Journal Pre-proof

Anticardiolipin and anti-beta-2 glycoprotein I antibodies in patients with moderate or severe COVID-19

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PII: S0002-9629(22)00453-0
DOI: https://doi.org/10.1016/j.amjms.2022.10.012
Reference: AMJMS 1782

To appear in: *The American Journal of the Medical Sciences*

Received date: 13 January 2022
Accepted date: 27 October 2022

Please cite this article as: Gisela Garcia-Arellano MD, PhD , Adrian Camacho-Ortiz MD, PhD , Ilse Andrea Moreno-Arquieta MD , Jesus Alberto Cardenas-de la Garza MD , Diana Carolina Rubio-Torres MD , Elvira Garza-Gonzalez MD , Paola Bocanegra-Ibarias MD , Dionicio Angel Galarza-Delgado MD, PhD , Anticardiolipin and anti-beta-2 glycoprotein I antibodies in patients with moderate or severe COVID-19, *The American Journal of the Medical Sciences* (2022), doi: https://doi.org/10.1016/j.amjms.2022.10.012

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Short title: Antiphospholipid antibodies in COVID-19

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Keywords: Antiphospholipid antibodies, COVID-19, SARS-CoV-2, immunoglobulin A, immunoglobulin G, blood coagulation disorders, thrombosis, pandemics, prevalence.

To the Editor:

The COVID-19 pandemic continues worldwide. A prothrombotic state has been associated with COVID-19, however, the pathogenesis remains unclear. Previous studies have reported elevated levels of antiphospholipid (aPL) antibodies in patients with COVID-19 which can lead to prolonged clotting times and thromboembolic events.[1,2] It is unclear if these antibodies represent an epiphenomenon or if they are involved in hemostatic abnormalities observed in COVID-19 patients.[3] Research has predominantly focused on lupus anticoagulant and other aPL antibodies although information is scarce.[4,5] We aimed to determine the prevalence of aPL antibodies, anticardiolipin (aCL) and anti-beta-2 glycoprotein I (anti-β2GPI), and their association with outcomes in patients with moderate/severe COVID-19.

We performed a case-control study involving hospitalized patients from the High Specialty and Advanced Medicine Hospital (HAEMA) - University Hospital, which was adapted as a COVID hospital at the beginning of the pandemic. We included patients aged 18-65 years old with confirmed COVID-19 pneumonia that required admission to the intensive care unit. We classified as moderate COVID the patients with pneumonia that required ICU care, and as severe those who required orotracheal intubation. Recruiting was done between August 6 to September 26 of 2020. The control group was healthcare workers without history of COVID-19, with a negative rRT-PCR and asymptomatic at inclusion. For both groups, we excluded subjects with previous thromboembolic events, previous history of

COVID-19, autoimmune disease, pregnancy or with history of a previous pregnancy-related complications.

Demographic and clinical data were obtained from the clinical files. For the control group we only collected data on age and sex. Serum samples were obtained at the time of admission and evaluated for anti-β2GPI (IgG, IgA, and IgM) and aCL (IgG, IgA, and IgM) antibodies using an enzyme-linked immunosorbent assay (Euroimmun, Lübeck, Germany). Results were expressed with a positive cutoff of >20 U/mL for anti-β2GPI and >12 U FL/mL for aCL, according to the manufacturer's instructions.

Comparisons between groups were performed using the Mann-Whitney U test, Chi-square test, and Fisher's exact test. All analyses were performed with SPSS v.25 (IBM Inc., Armonk, NY, USA). A p-value <0.05 was considered statistically significant. All patients gave their consent for participation. The study was approved by the institutional ethics and research committee (ID: RE20-00015).

Ninety-two subjects were included (46 COVID-19 patients and 46 controls), the majority were male (53/92, 57.6%) and the mean age was 52.5 (43.2-58 IQR) years old (Table 1). The prevalence of aPL antibodies was higher in COVID-19 patients than in controls: 76.1% versus 15.2%, respectively ($p < .001$). The most frequent aPL on the control group was aCL-IgG (6.5%, *p*= *.617*), and on the COVID-19 patients was anti-β2GPI-IgA (73.9%, *p*= *<.001*). Both groups were negative for aCL-IgA and anti-β2GPI-IgG (Table 2).

From the COVID-19 patients one (2.2%) patient developed a thromboembolic pulmonary event and another (2.2%) a hemorrhagic stroke. Since there were only two cases of these clinical events, we could not evaluate their association with aPL antibodies. A total of 13 (28.3%) patients required orotracheal intubation (OTI) of which one (7.7%, *p*=*>0.05*) had positive anti-β2GPI-IgM and 10 (76.9%, p=*>0.05*) had positive anti-β2GPI-IgA. Patients with hypertension (8/12, 66.7%) or OTI (7/12, 53.8%) had a higher mortality rate. We did not find an association with anti-β2GPI-IgA (9, 75.0%, *p*= *>0.05*) and mortality.

In this study, we were able to demonstrate a high rate of aPL antibodies (76.1%), most of which were anti-β2GPI-IgA (73.9%) in moderate to severe COVID-19 patients. This prevalence varies highly among series.[6,7] Our finding on anti-β2GPI-IgA is consistent with what was previously described by Van der Linden et al., where 20 (86%) out of 34 (100%) patients, had markedly elevated levels of this antibody. Yet, a recent meta-analysis showed a frequency of aPL antibodies in hospitalized patients with COVID-19 of 46.8%, although it did not include anti-β2GPI-IgA.[6]

The reason why anti-β2GPI-IgA was more prevalent in our case series is unknown. It might be explained by the anti-viral response at mucosal sites,[8] which could be supported by the higher frequency of gastrointestinal symptoms described in Mexican patients with COVID-19.[9]

A relationship between aCL-IgA and anti-β2GPI-IgA/IgG and coagulopathy or infarctions in COVID-19 has been reported.[1] Due to the small prevalence of thrombotic events, we could not analyze this relationship in our sample. Additionally, there was no association of aPL antibodies with OTI and mortality as was formerly described by Taha et al.[6] However, differences found between studies in prevalence and clinical outcomes may be due to several factors: variability in clinical conditions of patients' cohorts, different tests to measure aPL antibodies, and timing of the laboratory sample. Limitations of our report include the number of cases included and the prevalence of thrombotic events. Strengths include the inclusion of three serotypes of anti-β2GPI and aCL of patients with moderate and severe disease requiring oxygen supplementation, and that the study was performed before vaccine availability which limit confounding factors related to vaccine-induced antibodies.

In conclusion, we found a significantly higher prevalence of anti-β2GPI-IgA in moderate to severe COVID-19 patients, which was not associated with OTI or mortality. Research with a larger cohort and a longitudinal sampling on aPL antibodies including IgA class is necessary to establish their role in COVID-19 and its short and long-term complications.

Declarations

Ethics approval

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. Registration number of RE20-000015.

Consent to participate

All the data retrieved were anonymized. Consent was obtained before the procedure.

Author contributions

GGA, DAGD and ACO conceived the idea. Recruitment, statistical analyses, and interpretations was performed by IAMA, GGA, JACD, DCRT, EGG and PBI. Writing of the first draft was performed by DCRT, IAMA and JACD. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflicts of Interest and Funding

None of the authors of this study has any financial interest or conflict with industries or parties. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Table 1. Demographics, and SARS-CoV-2 patients' clinical data

Patients´ characteristics	COVID-19 patients n (%) = 46(100)	Controls n (%) = 46(100)
Age, mean (IQR)	55.5 (13.5)	48.21 (12.75)
Male, (%)	27 (58.7)	26 (56.5)
Pre-existing comorbidities, (%)		
T2DM	18 (39.1)	
Hypertension	15 (32.6)	
Obesity	12 (26.1)	
Hospitalization days, (IQR)	8.5 (6.7- 15)	
Clinical events, (%)		
Thrombotic event	1 (2.2)	
Hemorrhagic event	1 (2.2)	
OTI	13 (28.3)	
Outcome, (%)		
Discharged alive	34 (73.9)	
Died	12 (26.1)	

Orotracheal intubation (OTI), Type 2 diabetes mellitus (T2DM).

Table 2. Comparison of antiphospholipid antibodies between COVID-19 patients and controls.

	COVID-19 patients n (%) = 46 (100)	Controls n (%) = 46 (100)	P value
Age, years, median (IQR)	55(45.5-59)	48 (42 -55)	0.015
Male, (%)	27 (58.7)	26 (56.5)	0.833
Anti- β2 GPI, U/mL			
IgA, SAU	34 (73.9)	1 (2.2)	<0.001
IgM, SMU	3 (6.5)	2(4.3)	>0.05
IgG, SGU	0 (0)	0(0)	>0.05
aCL, U FL/mL			
IgA	0 (0)	0 (0)	>0.05
IgM	1 (2.2)	2 (4.3)	>0.05
IgG	1 (2.2)	3 (6.5)	0.617

Anti-beta-2 glycoprotein I (Anti- β2GPI); anticardiolipin (aCL).