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RESEARCH ARTICLE

Predictive effects of IgA and IgG combination to assess pulmonary exudation progression in COVID-19 patients

Mingshan Xue¹ | Teng Zhang² | Haisheng Hu¹ | Zhifeng Huang¹ | Yingjie Zhen¹ |
Yueting Liang¹ | Yifeng Zeng¹ | Tengchuan Jin³  | Luqian Zhou¹ |
Xiaohua D. Zhang² | Baoqing Sun¹ 

¹State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

²Faculty of Health Sciences, University of Macau, Taipa, Macau, China

³Hefei National Laboratory for Physical Sciences at Microscale, Laboratory of Structural Immunology, CAS Key Laboratory of Innate Immunity and Chronic Disease, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, China

Correspondence

Baoqing Sun, MD State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China.
Email: sunbaoqing@vip.163.com

Xiaohua Douglas Zhang, PhD Faculty of Health Sciences, University of Macau. Address: Avenida da Universidade, Taipa, Macau, China.
Email: douglaszhang@um.edu.mo

Funding information

Guangdong Science and Technology Fund, Grant/Award Number: 2020B1111300001; Zhejiang University special scientific research fund for COVID-19 prevention and control, Grant/Award Numbers: 2020XGZX001, 2020XGZX025

Abstract

Our study intended to longitudinally explore the prediction effect of immunoglobulin A (IgA) on pulmonary exudation progression in COVID-19 patients. The serum IgA was tested with chemiluminescence method. Autoregressive moving average model was used to extrapolate the IgA levels before hospital admission. The positive rate of IgA and IgG in our cohort was 97% and 79.0%, respectively. In this study, the IgA levels peaks within 10-15 days after admission, while the IgG levels peaks at admission. We found that the time difference between their peaks was about 10 days. Viral RNA detection results showed that the positive rate in sputum and feces were the highest. Blood gas analysis showed that deterioration of hypoxia with the enlargement of pulmonary exudation area. And alveolar-arterial oxygen difference and oxygenation index were correlated with IgA and IgG. The results of biopsy showed that the epithelium of lung was exfoliated and the mucosa was edematous. In severe COVID-19 patients, the combination of IgA and IgG can predict the progress of pulmonary lesions and is closely related to hypoxemia and both also play an important defense role in invasion and destruction of bronchial and alveolar epithelium by SARS-CoV-2.

KEYWORDS

COVID-19, IgA, IgG, prediction effects

1 | INTRODUCTION

Acute diffuse alveolar damage with extensive pulmonary exudation is the dominant feature of COVID-19, and it could progress to acute respiratory distress syndrome (ARDS) within a short time in some

severe patients.¹ It is not just caused by the virus mass replication but also associated with hyperactive immune responses.² Pathologically, bronchial inflammatory cells infiltration, epithelial and cilia desquamation, and hyaline membrane forming were observed.³ Meanwhile, the negative effects of COVID-19 lung exudation on

Mingshan Xue and Teng Zhang should be regard as co-first authors.

ventilation function deserve attention. In some patients, the improvement of ventilator-assisted ventilation is not significant, which eventually resulted in severe hypoxemia and death.^{4,5}

Serum antibodies play an important role in the defense against SARS-CoV-2 virus in the replication and infiltration of pulmonary epithelium.⁶ IgG, a known main component of serum, stands the first place with the 75% total amounts of Ig. By comparison, IgA accounts for only fewer than the content of IgG. Acting as the main and mild human antibody, IgA is present as secretory and serotype, wherein the latter accounts for as much as 85%. Both IgA and IgG in immunoglobulin are capable of eliminating non-inflammatory forms of antigens.⁷⁻¹⁰ Extensive pulmonary exudation in severe COVID-19 patients was noted in autopsy and case reports as one of its important features. Different from other viral pneumonia, lung effusion is related to prognosis.¹¹⁻¹³ IgA and IgG play an important role in the progress of SARS-COV-2 defense which as a major defensive barrier in the lung can lead to antigen-antibody reaction that cause lung exudation increasing, although they are not specific enough to assess the disease progression accurately.^{10,14} However, the association with local immune response and imbalance leading to pulmonary exudation is still a concern.

2 | METHODS

2.1 | Patient characteristics

This study included 21 COVID-19 patients diagnosed in the First Affiliated Hospital of Guangzhou Medical University from 1 February to 6 April 2020, including 14 severe COVID-19 patients and seven mild COVID-19 patients. Among them, 14 severe COVID-19 patients were transferred to our hospital after deterioration during the treatment period, so there is a time interval between the time of first symptom and the time of antibody detection in hospital. The average time interval was 8 days. This study was approved by the ethics Committee of First Affiliated Hospital of Guangzhou Medical University with approval number of 2020-77.

2.2 | Imaging detection

High-resolution computed tomography (HRCT) with 1 mm thin layer was used for imaging detection. We adopted quantitative analysis system and calculated the percentage of lesion area (patch shadow, fiber strip shadow, and ground-glass opacities) in the total lung window of each cross-section in jugular notch, sternoclavicular joint, aortic arch, and left atrium.

Owing to the limitation of physical condition in severe patients, their changes of lung condition were examined with bedside chest posteroanterior oblique and lateral views (PA&LAT). The time interval between each examination was about 1 or 2 days. Chest PA&LAT is faster and more efficient than HRCT in emergency

admission patients. In this study, chest PA&LAT was mainly used on severe patients, while the HRCT was still used on patients with milder clinical presentation.

2.3 | IgA antibody detection

In this study, we used the (SARS-CoV-2) IgA and IgG antibody detection kit (Kangrun Biotech, Guangzhou, China) and automatic chemiluminescent immune analyzer (KAESER 1000; Kangrun Biotech).

2.4 | Lung biopsy

A total of three patients had lung tissue biopsies performed and the samples were collected from lesioned areas (subcutaneous puncture).

2.5 | Data analysis

Autoregressive moving average (ARMA) model is an important model for the analysis of time series.¹⁵ This model has been applied to analyze and predict continuous monitoring of clinical and physiological data.^{16,17}

All quantitative data in this study were expressed as median (interquartile range) and the difference were assessed by non-parameter test (Mann-Whitney test). The significant level less than 0.05 ($P < .05$) indicated significant difference. All data analysis were performed in IBM SPSS (Statistics for Windows Version 22.0; IBM Corp, Chicago, IL), GraphPadPrism 5.0 (GraphPad Software, San Diego, CA) and R (Bell Laboratories Version 4.0.0).

3 | RESULTS

3.1 | Patient characteristics

A total of 21 COVID-19 patients were included in our study. The basic information of the patients is listed in Table 1. The information showed that the length of stay and the level of IgA in severe patients are significantly higher than in mild patients. In the early stage of admission, that is, during the acute onset, the IgG level is highest, and then decreased with disease remission. However, IgG did not show a significant difference between the common and the severe group. In general, the level of inflammation was higher in severe patients, with decrease of oxygenation index (OI), increase of alveolar arterial oxygen differential pressure alveolar-arterial oxygen difference (A-aDO₂), and increase of respiratory index. These indicators showed significant difference between severe patients and mild patients. In addition, inflammatory cytokines, interleukin-2 (IL-2), IL-6, IL-10, and tumor necrosis factor- α (TNF- α), were significantly increased in severe patients.

TABLE 1 Patient characteristics

	Common	Severe	P value
N	7	14	
Age, y	51.00 (49.00, 69.00)	59.50 (49.00, 69.00)	.350
Gender, male/female	3/4	12/2	.120
IgA, mg/L	10.34 (7.03, 31.49)	14.33 (6.82, 28.72)	.005
IgG, mg/L	21.06 (16.53, 37.24)	25.71 (15.43, 40.71)	.181
CRP, mg/L	2.54 (0.92, 12.29)	7.07 (2.71, 12.29)	.009
Hospital stays, days	34.00 (27.00, 39.00)	58.50 (44.00, 63.25)	.002
D-Dimer, $\mu\text{g/L}$	558.0 (263.00, 853.00)	4624.00 (2406.00, 9068.00)	.001
AST, U/L	27.40 (20.80, 37.20)	39.20 (27.60, 62.30)	.001
<i>Blood gas</i>			
PaO ₂ , mm Hg	98.10 (83.95, 107.20)	102.80 (82.28, 126.90)	.620
PaCO ₂ , mm Hg	48.35 (41.15, 48.35)	42.80 (39.80, 47.45)	.022
Oxygenation index	386.0 (253.30, 495.80)	203.00 (136.00, 276.50)	.001
Spiro-index	46 (-0.50, 133.50)	210.00 (125.50, 372.00)	.001
PA-aDO ₂ , mm Hg	53.70 (23.10, 161.90)	219.3 (158.60, 311.10)	.001
<i>Venous blood analysis</i>			
White blood cell, 10 ⁹ /L	4.80 (3.90, 6.20)	8.60 (6.80, 11.50)	.001
Neutrophil count, 10 ⁹ /L	2.90 (2.30, 4.00)	6.80 (4.80, 9.50)	.001
Lymphocyte count, 10 ⁹ /L	1.10 (0.90, 1.30)	0.80 (0.60, 1.20)	.001
Platelet count, 10 ⁹ /L	250.0 (169.00, 332.00)	172.5 (112.3, 233.0)	.001
IL-2, U/L	0.56 (0.44, 0.70)	0.73 (0.55, 1.09)	.001
IL-4, U/L	0.94 (0.72, 1.36)	1.13 (0.72, 1.88)	.264
IL-6, U/L	5.18 (2.63, 9.84)	34.62 (6.75, 108.10)	.001
IL-10, U/L	2.82 (2.10, 3.98)	4.68 (3.14, 7.67)	.001
TNF- α , U/L	0.75 (0.56, 1.01)	0.96 (0.70, 1.44)	.001
IFN- γ , U/L	0.78 (0.51, 1.10)	0.95 (0.60, 1.41)	.022

Note: The statistics in the table are the median (IQR) of the indices collected at all the time points during hospitalization.

Abbreviations: A-aDO₂, alveolar-arterial oxygen difference; AST, aspartate aminotransferase; CRP, C-reactive protein; IFN, interferon; IgA, immunoglobulin A; IL, interleukin; PaO₂, partial pressure of oxygen; PaCO₂, partial pressure of carbon dioxide; TNF, tumor necrosis factor.

3.2 | Dynamics of serum IgA and IgG levels

In our study, the positive rate of IgA and IgG was 97% and 79.0%, respectively. We collated all IgA and IgG data of severe patients within 65 days of hospitalization and used ARMA model to predict the IgA level between the symptom onset and admission time. The changes in IgA and IgG levels are shown in Figure 1. We found that IgA levels peaked around 10 to 15 days of hospitalization, while IgG peaked at admission and then declined progressively with disease remission.

3.3 | Assessment of pulmonary effusion and correlation analysis of IgA

We collated the data of lung involvement area (chest PA&LAT and CT) in severe COVID-19 patients. The results in Figure 2 show that the lung involvement area in severe COVID-19 patients was present on admission and began to rise. The lung involvement area peaked in

20 to 25 days and then began to decrease (Figure 2A). According to the patient records, four patients were still in intensive care, without any decrease in lung involvement area and remission in condition. Therefore, these four patients were assigned into a deterioration group, while the rest were in remission group. The results showed that the area of pulmonary involvement in the remission group decreased gradually with the improvement of the disease (Figure 2C). There was no significant change in lung lesion area in deterioration group (Figure 2D).

We also calculated the cross correlation between IgA level and chest PA&LAT and CT lung involvement area (Figure 2B). The results showed that there is a positive correlation between IgA level and lung involvement area. In particular, the correlation between IgA level and lung involvement area was high (chest PA&LAT: $r = .6663$, $P < .05$; CT: $r = .7681$, $P < .05$) when the lag days of IgA was 10 days. The results showed that the IgA levels reach the peak value earlier than the lung involvement area, about 10 days in advance.

It is important to note that a decrease/increase in the area of the lung lesions can be used as a sign of remission/exacerbation, and a

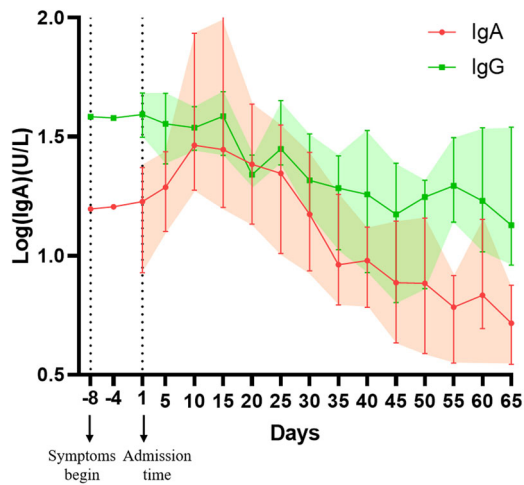


FIGURE 1 The dynamics of IgA and IgG levels in severe COVID-19 patients. The red points represented the median of IgA levels and the error bar represented the upper and lower quartiles. The trend of IgA levels before admission time was predicted by ARMA (1,1,0). The green points represented the median of IgG levels and the error bar represented the upper and lower quartiles. ARMA, autoregressive moving average; IgA, immunoglobulin A

decrease/increase in the density of the lung lesion can also be considered as remission/exacerbation. As a result, we evaluated the changes in HRCT values. We found 25 to 30 days HRCT value up to -258.8 (-475.0 , -108.1), 55 to 60 days HRCT value of the minimum -642.9 (-736.9 , -582.5), from 5 days to peak time and IgA level high. And significant correlation ($P < .05$) was observed at 20 days. However, due to the small amount of HRCT data, we could not make a fitting prediction.

3.4 | Viral nucleic acid analysis

The highest positive rate was found in the sputum (severe: 62.5% and mild: 54.1%). The total positive rate of pharyngeal swabs and nasopharyngeal swabs results were also summarized (severe: 39.6%, mild: 0%; severe: 26.8%, mild 10.3%). And also feces (severe: 51.0% and mild: 2.9%) and anal swab (severe: 28.9% and mild: 0%) detection results. Moreover, the positive rate of gastric juice in severe patients was 17.5%.

3.5 | Blood gas index and the correlation analysis of IgA and IgG

The results of correlation analysis between IgA-IgG and blood gas index showed that both IgA and IgG was significantly correlated with $A-aDO_2$, oxygenation index, and respiration index, all $P < .05$ (Figure 3A). In addition, the oxygenation index reflects the degree of hypoxia in severe patients. The results showed that the higher the degree of hypoxia in patients, the larger the lung involvement area and the higher the levels of IgA and IgG (Figure 3B).

3.6 | The biopsy results

In this study, three patients with severe COVID-19 underwent lung biopsy (lower lobe of the lung), all of which indicated that part of the lung epithelium was exfoliated, submucosal edema with few lymphocytes infiltrated, presenting chronic inflammatory changes of the mucosa.

4 | DISCUSSION

In our study, the level of IgG increased to maximum when the first symptoms began, and IgA peaked after admission 15 to 20 days, which were earlier than the chest PA&LAT and CT to the maximum lung exudation area, and both showed significant correlation with the oxygen partial pressure difference ($A-aDO_2$) and OI of arterial alveolar blood. At the same time, lymphocytes were significantly decreased and IL-2, IL-6, IL-10, and TNF- α levels were significantly increased in severe patients of COVID-19. We considered the excessive activation of immune systems, which induced the release of a large quantity of inflammatory mediators and the latter led to destruction of lung epithelial cells. Therefore, the clinical manifestations (fever and respiratory symptoms) of the severe COVID-19 patients are worse, and some of them need to be admitted to ICU for treatment with mechanical ventilation.

In this study, it was also found that whole lung lobes were involved and there was no central tendency in any particular lobe in severe patient, which was different from other epidemic pneumonia. Under the influence of virus invasion and inflammatory factor storm, the patients may develop acute bronchitis and diffuse alveolar damage. The damage of lung epithelium, capillary endothelium, and connective tissue caused significant increase in lung exudation and/or formation of a transparent membrane. The imaging showed pulmonary patchy shadows, ground glass, and consolidation. IgA and IgG are the first line of defense against virus invasion in bronchoalveolar epithelium, which appear earlier than the expansion of the lung exudation, and there is a significant correlation between the two. Therefore, we conclude that a combination of IgA and IgG could be used as a predictive indicator to evaluate the level of lung exudation in patients with severe COVID-19.

Interestingly, the sputum and fecal nucleic acid positive rate of severe patients was higher (62.5% and 51.0%). To rule out the possibility of intestinal nucleic acid test positive for swallowing sputum, we also tested gastric juice and found that the positive rate was only 17.5%. Therefore, it can be proved that COVID-19 virus mainly invades the respiratory and intestinal tract. Meanwhile, the virus-induced immunomodulatory imbalance caused inflammatory storm and further aggravates the lung damage, which would trigger alveolar epithelial detachment, mucosal edema, and even lung exudation, eventually evolving into the worsening of hypoxemia and life-threatening situation. IgA and IgG were shown to be significantly correlated with $A-aDO_2$ and OI. The oxygenation index grouping could reflect the degree of hypoxia and we found that the

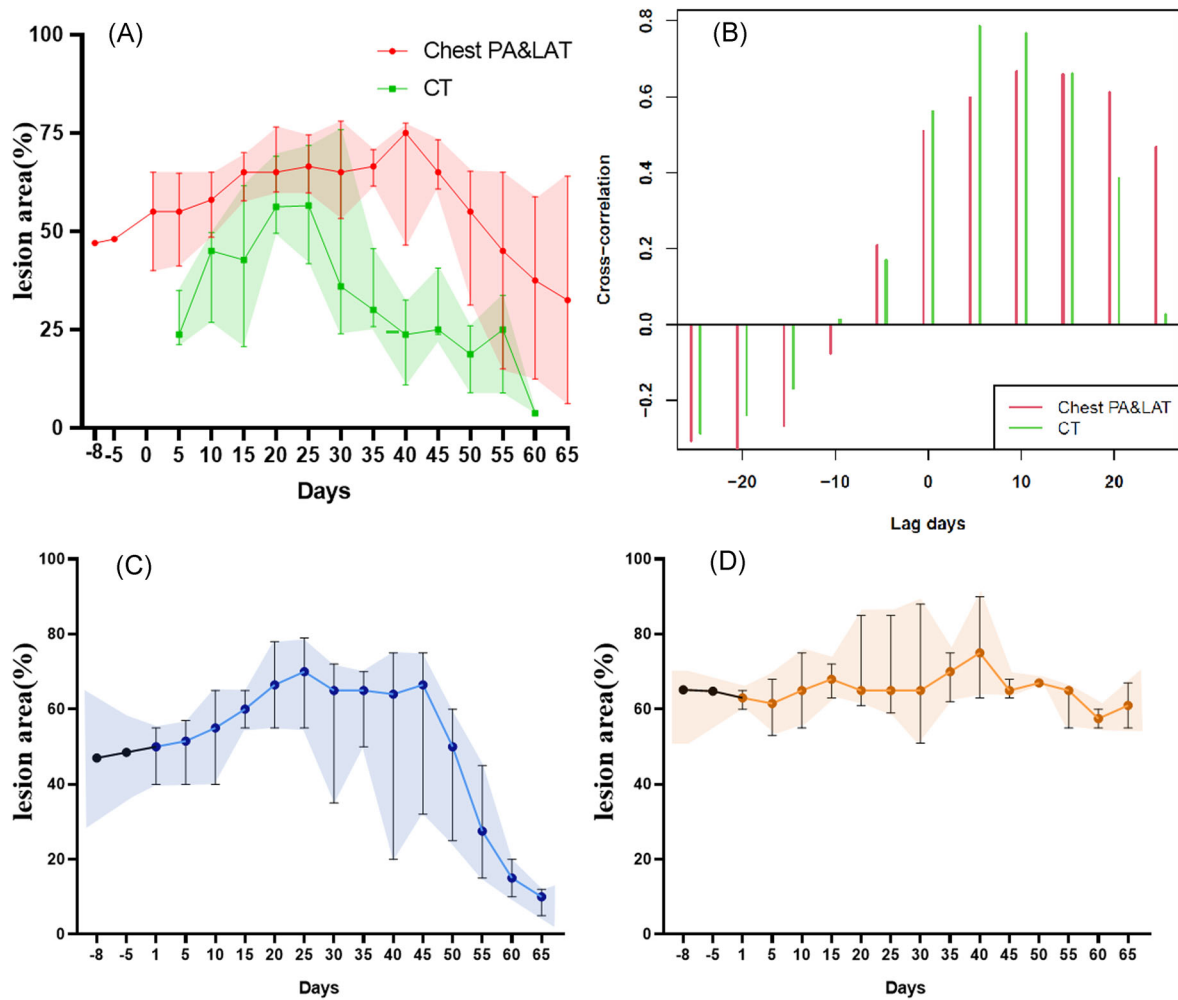


FIGURE 2 The trend of chest PA&LAT lung involvement analysis. A, The trend of chest PA&LAT and CT lung involvement area in severe COVID-19 patients. The trend of chest PA&LAT before admission time was predicted by ARMA (0,2,0). The CT data in 1 to 5 days were missing, and no modeling and prediction of ARMA was carried out. B, The cross correlation between lung involvement area (chest PA&LAT and DR) and IgA levels. When the lag days of IgA was 10 days, the correlation was high (correlation coefficient is .6663, $P < .05$). C, The change of chest PA&LAT lung involvement area in severe COVID-19 patients with remission of pulmonary lesions. The black points represent the predicting values of ARMA (0,2,0) before admission time. D, The change of chest PA&LAT lung involvement area in severe COVID-19 patients with deterioration of pulmonary lesions. The black points represent the predicting values of ARMA (0,1,0) before admission time. ARMA, autoregressive moving average; CT, computed tomography; IgA, immunoglobulin A; LAT, lateral PA, posteroanterior

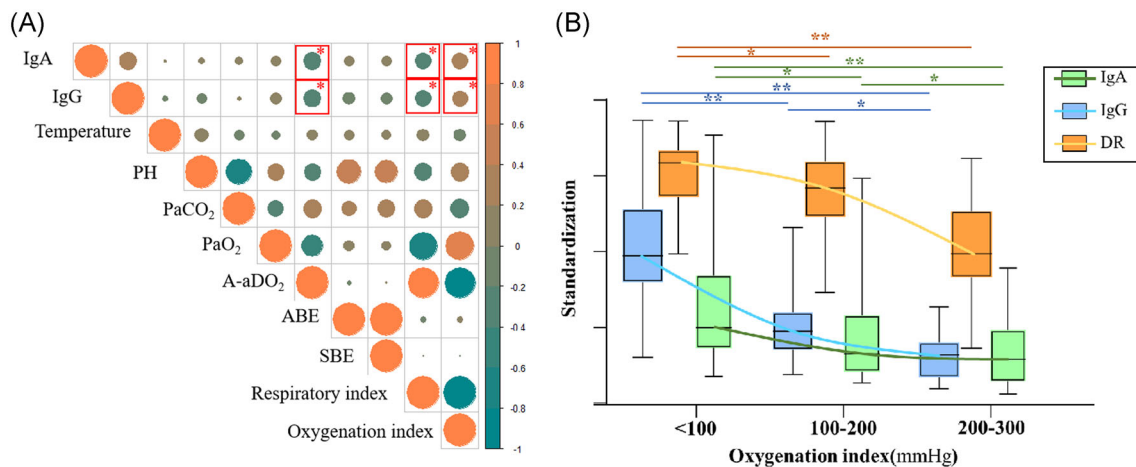


FIGURE 3 The trend of hypoxia degree and the affected area of IgA and lung lesions. A-aDO₂, alveolar-arterial oxygen difference; ABE, actual base residue; IgA, immunoglobulin A; DR: Digital X-ray photography system (This is the same as what was mentioned above chest posteroanterior oblique and lateral views); PaO₂, partial pressure of oxygen; PaCO₂, partial pressure of carbon dioxide; SBE, standard base residue. * $P < .05$, ** $P < .01$

oxygenation index decreases along with the increase of the lung exudation. In combination with lung biopsy results, we consider that the virus infiltrated and destroyed the bronchial and alveolar epithelium in severe patients of COVID-19, and that IgA and IgG play a major immunomodulatory role in submucosal circulation.

5 | CONCLUSION

As an important antibody to the airway epithelium of the lung, IgA and IgG play an important defense role against invasion and destruction of bronchial and alveolar epithelium by SARS-CoV-2, and can be used as a predictor to reflect the degree of lung exudation in critical COVID-19 patients to evaluate the progress and prognosis.

ACKNOWLEDGMENT

We would like to thank Editage (www.editage.com) for English language editing. This work was supported by Guangdong Science and Technology Fund (No. 2020B1111300001), Zhejiang University special scientific research fund for COVID-19 prevention and control (Nos. 2020XGZX001, 2020XGZX025). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding was received for this study.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

BS and XDZ designed and funded this study project. MX and TZ completed the article writing. HH and ZH participated in the data collation. YZ and YL participated in clinical information collection. YZ completed the detection of IgA and IgG in this study. TJ and LZ further improved the content of this article.

DATA AVAILABILITY STATEMENT

All the data in this article were collected from clinical or laboratory of our hospital sources and partly was still being studied. Therefore, the data cannot be fully shared for the time being.

ORCID

Tengchuan Jin  <http://orcid.org/0000-0002-1395-188X>

Baoqing Sun  <http://orcid.org/0000-0002-1671-0723>

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How to cite this article: Xue M, Zhang T, Hu H, et al. Predictive effects of IgA and IgG combination to assess pulmonary exudation progression in COVID-19 patients. *J Med Virol.* 2021; 93:1443-1448. <https://doi.org/10.1002/jmv.26437>