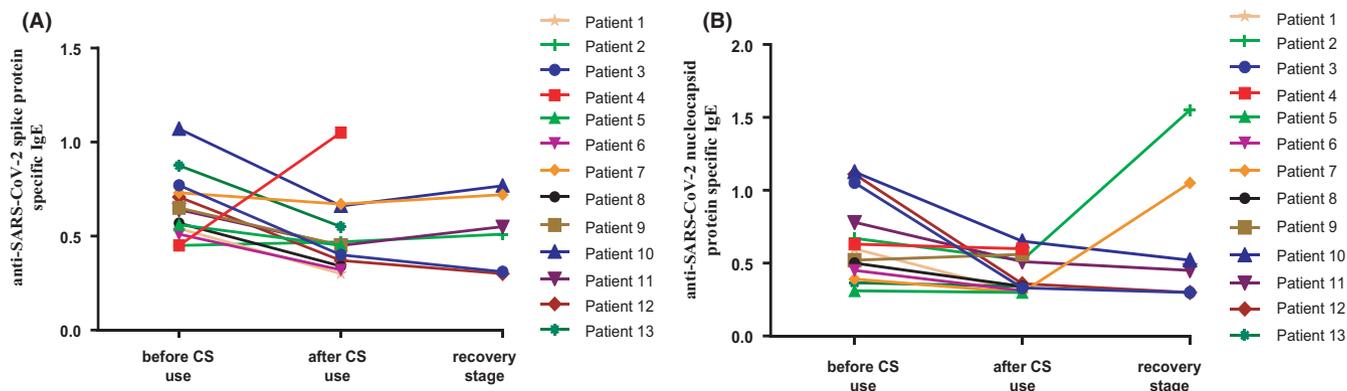


FIGURE 3 The change of SARS-CoV-2-specific IgE in different stages in severe COVID-19 patients who were treated with CS. The change of anti-SARS-CoV-2 spike protein-specific IgE (A) and anti-SARS-CoV-2 nucleocapsid protein-specific IgE (B). CS: corticosteroid

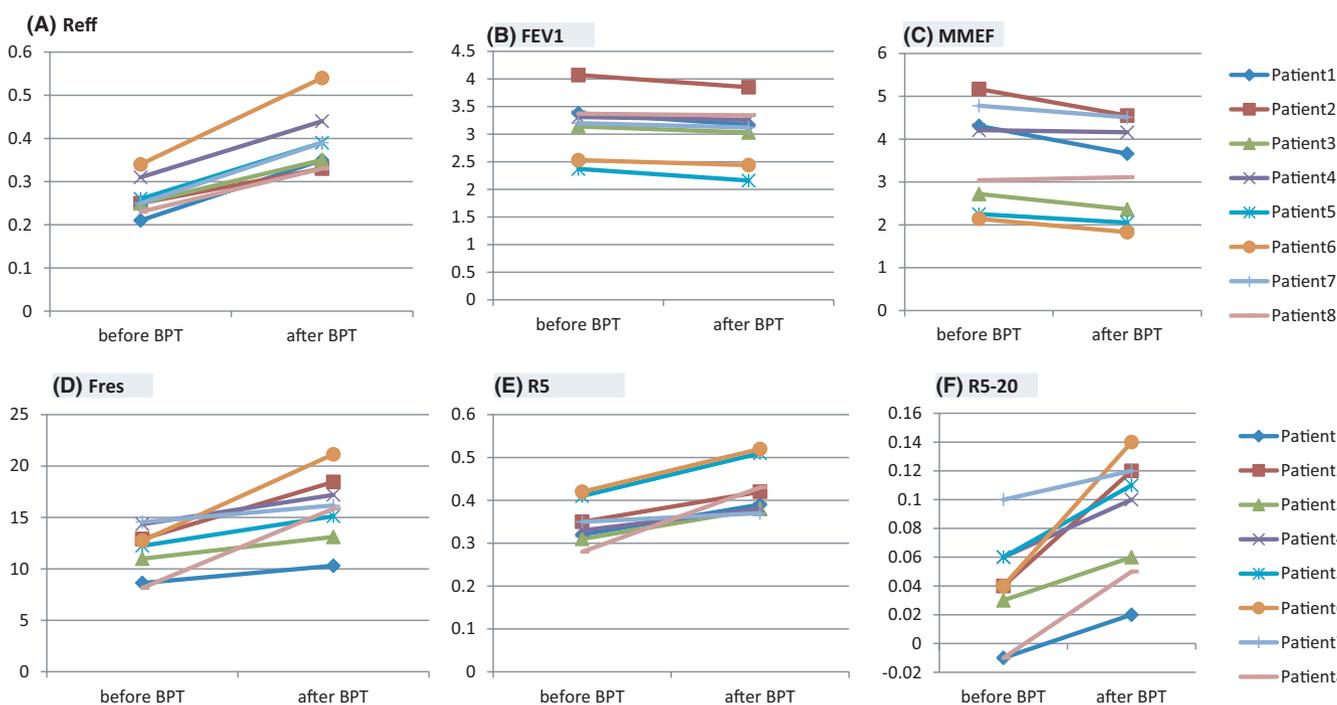


FIGURE 4 Airway hyperresponsiveness (AHR) exists in early convalescence of COVID-19. Before and after BPT, the worsen index of lung function included Reff (A), FEV1 (B), MMEF (C), Fres (D), R5 (E) and R5-20 (F) in early convalescence of 8 COVID-19 patients

(FcεRIα) expression.⁴⁴ These results support a potential mechanism that allergic sensitization is associated with increased susceptibility to virus-induced asthma exacerbations. We noticed some articles focus on the use of omalizumab in COVID-19 patients who have to co-exist asthma or urticaria. The application of omalizumab can prevent exacerbation of asthma and even the existence of pneumonia during SARS-CoV-2 infection,⁴⁵ and also alleviate symptoms of exacerbated urticarial rash and angioedema due to COVID-19 in a patient with chronic spontaneous urticaria.⁴⁶ As a recombinant human anti-IgE antibody, omalizumab is originally designed to reduce sensitivity to allergens and blocks IgE binding to FcεRI. The excellent response to omalizumab may give evidence to how IgE work on COVID-19.

Furthermore, we detected anti-SARS-CoV-2-specific IgE on the surface of activated CD63+ cells (most likely mast cells) in the bronchial and gastrointestinal mucous membranes of patients with COVID-19. NP-specific activated mast cells in gastrointestinal mucous induced hypersensitivity, which may be an explanation of digestive system symptoms partially in COVID-19. In addition, although there is no data about pulmonary function test in an acute stage, airway hyperresponsiveness proved by BPT is common in a convalescent stage in COVID-19 patients. Considering all of this evidence together, it is reasonable to infer that SARS-CoV-2-induced IgE production, which primes IgE-mediated immediate hypersensitivity reactions, results in hypoxemic respiratory failure due to inflammatory

storm. Detection of SARS-CoV-2 antigen-IgE antibody on the mast cell surface represents the typical type I hypersensitivity reaction. In conclusion, we propose that anti-SARS-CoV-2-specific IgE-mediated hypersensitivity reactions are the chief mechanism of COVID-19-related hypoxemic respiratory failure and other organ dysfunction.

Glucocorticoids are well-established as the first-line therapy for type I hypersensitivity reactions and are also effective for inhibiting the inflammatory storm. It is reported that COVID-19-associated CSS could be treated with high-dose of methylprednisolone. The use of glucocorticoids may accelerate respiratory recovery, lower hospital mortality and reduce the likelihood of invasive mechanical ventilation in COVID-19-associated CSS.⁴⁷ The same phenomenon was observed by our team.¹⁵ In our study, we found that timely and appropriate application of glucocorticoid could avoid the need for invasive mechanical ventilation and improve the outcomes of critical COVID-19 patients, compared with the outcomes in the reported studies. In the later stage in the "cytokine storm," when the immune system becomes exhausted, continuous administration of glucocorticoid may lead to subsequent co-infections and slows down the viral clearance. However, this did not happen in our patients. No obvious side effect has been observed during the half-year follow-up, probably because of the strictly controlled total dosage of methylprednisolone and the proper time of administration. The excellent response to glucocorticoids provides further evidence for the hypothesis that hypersensitivity reactions mediated by SARS-CoV-2-induced specific IgE production might be the chief pathological mechanism of severe COVID-19. Larger studies in more diverse populations are warranted to confirm this hypothesis.

The study has several limitations. First, due to the difference in the progression of COVID-19 between patients, it is hard to determine an appropriate time to measure IgE, TLSS and PaO₂/FiO₂ ratio. Therefore, these patients were examined two weeks later, after admission and CS use. Second, the sample size of our study remains small. Third, some studies also found that previous allergic history (such as asthma, atopic rhinitis and allergic dermatitis) has little effect on the occurrence and severity of COVID-19,^{36,48} which need further research. Although our data suggested that IgE could be used to evaluate the severity of COVID-19, more research is required to validate our findings. In addition, limited data were available and the usefulness of specific IgE in predicting mortality remains unknown. However, this research may have a good application prospect.

In COVID-19 patients, anti-SARS-CoV-2-specific IgE, which mediated activated mast cells in the bronchial and intestinal mucosa, had been detected and significant correlations with the severity of patients were found. After the application of CS in severe COVID-19, anti-SARS-CoV-2-specific IgE decreased but was maintained at a high level in early convalescence, which maybe is the reason for airway hyperresponsiveness (AHR) in these patients partly. Overall, our study indicated that hypersensitivity might be involved in severe COVID-19.

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Not applicable.

CONFLICT OF INTEREST

The authors of this manuscript declare no relationships with any companies whose products or services might be related to the subject matter of the article.

AUTHOR CONTRIBUTIONS

Jin Huang and Jing Liu had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. Cuiyan Tan, Xiaobin Zheng, Fengfei Sun, Jianzhong He, Honglei Shi, Meizhu Chen, Changli Tu, Yiyang Huang, Zhenguo Wang, Yingjian Liang, Jian Wu, Ye liu, Jing Liu and Jin Huang contributed substantially to the study design, data analysis and interpretation and the writing of the manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Committee of The Fifth Affiliated Hospital of Sun Yat-sen University (approval series number K30-1). The registration number of clinical trials: ChiCTR2000032895, Jing Liu.

CONSENT FOR PUBLICATION

All data published here are under the consent for publication.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the present study are available from the corresponding author on a reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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