

Antiphospholipid and other autoantibodies in COVID-19 patients: A Singapore series

Dear Editor,

Thrombosis is an unexpected complication of COVID-19 initially reported in 3 patients from China.¹ These patients tested positive for immunoglobulin (Ig) A anticardiolipin (ACA), IgG anti- β 2-glycoprotein I antibodies (a β 2GPI) and IgA a β 2GPI, though not for the lupus anticoagulant (LAC).

In a Singapore study comprising 47,527 patients, 19 (0.04%) developed acute cerebral arterial and 4 (0.01%) developed venous thrombosis.² Only 3 patients in the former group and 1 in the latter group were found to have LAC, ACA or a β 2GPI (personal communication). In a meta-analysis of 28 studies, among 2,928 intensive-care COVID-19 patients, deep venous thrombosis occurred in 16.1% and pulmonary embolism in 12.6%.³

We sought to identify autoantibodies (antinuclear antibody [ANA], rheumatoid factor [RF], ACA and a β 2GPI) in the serum of COVID-19 patients admitted to our hospital from 21 April to 2 September 2020. During this period, the original virus strain was prevalent and all COVID-19 patients were managed in the hospital. Leftover serum samples from completed routine tests in the laboratory were used. This work is approved by the institutional review board (DSRB 2020/00741).

Healthy control sera had been collected before the pandemic for our biobank. These were from 100 volunteers (80% men) comprising Filipinos (50%), Chinese (30%), Malays (10%) and Indians (10%).

The ANA, IgA and IgM RF, and IgG and IgA a β 2GPI kits were obtained from EUROIMMUN Medizinische Labordiagnostika AG (Lübeck, Germany). The IgG and IgA ACA kits were from Inova Diagnostics Inc (San Diego, US). The method for detection was indirect immunofluorescence for ANA, and enzyme-linked immunosorbent assay for the rest. Reference values were: ANA <1/80 titre; IgA and IgM RF, IgG and IgA ACA, and IgG and IgA a β 2GPI, <20 units/mL.

There were 52 COVID-19 patients (92.3% men). The mean age was 43.8 $\pm$ 13.3 years, ranging 22.5–95.5 years. There were 16 Bangladeshis, 24 Indians, 9 Chinese, 2 Malays and 1 Burmese. Based on clinical record charting and chest imaging, we categorised the severity of COVID-19 infection into 3 categories: asymptomatic, mid/moderate and severe/critical. For symptomatic

patients, the interval between the first symptoms and blood draw was 14.3 $\pm$ 18.6 days (range 3–93 days, 95% confidence interval 8.1–20.4).

Twenty-one patients experienced no respiratory symptoms (including 1 hospitalised for aortic thrombus), 17 developed upper respiratory tract infection, and 14 developed pneumonia. One patient died of pneumonia. Four patients (3 with pneumonia and 1 otherwise asymptomatic) were considered to have COVID-19-associated coagulopathy, based on emerging literature at the time.⁴

There is statistically significant higher prevalence of IgA RF, IgG ACA, IgA ACA, IgG a β 2GPI and IgA a β 2GPI in our COVID-19 patients compared with healthy controls (Table 1). The odds ratios are highest for IgA a β 2GPI, IgG ACA and IgG a β 2GPI. There is no difference in the prevalence of ANA and IgM RF. Almost two-thirds of the patients (63.4%) and 11% of the controls developed at least ACA or a β 2GPI antibodies. There was no association between disease severity and presence or the concentration of any autoantibodies (Table 1).

Four patients developed arterial thrombosis—2 with cerebral infarcts, 1 with right suprarenal aortic thrombosis, and 1 with abdominal aorta and right external iliac artery thrombosis. Separately, 1 patient developed pulmonary embolism. These occurred acutely, apart from the cerebral infarcts that developed 1- and 3-month post-recovery. Per the aforementioned sequence, the first patient tested positive for IgA ACA; the second IgA RF; the third IgG ACA, IgG a β 2GPI and IgA a β 2GPI; and the fourth IgA ACA, IgG a β 2GPI, IgA a β 2GPI and IgA RF. The patient with pulmonary embolism tested positive for ANA and IgA a β 2GPI. Four of the 5 patients tested positive for $\geq$ 1 antiphospholipid antibodies.

This controlled study refines our understanding of antiphospholipid antibodies in COVID-19. We showed that COVID-19 patients are highly likely to develop antiphospholipid antibodies compared with healthy controls. Autoantibodies were not generated across the board as the prevalence of ANA and RF IgM positivity was no different between patients and controls.

The lack of association between disease severity and the development of autoantibodies is consistent with

Table 1. Frequency of the autoantibodies in COVID-19 patients and healthy controls

	COVID-19 patients				Controls (n=100)	OR (95% CI) of presence of antibody in patients of all severity compared with controls	P value
	Asymptomatic (n=21)	Mild and moderate (n=17)	Severe and critical (n=14)	Total (n=52)			
Antinuclear antibody, no. (%)	2 (9.5)	2 (11.8)	3 (21.4)	7 (13.5)	13 (13)	1.04 (0.4–2.8)	0.936
IgA rheumatoid factor, no. (%)	9 (42.9)	6 (35.3)	6 (42.9)	21 (40.4)	10 (10)	6.10 (2.6–14.4)	<0.001
IgM rheumatoid factor, no. (%)	0	2 (11.8)	1 (7.1)	3 (5.8)	15 (15)	0.35 (0.1–1.3)	0.095
IgG anticardiolipin antibody, no. (%)	8 (38.1)	6 (35.3)	4 (28.6)	18 (34.6)	0	108 (6.3–1837)	<0.001
IgA anticardiolipin antibody, no. (%)	10 (47.6)	8 (47.1)	8 (57.1)	26 (50)	9 (9)	10.1 (4.2–24.2)	<0.001
IgG anti-β2-glycoprotein I, no. (%)	11 (52.4)	6 (35.3)	6 (42.9)	23 (44.2)	0	160 (9.4–2716)	<0.001
IgA anti-β2-glycoprotein I, no. (%)	12 (57.1)	8 (47.1)	11 (78.6)	31 (59.6)	2 (2)	72.3 (16.0–326)	<0.001

CI: confidence interval; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; OR: odds ratio
Chi-square test was carried out for each comparison

some reports⁵ and contrasts with others.⁶ A meta-analysis of 21 studies completed in January 2021 concluded that the presence of ACA (IgM or IgG) and anti-β2GPI (IgM or IgG) was significantly more prevalent in critically ill COVID-19 patients, although a source of bias could be that non-critically ill patients were included in only 3 studies.⁷

Viral infections in the acute stage are associated with the generation of autoantibodies; these antibodies tend to be transient and do not cause pathology. The relative risk of developing ACA was 10.5% in individuals with human immunodeficiency virus, 6.3% with hepatitis C (HCV), 4.2% with hepatitis B (HBV) and 10.9% with Epstein-Barr virus infection. Only HCV infection was associated with aβ2GPI antibodies. Thrombosis occurs in HBV and HCV patients only.⁸ Severe acute respiratory syndrome, caused by the closely related SARS-CoV virus, is also known to be associated with autoantibodies.⁹ A review suggested that although antiphospholipid antibodies may be found in COVID-19 patients, they are not closely related to thrombotic events.¹⁰

The strengths of our study are the classification of patients by disease severity, and comparison with healthy controls. The weaknesses are the small number of patients in our convenience sample, which may have contributed to bias. The use of anticoagulation in our patients could have reduced the incidence of clinically detected thrombosis. Our findings may not be generalisable to infection by subsequent variants, such as the Delta and Omicron strains.

In conclusion, almost two-thirds of the COVID-19 patients in our cohort developed antiphospholipid autoantibodies regardless of the disease severity. These autoantibodies are not associated with thrombosis in COVID-19.

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