



# Antiphospholipid antibodies in patients hospitalized with COVID-19 infection in conventional units

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## Abstract

**Objectives:** This study aimed to evaluate the status of antiphospholipid antibodies (APL) in hospitalized patients with COVID-19 infection.

**Methods:** This case-control study was conducted at Shahid Beheshti Hospital of Hamadan University of Medical Sciences from 2020 to 2022. Participants were divided into two groups; COVID-19 (n=35 recovered cases) and non-COVID-19 (n=34). The study assessed anti-cardiolipin antibodies (ACA) including IgM, IgG, and antibodies against  $\beta$ 2-glycoprotein (anti-B2GPI). Additionally, the lupus anticoagulant (LAC) test was conducted on patients not using anticoagulant medications. Enzyme-linked immunosorbent assay (ELISA) was employed for ACA and anti-B2GPI testing, while the LAC test was conducted using a fully automated coagulometer.

**Results:** The mean LAC among participants was  $33.8 \pm 7.53$ s for females and  $36.39 \pm 5.54$ s for males. Results of antibody tests indicated that ACA IgM was positive in 2 out of 33 cases, with none in the non-recovered COVID-19 and COVID-19 patients (P value = 0.15). Furthermore, the LAC test showed positive results in 5 and 2 patients in the case and control groups, respectively (P value = 0.26). Correlation analyses of LAC with other antibodies revealed no significant associations for non-COVID-19 patients, while a significant association was observed between LAC and  $\beta$ 2GPI IgG and ACA IgG ( $r = 0.52$ ;  $P < 0.001$  and  $r = 0.51$ ;  $P < 0.001$ , respectively). Negative correlations were found between LAC and ACA IgM and hospitalization in the case group ( $r = -0.22$  and  $r = -0.04$ , respectively). For the control group, negative correlations were observed between LAC and  $\beta$ 2GPI IgM, ACA IgG, and ACA IgM ( $r = -0.25$ ,  $r = -0.03$ , and  $r = -0.03$ , respectively). Additionally, Anti- $\beta$ 2GPI IgG and anti- $\beta$ 2GPI IgM tests were positive in both COVID-19 and non-COVID-19 groups (two positives in total) (P value = 0.98 vs P value = 0.31).

**Conclusion:** We recommend routine testing for aPL antibodies in recovered COVID-19 patients to assess their normal condition. Our findings suggest that APS antibodies in COVID-19 patients can serve as a negative prognostic indicator, guiding decisions on the need for intensive care based on antibody levels.

**Keywords:** COVID-19, SARS-CoV-2, Antiphospholipid antibodies, Anticardiolipin, Hospitalization.

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the viral agent responsible for COVID-19, a life-threatening infection that can range from pulmonary disorders to multiple organ complications accompanied by hypercoagulability.<sup>[1-4]</sup> While the clinical course of

COVID-19 is generally favorable, some cases may progress to acute respiratory insufficiency, necessitating intensive care unit (ICU) hospitalization.<sup>[5,6]</sup> Recent studies have explored antiphospholipid antibodies (APL) in individuals with SARS-CoV-2 infection.<sup>[7-10]</sup> Antiphospholipid syndrome (APS), a systemic autoimmune disorder, leads

to the production of APL antibodies that target body cells, causing blood clots, miscarriages, and other complications such as low platelet counts.<sup>[11]</sup> Diagnosis of APS requires clinical symptoms and positive antibody findings since some individuals may be asymptomatic despite testing positive for APL and may not develop APS.<sup>[12]</sup> These antibodies can persist for more than three months.<sup>[13-15]</sup> Critically ill patients, including those with various infections, may exhibit a temporary increase in APL levels. Although rare, the presence of these antibodies can lead to thrombotic events, posing challenges in differentiating them from other causes of multifocal thrombosis in severely ill patients.<sup>[9]</sup> Comprehensive data on all APL indicators, including lupus anticoagulant (LAC), anticardiolipin (aCL), and antibeta2-glycoprotein I antibodies (a $\beta$ 2GPI), are crucial to enhance our understanding of the role of APL in SARS-CoV-2 cases.<sup>[16]</sup>

A study by Benjamina et al. (2021) involving 106 adult subjects, including 30 hospitalized COVID-neurological patients, 47 hospitalized COVID-19 patients without neurological disorders, and 29 hospitalized COVID-19 patients as the control group, revealed that anti-phosphatidylserine/prothrombin antibodies IgG levels correlated with aPS/PT IgG levels in COVID-19 patients with acute disseminated encephalomyelitis.<sup>[9]</sup> Conversely, significantly elevated levels of aCL IgA and IgG were observed in non-neurological hospitalized COVID-19 cases. Understanding the persistence and potential clinical impact of antiphospholipid antibodies is crucial for appropriate management.

Devreese et al., reported LAC positivity during the acute phase in individuals with COVID-19. They also highlighted triple APL positivity and high aCL/a $\beta$ 2GPI titers as rare occurrences.<sup>[17]</sup> Repeat testing indicated transient APL positivity in most cases. Further research and international collaboration on APL are essential to expand our knowledge of their role in thrombotic events associated with COVID-19. Limited case series have reported frequent antiphospholipid antibodies in COVID-19 cases.

## Objectives

This study aims to investigate the detection of common antiphospholipid antibodies managed within the antiphospholipid antibody syndrome classification in individuals with COVID-19 and compare the findings with severely ill non-COVID-19 cases.

## Methods

A case-control study was conducted on adult patients

over 18 years of age who were referred to Shahid Beheshti Hospital of Hamadan University of Medical Sciences between March and June 2020. These patients had confirmed COVID-19 through positive real-time PCR (RT-qPCR) and exhibited a moderate level of infection based on lung involvement and SPO<sub>2</sub> levels. In the case group, consisting of 35 recovered COVID-19 patients (10 men and 25 women), venous blood samples were collected for analysis one month after infection. Anti-cardiolipin antibodies (ACA) IgM, IgG, anti-B2GPI, and LAC tests were performed on patients who were not taking anticoagulant medications. Patients with autoimmune diseases that could lead to a positive antiphospholipid antibody (APL) test were excluded from the study. Exclusion criteria also included viral infections (HIV, HBV, HCV, EBV, and CMV), primary antiphospholipid syndrome (APS), and medications known to induce APA formation, such as certain epilepsy treatments and combined oral contraceptive pills. Suspected cases of cancer and respiratory system inflammation were also excluded. The control group, matched in gender and age to the case group, consisted of individuals hospitalized for reasons other than COVID-19 in different departments of the hospital (non-COVID group, n=34 patients, 10 men, 24 women), selected consecutively.

Blood samples were collected from all participants and controls, centrifuged, and serum was stored at -20°C until laboratory testing. Serum levels of IgG and IgM antibodies to cardiolipin and  $\beta$ 2-glycoprotein I were determined using an enzyme immunoassay method with commercial enzyme-linked immunosorbent assay kits (GA GENERIC ASSAYS, Dahlwitz, Germany) following the manufacturer's instructions. The detection limit for both autoantibodies was set at 10 IU/mL, with all samples run in duplicate. Heparinized plasma samples were used to measure LAC level with the HEMOCLOT™ LA-S assay (HYPHEN Biomed), a clotting method specifically designed for LAC detection. Results exceeding 45 seconds were considered positive.

Data analysis included frequency, percentage, total percentage, and demographic tables. The Shapiro-Wilk test was used to evaluate data normality. Intergroup comparisons were made using the chi-square test or Fisher's exact test, with a comparison table utilized for significant variables. The Mann-Whitney U test was applied for non-normally distributed continuous variables, while the Student t-test was used for normally distributed continuous variables. Statistical analysis was conducted using SPSS version 25.0 (IBM, Armonk, NY, USA), with significance set at  $p < 0.05$ .

The study was conducted in accordance with the Declaration of Helsinki. All experiments were conducted in accordance with the guidelines of the local Ethics Committee of HUMS, and the study protocol was approved (IR.UMSHA.REC.1399.883). The present study did not interfere with the process of diagnosis and treatment of patients and all participants signed an informed consent form.

## Results

The group of recovered COVID-19 patients included 10 (28.6%) men, while the non-COVID-19 group had 11 (32.4%) men (COVID-19 group vs. non-COVID-19 group, chi-square = 0.2 and P-value = 0.73). Tables 1 and Table 2 summarize the results of antibody testing and the relative differences between the case and control groups based on age. In the group of recovered COVID-19 patients, ACA IgM tested positive in 2 out of 33 cases, with no positives in the non-COVID-19 patients (P-value =

0.15). The LAC test was positive in 5 patients in the case group, compared to 2 in the control group (P value=0.26). Additionally, ACL was positive in 2 patients in the case group (IgG in zero, IgM in 2 patients), while there was one positive case (ACL IgG) in the healthy controls (P value=0.57). Anti-β2GPI (IgG and IgM) tests were positive in 2 patients.

The mean LAC levels among the study participants were  $33.8 \pm 7.53$ s for females and  $36.39 \pm 5.54$ s for males. Correlation tests of LAC with other antibodies [Table 3] revealed no significant associations with other antibodies for non-COVID-19 patients. However, a significant association was found between LAC and β2GPI IgG and ACA IgG ( $r = 0.52$ ;  $P < 0.001$  and  $r = 0.51$ ;  $P < 0.001$ , respectively). Negative correlations were observed between LAC and ACA IgM and hospitalization in the case group ( $r = -0.22$  and  $r = -0.04$ , respectively). For the control group, negative correlations were found between LAC, β2GPI IgM, ACA IgG, and ACA IgM in the case group ( $r = -0.25$ ,  $r = -0.03$ , and  $r = -0.03$ , respectively).

**Table 1.** Antibody testing and relative differences between the case and control groups based on age

Evaluated factors	Mean ± SD		T Value (Group Case VS. Group control)	P value (Group Case VS. Group control)
	Group Case	Group control		
Age(year)	49.34 ± 16.5	50.06 ± 14.78	0.19	0.85
LAC(s)	37.11 ± 5.2	31.99 ± 7.74	- 3.23	0.002**
B2GPI IgM(IU/mL)	2.02 ± 2.24	1.69 ± 0.99	- 0.79	0.43
B2GPI IgG(IU/mL)	1.85 ± 2.65	1.26 ± 1.96	- 1.06	0.29
ACA IgG(IU/mL)	1.04 ± 0.89	1.28 ± 1.16	0.98	0.33
ACA IgM(IU/mL)	4.73 ± 4.71	3.24 ± 1.79	- 1.73	0.09
Hospitalization duration(day)	29 ± 1.31	28.4 ± 1.54	-1.63	0.11

Lupus anticoagulants (LAC); Anti-cardiolipin antibodies (ACA); B2GPI: anti-β2-glycoprotein-I (anti-β2GPI) antibodies. \*\* Significant

**Table 2.** Antibody testing and relative differences between the case and control groups based on sex

Evaluated factors	mean ± SD		T Value (female VS. male)	P value (female VS. male)
	Female	Male		
age(y)	49.6 ± 15.85	49.09 ± 15.27	-0.7	0.94
LAC(s)	33.8 ± 7.53	36.39 ± 5.4	-1.42	0.16
B2GPI IgM(IU/mL)	2.05 ± 1.95	1.42 ± 1.04	1.38	0.17
B2GPI IgG(IU/mL)	1.81 ± 2.77	0.99 ± 0.2	2.04	0.04**
ACA IgG(IU/mL)	1.22 ± 1.18	1.01 ± 0.54	0.79	0.43
ACA IgM(IU/mL)	3.94 ± 3.59	4.11 ± 3.81	-0.18	0.86

Lupus anticoagulants (LAC); Anti-cardiolipin antibodies (ACA); B2GPI: anti-β2-glycoprotein-I (anti-β2GPI) antibodies. \*\* Significant

**Table 3.** Values of Pearson's correlation coefficient and associated probability between the measured variables

Groups	Pearson Correlation Coefficients	LAC	B2GPI IgM	B2GPI IgG	ACA IgG	ACA IgM	hospitalization	age
Control	LAC	1	-0.25	0.02	-0.03	-0.03	0.14	0.07
			0.15	0.91	0.88	0.86	0.42	0.68
	B2GPI IgM			-0.11	-0.07	0.44	0.04	-0.08
				0.53	0.7	0.01**	0.81	0.66
	B2GPI IgG				0.93	-0.02	-0.05	0.23
					<0.001**	0.91	0.76	0.2
	ACA IgG					-0.06	-0.07	0.1
						0.73	0.68	0.57
	ACA IgM						0.28	-0.13
							0.11	0.46
Case	hospitalization							0.08
								0.64
	hospitalization							0.09
								0.59
	ACA IgM						0.13	0.26
							0.45	0.13
	ACA IgG					0.12	0.03	0.54
						0.49	0.88	<0.001**
	B2GPI IgG				0.68	0.01	-0.07	0.44
					<0.001**	0.95	0.7	0.01**
B2GPI IgM			0.19	0.1	0.2	0.29	0.04	
			0.26	0.59	0.24	0.09	0.8	
LAC	1	0.09	0.52	0.51	-0.22	-0.04	0.27	
		0.6	<0.001**	<0.001**	0.2	0.8	0.11	

Lupus anticoagulants (LAC); Anti-cardiolipin antibodies (ACA); B2GPI: anti- $\beta$ 2-glycoprotein-I (anti- $\beta$ 2GPI) antibodies. \*\* significant correlation

## Discussion

This study aimed to investigate the antiphospholipid antibody status in recovered COVID-19 patients. The Anti- $\beta$ 2GPI IgG and Anti- $\beta$ 2GPI IgM tests yielded positive results for one case in the COVID-19 group and one case in the non-COVID-19 group, totaling two positives (P-value= 0.98 vs. P-value= 0.32). In a study by Nosrati et al., they examined antiphospholipid antibodies in 40 COVID-19 patients with coagulopathy and found no significant correlation between thromboembolic events and antiphospholipid antibody levels in COVID-19 patients, aligning with our findings.<sup>[18]</sup>

Our results for the recovered COVID-19 patient group revealed that the LAC test was positive in two out of 33 cases, compared to one out of 34 patients in the non-COVID-19 group (P value=0.57). Statistical analysis indicated significant differences in Anti- $\beta$ 2GPI IgG based on sex (female vs. male). Taha and Samavati conducted a meta-analysis and systematic review on APL prevalence and its clinical impact in COVID-19 patients, showing a high prevalence of APL in severe disease but no significant correlation between APL positivity and adverse outcomes

(such as thrombosis, invasive ventilation, and mortality).<sup>[19]</sup> Similarly, Devreese et al. conducted a cohort study illustrating antiphospholipid antibody status in COVID-19 patients, demonstrating positive APL in COVID-19-positive patients. They highlighted the unclear connection between APL status and thrombosis, calling for further research.<sup>[17]</sup>

In our study, correlation tests between LAC and other antibodies showed no significant associations for non-COVID-19 patients [Table 3], while a significant association was found between LAC and  $\beta$ 2GPI IgG and ACA IgG (r=0.52; P<0.001 and r=0.51; P<0.001, respectively), consistent with previous studies.<sup>[20-25]</sup> Karahan et al. performed APA tests post-recovery from COVID-19 and other critical illnesses, reporting three deaths within four weeks, with one APA-positive case in the COVID-19 group and two in the control group.<sup>[16]</sup>

Regarding our study, negative correlations were observed between LAC and ACA IgM and hospitalization in the case group (r = -0.22 and r = -0.04, respectively), while for the control group, negative correlations were found between LAC,  $\beta$ 2GPI IgM, ACA IgG, and ACA IgM in the case

group ( $r=-0.25$ ,  $r=-0.03$ , and  $r=-0.03$ , respectively). Pakzad et al., investigated the correlation between antiphospholipid antibodies and COVID-19 severity, finding no significant association between APL and ICU admission outcomes, suggesting that APL may not be reliable predictors for ICU admissions in COVID-19 patients based on their logistic regression analysis results.<sup>[26]</sup>

## Conclusions

Our results revealed an equal rate of positive cases in both groups. Our study found no significant difference in antiphospholipid antibodies between individuals who had recovered from COVID-19 one month after testing positive and the control group. These findings suggest that the serum levels of antiphospholipid antibodies in patients after four weeks of infection are comparable to those in the general population. This information could be valuable for guiding prophylactic treatment with anticoagulants in these patients.

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## Competing interests

The authors declare that they have no competing interests.

## Abbreviations

Coronavirus disease 2019: COVID-19;  
Severe acute respiratory syndrome coronavirus 2: SARS-CoV-2;  
Antiphospholipid antibody: APL;  
Antiphospholipid syndrome: APS;  
Anti-cardiolipin antibodies: ACA;  
Antibodies against  $\beta$ 2-glycoprotein: anti-B2GPI;  
Antibeta2-glycoprotein I antibodies: a $\beta$ 2GPI;  
Lupus anticoagulant: LAC;  
Anticardiolipin: Acl;  
Anti-cardiolipin antibodies: ACA;  
Enzyme-linked immunosorbent assay: ELISA;  
Intensive care unit: ICU;  
Real-time PCR: RT-qPCR.

## Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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## Role of the funding source

None.

## Availability of data and materials

The data used in this study are available from the corresponding author on request.

## Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. All experiments were conducted in accordance with the guidelines of the local Ethics Committee of HUMS, and the study protocol was approved (IR.UMSHA.REC.1399.883). The present study did not interfere with the process of diagnosis and treatment of patients and all participants signed an informed consent form.

## Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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