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SHORT COMMUNICATION

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Multiplexed analysis of circulating IgA antibodies for SARS-CoV-2 and common respiratory pathogens in COVID-19 patients

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Abstract

Previous studies have revealed a diagnostic role of pathogen-specific IgA in respiratory infections. However, co-detection of serum specific IgA for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and common respiratory pathogens remains largely unexplored. This study utilizes a protein microarray technology for simultaneous and quantitative measurements of specific IgAs for eight different respiratory pathogens including adenovirus, respiratory syncytial virus, influenza virus type A, influenza virus type B, parainfluenza virus, mycoplasma pneumoniae, chlamydia pneumoniae, and SARS-CoV-2 in serum sample of patients with coronavirus disease 2019 (COVID-19). A total of 42 patients with COVID-19 were included and categorized into severe cases (20 cases) and nonsevere cases (22 cases). The results showed that co-detection rate of specific-IgA for SARS-CoV-2 with at least one pathogen were significantly higher in severe cases than that of nonsevere cases (72.2% vs. 46.2%, $p = .014$). Our study indicates that co-detection of IgA antibodies for respiratory pathogens might provide diagnostic value for the clinics and also be informative for risk stratification and disease management in patients with COVID-19.

KEYWORDS
circulating IgA, co-infection, COVID-19, respiratory pathogens, SARS-CoV-2

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1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), a global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was transmitted rapidly more so than SARS-CoV and Middle East respiratory syndrome-related coronavirus.[1] At present, there are no specific means to prevent or treat COVID-19, highlighting an urgent need for efficacious therapies and vaccines. Moreover, further understanding of its pathogenesis and improvement of the diagnostic efficacy are critical to identify the potentially infected people and block the disease progression.

Laboratory diagnosis of COVID-19 includes viral nucleic acid detection and specific serological antibody assay. The sensitivity of nucleic acid detection may be low due to unskilled sample collection, varying mucosal sites, and time periods of viral release in patients. In contrast, serological testing is advantageous with less workload and faster turn-around time. In the case of pathogen infections, while IgM and IgG isotypes have been the primary emphasis, increasing evidence has indicated that systemic IgA responses played important roles in patients with COVID-19.[2,3] Our previous study has shown an early response of SARS-CoV-2 specific IgA in serum samples, occurring 2 days post symptoms onset and earlier than specific IgM and IgG antibodies.[4] In addition to SARS-CoV-2 infection, human serum specific IgA responses have been observed in multiple infections, for example, chlamydia pneumoniae,[5] mycoplasma pneumoniae,[6] respiratory syncytial virus,[7] parainfluenza virus,[8] and influenza infections.[9] Also, coexistence of SARS-CoV-2 and other respiratory pathogens in patients with COVID-19 through the nucleic acid tests have been reported.[10,11] However, co-detection of serum specific IgA for SARS-CoV-2 and common respiratory pathogens remains, at present, unexplored. Considering also the potential ramification of co-infection with other respiratory pathogens in precise diagnosis and management of patients with COVID-19.

2 | METHODS

This study was conducted to investigate the circulating IgAs for SARS-CoV-2 and common respiratory pathogens in a cohort of patients with COVID-19. Our cohort has been recently reported elsewhere,[4] of which the patients were recruited from three hospitals in Guangdong province, China. A total of 42 patients with COVID-19 were enrolled during January 26, 2020 to February 25, 2020. The patients were categorized into two groups, 20 (47.6%) severe cases and 22 (52.4%) nonsevere cases. All patients were confirmed by positive testing for viral nucleic acid of SARS-CoV-2 with respiratory specimens (Real-Time Fluorescent RT-PCR Kit, DaAn Gene Co., Ltd.).

A protein microarray technology for simultaneous and quantitative measurements of specific IgAs for eight different respiratory pathogens in human serum was utilized. The pathogens included adenovirus (ADV), respiratory syncytial virus (RSV), influenza virus type A (IFV-A), influenza virus type B (IFV-B), and parainfluenza virus (PIV, type I, II, and III mixed), mycoplasma pneumoniae (MP), chlamydia pneumoniae (CP), and SARS-CoV-2 (Figure S1). In this assay kit, the microarray chip was made of a glass slide on which eight recombinant antigens of respiratory pathogens (ADV, RSV, IFV-A, IFV-B, PIV, MP, CP, and SARS-CoV-2 nucleocapsid (N) protein) were spotted and immobilized, respectively, by an automatic machine smart Arrayer TM 136 (Capital Bio corporation Ltd.). Briefly, a total of 300 μl diluted (a dilution factor of 40) samples were added to each chip grid. After washing three times, 200 μl of HRP-conjugated secondary antibodies (1:5000) were added and incubated for 30 min at 37°C, followed by the addition of 150 μl substrate and incubated for 15 min at 37°C for color development. Finally, the reaction intensity was read by a biochip reader-SCIARRAY-I (Shenzhen Sciarray Biotech Co., Ltd.) and expressed as relative unit after subtracting the background. Sensitivity and specificity of circulating IgAs for SARS-CoV-2 and common respiratory pathogens is provided in Table S1. The χ^2 test was used to compare positive rates between groups with SPSS software version 23.0.

3 | RESULTS

As shown in Table 1, the enrolled patients with COVID-19 consisted of 27 males (64.3%) and 15 females (35.7%). The severe group with mean age of 57.4 (51.5–63.3) years was older than the nonsevere group of 46.8 (38.5–55.2) years, displaying an age-dependent severity ($p = .040$). There was no significant difference in comorbidity rate between the two groups ($p > .05$), except for the increasing trend of comorbid T2DM in the severe group, although the difference did not reach the significance level ($p = .052$). The overall positive rate of SARS-CoV-2 specific IgA was 83.3%, while no significant difference could be seen between severe and nonsevere groups (90.0% vs. 77.3%, $p = .269$). However, the level of SARS-CoV-2 specific IgA was significantly higher in severe group than that of nonsevere group (Figure S2). Serum specific IgA antibodies for common respiratory pathogens were detected for IFV-B, CP, IFV-A, and RSV, with the positive rates of 38.1%, 23.8%, 21.4%, and 21.4%, respectively (Table 1). Additionally, we observed significantly higher positive rates of IFV-A and CP specific IgAs in severe patients as compared to those in nonsevere patients ($p < .05$). Further analysis showed that the positive rate of SARS-CoV-2 with at least one pathogen specific IgAs in hospitalized patients with COVID-19 was 61.3%, while the co-positive rate in severe cases were significantly higher than that of nonsevere cases (72.2% vs. 46.2%, $p = .014$).

4 | DISCUSSION

Pathogen associated nucleic acid assay has been commonly regarded as gold standard for a confirmative infection. The present study showed that the positive rate of serum specific IgA for SARS-CoV-2 reached 90.0% in severe patients, indicating its potential utility in identifying severe COVID-19. By co-detecting IgA responses, this study showed that common respiratory virus, such as influenza (A

TABLE 1 Demographic features and positive numbers (rates) of serum specific IgAs in patients with COVID-19

	All patients	Non-severe patients	Severe patients	*p*-value
Baseline variables				
No.	42	22	20	–
Age, year[a]	51.9 (46.6–57.1)	46.8 (38.5–55.2)	57.4 (51.5–63.3)	.040
Female, *n* (%)	15 (35.7%)	10 (45.5%)	5 (25.0%)	.167
Type II Diabetes, *n* (%)	11 (26.2%)	3 (13.6%)	8 (40.0%)	.052
Hypertension, *n* (%)	14 (33.3%)	6 (27.3%)	8 (40.0%)	.382
COPD, *n* (%)	6 (14.3%)	2 (9.1%)	4 (20.0%)	.313
CHB, *n* (%)	2 (4.8%)	1 (4.5%)	1 (5.0%)	.945
CHD, *n* (%)	4 (9.5%)	2 (9.1%)	2 (10.0%)	.920
Comorbidities[b]	17 (40.5%)	6 (27.3%)	11 (55.0%)	.067
Pathogen-specific IgAs				
SARS-CoV-2, *n* (%)	35 (83.3%)	17 (77.3%)	18 (90.0%)	.269
IFV-B, *n* (%)	16 (38.1%)	7 (31.8%)	9 (45.0%)	.380
CP, *n* (%)	10 (23.8%)	2 (9.1%)	8 (40.0%)	.019
IFV-A, *n* (%)	9 (21.4%)	2 (9.1%)	7 (35.0%)	.041
RSV, *n* (%)	9 (21.4%)	4 (18.2%)	5 (25.0%)	.591
ADV, (%)	0	0	0	–
PIV, (%)	0	0	0	–
MP, (%)	0	0	0	–
Co-detections[c], *n* (%)	19 (45.2%)	6 (46.2%)	13 (72.2%)	.014

Abbreviations: ADV, adenovirus; CHB, chronic hepatitis B; CHD, coronary heart disease; CI, confidence interval; CP, chlamydia pneumoniae; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; IFV, influenza virus; PIV, parainfluenza virus; MP, mycoplasma pneumoniae; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Data are presented as mean (95% CI).

[b]COVID-19 patients with at least one comorbidity.

[c]Denotes the co-detection of SARS-CoV-2 and at least one pathogen specific IgAs.

and B), were present in patients with COVID-19, which was in line with the findings revealed by polymerase chain reaction (PCR) assays in a case report.[12] Intriguingly, the present study revealed a relatively higher seroprevalence of CP and RSV specific IgAs in patients with COVID-19, as compared with those observational studies using PCR assays.[10,11] The reasons accounting for the discrepancies may be multifactorial, for example, varying study populations, small sample sizes and high sensitivity of protein microarray detections. High seroprevalence of pathogens specific IgAs may also be related to a past history of infections of those patients with COVID-19. Nevertheless, further study of a larger sample size is warranted. We also found that seroprevalence of SARS-CoV-2 and other respiratory pathogens was associated with the disease severity. Since the disease progression and outcome of patients with COVID-19 are dependent on the host immune response, we hypothesized that compromised immune response (e.g., diabetes) with older ages may predispose to an increased risk of co-infection in severe patients with COVID-19. Nevertheless, this study provides evidence that co-detection of serum IgA antibodies for SARS-CoV-2 and other respiratory pathogens is feasible using a microarray assay with advantages of time saving and high efficiency. Moreover, our findings that higher rates of positive IgA response to multiple pathogens were noted, particularly in severe COVID-19 cases, which may suggest a heightened memory B cell response in severe patients with COVID-19 coinfected with different pathogens or suffered from recent infections with non-SARS-CoV-2 viruses. While these possibilities cannot be discerned at this time, future efforts investigating the likelihood of viral interference or nonspecific modulation of immunity in disease progression or severity of COVID-19 may need to be considered.

In summary, this study suggests that serological detection of pathogen-specific IgAs during the COVID-19 pandemic may offer diagnostic benefits for laboratory testing. Furthermore, testing for non–SARS-CoV-2 respiratory pathogens during the COVID-19 pandemic may be warranted and informative for risk stratification and disease management in patients with COVID-19. We acknowledge limitations of this study, including lower sensitivity and specificity of serologic assay as compared to the gold standard method and limited number of patients. Future investigative work is certainly needed to refine the detection methodology and confirm its utility in large scale studies. Also, further understanding of the role of pathogen-specific IgA antibodies in COVID-19 patients and the likely co-infection with other respiratory pathogens would be prudent for achieving more precise diagnosis and therapy for severe COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

The study was approved by the Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University (YYLS 2020-77).

AUTHOR CONTRIBUTIONS

Zhang-fu Fang, Bao-qing Sun, Ai-ru Zhu, and Lian-cheng Lin contributed equally to this study. Zhang-fu Fang, Bao-qing Sun, Ai-ru Zhu, and Lian-cheng Lin undertook the experiments. Jin-cun Zhao and Song He took part in sample collection and technical support. All authors contributed to the conception of the work and interpretation of the findings. Zhang-fu Fang and Zhi-gang Liu drafted the manuscript. Song He and Nan-shan Zhong critically revised the manuscript. All authors read the manuscript and approved the final version. Nan-shan Zhong, Shau-Ku Huang, and Zhi-gang Liu act as guarantors. The corresponding author attests that all listed authors meet authorship criteria and those did not meet the criteria have been omitted.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

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