

ORIGINAL ARTICLE

Antiphospholipid antibodies in COVID-19-associated pneumonia patients in intensive care unit

Samet Karahan^a, Kemal Erol^b, Recep Civan Yuksel^c, Cem Artan^d and İlhami Celik^e

^aDepartment of Internal Medicine, Division of Rheumatology, Kayseri City Education and Research Hospital, Kayseri, Turkey; ^bDepartment of Physical Medicine and Rehabilitation, Division of Rheumatology, Kayseri City Education and Research Hospital, Kayseri, Turkey; ^cIntensive Care Unit, Department of Internal Medicine, Kayseri City Education and Research Hospital, Kayseri, Turkey; ^dDepartment of Microbiology, Kayseri City Education and Research Hospital, Kayseri, Turkey; ^eDepartment of Infectious Diseases and Clinical Microbiology, Kayseri City Education and Research Hospital, Kayseri, Turkey

ABSTRACT

Objectives: Antiphospholipid antibodies (APAs) increase the risk of excessive blood clotting, but their role in COVID-19 remains unclear. We aimed to investigate the presence of conventional APAs used in the classification of antiphospholipid antibody syndrome in patients with severe lung infection with SARS-CoV-2 and to compare these results with non-COVID-19 critically ill patients

Methods: Thirty-one COVID-19 patients (COVID group) and 28 non-COVID-19 critically ill patients (non-COVID group), were included in the study. Anti-cardiolipin (ACA) (IgG, IgM), anti-β2-glycoprotein 1 (Anti-β2GPI) (IgG, IgM, and IgA), and if the patient had not received any anti thrombotic agent before blood collection, lupus anticoagulant (LAC) tests were studied from the plasma of the patients. For testing ACA and Anti-β2GPI, ELISA method was used, while fully automated coagulometer device was used for LAC test.

Results: APAs were positive in 25.81% in the COVID group (8/31) and 25% in the non-COVID group (7/28). LAC was the most common APA present in 23.08% of the COVID-19 group, who underwent measurement (6/26), while 3.57% of the non-COVID group was LAC positive (1/28) ($p = .047$). In the COVID group, ACA IgM, and IgG were positive in 6.45% and 0%, respectively (2/31 vs 0/31). In the non-COVID group, ACA IgM was not positive in any patient, while ACA IgG was positive in 7.14% (2/28). Anti-β2GPI IgG and IgM tests were not positive in any patient in either the COVID or the non-COVID group. Anti-β2GPI IgA were positive in 6.45% and 14.29%, respectively (2/31 vs 4/28).

Conclusion: In this study, APAs were equally positive in critically ill patients among COVID-19 or non-COVID-19 patients. Only LAC was more observed in COVID-19 patients.

KEY MESSAGES

- In this study, APAs were equally positive in critically ill patients among COVID-19 or non-COVID-19 related conditions. Only LAC was more observed in COVID-19 patients. ‘COVID-19-induced-APS-like-syndrome’ is a new term and it is important in long term follow-up of COVID-19 patients with positive APAs.

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Introduction

The virus named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which appeared for the first time in Wuhan, the capital of the Hubei region of China on December 1, 2019, spread to the entire world in a very short time and was named as a pandemic by the World Health Organization [1]. Although the disease is asymptomatic in most people, it is seen that intensive care and intubation is required because of pneumonia, especially in elderly patients (>65 years old) [2]. New studies show that severe Coronavirus Disease 2019 (COVID-19) may be complicated by coagulopathy and disseminated intravascular coagulation [3]. In addition, it was reported that pulmonary thromboembolism might be seen as a part of lung involvement in

patients at the time of initial diagnosis. The reason this embolization has been reported is that SARS-CoV-2 infection may be complicated by coagulopathy. One of the most important causes of lung damage in COVID-19 is thought to be pneumonic infiltrations that may be caused by micro-embolisms [3]. For these reasons, some experts from China decided to initiate the treatment of unfractionated heparin or low molecular weight heparin (LMWH) as soon as the disease is suspected or diagnosed [4].

Anti-phospholipid antibodies (APAs) are autoantibodies developed by mistake against phospholipids, after autoimmune processes and can be used in the diagnosis of antiphospholipid antibody syndrome (APS). These autoantibodies, in a way that is not well understood, increase the risk of excessive blood clotting and thrombocytopenia.

APAs have been well reported in most autoimmune diseases, especially systemic lupus erythematosus (SLE), and in the course of various viral diseases. The formation of APAs, which can predispose patients to thromboembolism, is observed in APS and these antibodies can be temporary or persist for a long time [5].

In this study, we aimed to investigate the presence of conventional APAs in patients with SARS-CoV-2 lung infection and to compare the results with critically ill non-COVID-19 patients. Our results indicated that APAs were equally positive in critically ill patients among COVID-19 or non-COVID-19 patients, with LAC positivity being the most prevalent among COVID-19 patients.

Material and methods

Patients and method

From April to June 2020, a series of 33 COVID-19 pneumonia patients (18 men, 15 women) referred to the internal medicine intensive care unit of a tertiary hospital (Kayseri City Training and Research Hospital), were consecutively studied (COVID Group). The diagnosis of pneumonia was made by low dose computed tomography (CT) and categorized according to expert consensus statement on reporting chest CT findings related to COVID-19 by the Radiological Society of North America [6].

In these COVID patients, ACA IgG, ACA IgM, Anti- β 2GPI IgG, Anti- β 2GPI IgM and Anti- β 2GPI IgA, and if the patient did not receive any anti-thrombotic agent before blood collection, LAC tests were performed. In addition, the same tests were done in patients hospitalized with diseases other than COVID-19 in other internal disease intensive care units of the same hospital (non-COVID Group, $n = 31$ patients, 12 men 19 women). These non-COVID-19 patients are consecutively selected. Ten of these non-COVID patients were urosepsis, seven were upper gastrointestinal bleeding, seven were pneumonia, three were hyperosmolar coma/ketoacidosis, two were cholangitis, one was methyl alcohol intoxication and one was cellulitis. Autoimmune diseases such as rheumatoid arthritis, SLE, Sjögren's disease, dermatomyositis, mixed connective tissue disease, and undifferentiated connective tissue diseases, in which APAs tests are likely to be positive, were excluded in both groups. To further eliminate the possibility of autoimmunity in our subjects, antinuclear antibodies (ANA), rheumatoid factor (RF), and anti-cyclic citrullinated peptide were determined in both groups and positive patients were excluded from the study. Patients in both groups who had human immunodeficiency virus, hepatitis B and C virus, Epstein-Barr virus, cytomegalovirus infections, primary antiphospholipid antibody syndrome, vasculitis, and who were taking any drug that induces APA formation, such as anti-epileptic medicine or oral contraceptive pills were excluded. Other exclusion criteria in both groups were malignancies (lymphoproliferative or solid organ tumors) and chronic infective/inflammatory pathologies that may cause systemic inflammation (such as tuberculosis, sarcoidosis, brucellosis, histoplasmosis, leptospirosis, etc.). Two female patients in the COVID-19

group had ANA positivity, while one male patient in the non-COVID-19 group had RF positivity and one female patient had ANA positivity and one male patient had colonic cancer, and these patients were excluded from the study for these reasons. Therefore, the study was continued with 31 patients (18 men, 13 women) in the COVID-19 group and 28 (10 men 18 women) patients in the non-COVID group.

Detection of SARS-CoV-2 virus isolates from patients' nasopharyngeal aspirate/lavage and rapid diagnosis with the Bio-Speedy[®] SARS-CoV-2 Kit is achieved via one-step reverse transcription and real-time PCR (RT-qPCR) targeting SARS-CoV-2 specific RNA-dependent RNA polymerase gene fragment.

For ACA detection Orgentec[®] Diagnostika ACA ELISA test kits were used in a Seac-Radim Company ALISEI Q.S.[®] (Next Level Strumenti Diagnostici, Calenzano, Italy) fully automatic ELISA Analyzer. For ACA detection patient's serum was used. Anti- β 2GPI antibodies were determined with the Triturus[®] Analyzer (Diagnostics Grifols, S.A. Barcelona, Spain) device using the ELISA method. Measurement was done in singlet. Our local cut off value for ACA test was set as validated manufacturer's cut-offs. The LAC test was performed with a STA R MAX[®] (Stago) fully automatic coagulometer device which has an integrated system including screening and confirmation step. APTT-based reagent is used for antiphospholipid-dependent coagulation technique of LAC test. Confirmation tests were performed in aPTT with hexagonal phase phospholipids (Staclot[®] LA, Stago) and in dRVVT with a phospholipid-rich dRVVT reagent (STA-Staclot[®] DRVV confirm, Stago). Our local cut off value for LAC test was set as >99th percentile of distribution.

The cut-off value for ACA IgG and ACA IgM were 10 Units/ml and 7 Units/ml, respectively. For anti- β 2GPI IgG, anti- β 2GPI IgM, and anti- β 2GPI IgA the cut-off value was 12 Units/ml and for LAC, it was 1.20. APA antibodies were retested from patients who survived after the patients' diseases were treated.

Doppler ultrasound imaging was used for the evaluation of lower and upper extremity deep vein thrombosis. Contrast-enhanced computed tomography was used for pulmonary thromboembolism scans. In addition, brain magnetic resonance imaging was performed to diagnose stroke in patients with neurological deficits.

All patients or their first-degree relatives gave written informed consent and the study was approved by the local Ethics Committee in Kayseri City Training and Research Hospital (Date: 14.5.2020 – Number: 76397871/69).

Statistical analysis

For frequencies, percentage rates and total percentage rates were used. Demographic tables were created for these rates. The data grouping method was chosen to capture agglomerations in certain variables. Shapiro-Wilk test was used to check the normal distribution of data. Intergroup prevalence rates were compared by the Chi-square test or Fisher's exact

test. Comparison tables were made for variables containing significance. To compare two groups of non-normally distributed continuous data, the Mann-Whitney *U* test was used and for normally distributed continuous data, the *student t* test was used. All *p* values were two-tailed. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 25.0 (IBM, Armonk, NY, USA). Probability values $<.05$ were considered significant.

Results

There were 31 patients in the COVID-19 group, of which 13 (41.9%) were women. Of the 28 patients in the non-COVID-19 group, 16 (57.1%) were female (COVID-19 group vs non-COVID-19 group, $p = .243$). Demographic and clinical data between the two groups are summarized in Table 1. No differences were noted between the two groups with regard to demographics and clinical data.

In the COVID-19 group, ACA IgM and IgG were positive in 2 of 31 patients (6.45%) and none of 31 patients, respectively. On the other hand, in the non-COVID-19 group, ACA IgM was not positive in any patient, while ACA IgG was positive in 2 (7.14%) patients ($p = .493$ and $.221$, respectively). Anti- β 2GPI IgG and anti- β 2GPI IgM tests were not positive in any patient in either in the COVID-19 group or the non-COVID-19 group. In the case of anti- β 2GPI IgA, the test was positive in 2 of 31 patients (6.45%) in the COVID-19 group, while it was positive in 4 of 28 patients (14.29%) in the non-COVID-19 group ($p = .409$). Low molecular weight heparin was started in 4 patients in the COVID group before being taken to the Intensive Care Unit. In addition, one patient was taking a vitamin K antagonist because of cardiac valve pathology. For these reasons, LAC could not be tested in 5 patients in the COVID-19 group. In the COVID-19 group, the LAC test was positive in 6 of 26 patients (23.08%), who underwent measurement. In the non-COVID-19 Group, none of whom received anti-thrombotic therapy, 1 patient (3.57%) had positivity for the LAC test ($p = .047$). Considering all the conventional tests, APAs were positive in 9 (29.03%) patients in the COVID-19 group and 7 (25%) patients in the non-COVID-19 group ($p = .728$) (Table 2). In the COVID-19 group, in one female patient, both the LAC and anti β 2GPI IgA tests were positive and she was the only patient evaluated as 'double positive'. There were no 'triple positive' patients. APA titers of positive patients in both groups are shown in Table 3.

Thrombotic processes in 28 days after hospital admission in the COVID-19 group were deep venous thrombosis without pulmonary embolism in one case, myocardial infarction in two, ischemic stroke in one case. The LAC test was positive in one patient with myocardial ischemia and in one patient with ischemic stroke. Two other COVID-19 patients with thromboembolic events were not positive for APAs. On the other hand, two thromboembolic events developed in the non-COVID-19 group, and these were an ischemic stroke and a case of pulmonary embolism. However, both

Table 1. Demographic and clinical data between COVID and non-COVID groups.

	COVID (n = 31)	Non-COVID (n = 28)	<i>p</i> Value
Age, years, mean \pm SD	56.77 \pm 17.17	60.64 \pm 18.18	.723
Age \geq 65 years, no (%)	12 (38.70)	15 (53.57)	.253
Female, no. (%)	13 (41.93)	16 (57.14)	.243
BMI, kg/m ² , mean \pm SEM	26.56 \pm 6.11	27.62 \pm 5.36	.484
Smoking, no. (%)	18 (58.06)	12 (42.85)	.243
ICU days, mean \pm SEM	10.36 \pm 10.0	9.87 \pm 7.35	.744
Intubation, no. (%)	9 (29.03)	7 (25)	.728
Intubation, days, mean \pm SEM	8.44 \pm 8.77	15.57 \pm 10.03	.152
Hemodialysis, no. (%)	5 (16.12)	4 (14.28)	.844
Thromboembolic event, no. (%)	3 (9.68)	2 (7.14)	.727
Cardiopulmonary arrest, no (%)	8 (25.81)	5 (17.86)	.462
28 day mortality, no. (%)	7 (22.58)	8 (28.57)	.598
Co-morbidities, no. (%)			
DM	8 (25.81)	12 (42.86)	.167
HT	15 (48.39)	11 (39.29)	.482
ASHD	5 (16.13)	5 (17.86)	.860
Asthma	1 (3.22)	2 (7.14)	.599
COPD	11 (35.48)	6 (21.43)	.234
CKD	5 (16.13)	9 (32.14)	.149
CHF	1 (3.22)	3 (10.71)	.337

COVID: coronavirus disease; BMI: body mass index; ICU: intensive care unit; DM: diabetes mellitus; HT: hypertension; ASHD: atherosclerotic heart disease; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; CHF: congestive heart failure.

Table 2. APAs in the COVID and non-COVID Groups.

	COVID	NON-COVID	<i>p</i> Value
ACA IgM, no., %	2/31(6.45)	0/28	.493
ACA IgG, no., %	0/31	2/28 (7.14)	.221
Anti- β 2GP IgM, no., %	0/31	0/28	Null
Anti- β 2GP IgG, no., %	0/31	0/28	Null
Anti- β 2GP IgA, no., %	2/31 (6.45)	4/28 (14.29)	.409
LAC, no., %	6/26 (23.08)	1/28 (3.57)	.047
All APAs, no., %	9/31 (29.03)	7/28 (25)	.728

APAs: antiphospholipid antibodies; COVID: coronavirus disease; ACA: anti-cardiolipin; Anti- β 2GP: anti-beta-2 glycoprotein 1; LAC: lupus anticoagulant.

patients were negative for APAs. In summary, there were 4 of 31 (12.90%) patients in the COVID-19 group and 2 of 28 (7.14%) patients in the non-COVID-19 group, who experienced thromboembolic events ($p = .673$). Thromboembolic events during 28 days of follow-up were detected in 2 (12.5%) of 16 patients, who were positive for APAs, while 4 (9.1%) of 44 patients with negative APAs were encountered ($p = .697$) (Table 4). Considering 28-day mortality, 3 out of 16 (18.8%) APAs positive patients died. On the other hand, mortality rate in APAs negatives was 8 out of 44 (18.18%) ($p = .960$).

After recovery of COVID-19 and other diseases requiring intensive care follow-up, APA tests were performed again. However, one of the 9 APAs positive patients in the COVID-19 group and 2 of the 7 APAs positive patients in the non-COVID group died within 28 days ($p = .375$). After 28 days one of the APAs positive patients in the COVID-19 group and one of the APAs positive patients in the non-COVID group also died. Among the retests taken from the remaining 11 APAs positive patients, only one patient in the non-COVID group had a positive β 2GPI IgA test. Patient 3 in the non-COVID group indicated in Table 3, had a tested β 2GPI IgA titer of 24.2 U/ml after 12 weeks and was found to be significantly lower than baseline.

Table 3. APA Titers and its location if there is thrombosis in both groups.

	Sex	Age (year)	APA Type	Titer	Thrombosis (if any)
COVID Group, n = 31					
Patient 1	Male	71	ACA IgM, U/ml	14.02	None
Patient 2	Male	62	ACA IgM, U/ml	12.60	None
Patient 3	Female	65	β 2GPI IgA, U/ml	12.82	None
Patient 4	Female	81	β 2GPI IgA, U/ml	>300	None
			LAC	1.62	
Patient 5	Male	67	LAC	1.50	Myocardial infarction
Patient 6	Male	43	LAC	1.40	None
Patient 7	Male	78	LAC	1.55	None
Patient 8	Female	67	LAC	1.32	None
Pateint 9	Male	72	LAC	1.58	Stroke
Non-COVID Group, n = 28					
Patient 1	Male	72	ACA IgG, U/ml	16.82	None
Patient 2	Female	87	ACA IgG, U/ml	17.36	None
Patient 3	Female	72	β 2GPI IgA, U/ml	116.02	None
Patient 4	Female	58	β 2GPI IgA, U/ml	25.70	None
Patient 5	Female	66	β 2GPI IgA, U/ml	22.30	None
Patient 6	Male	69	β 2GPI IgA, U/ml	71.10	None
Patient 7	Male	82	LAC	1.40	None

APA: antiphospholipid antibodies; COVID: coronavirus disease; ACA: anti-cardiolipin; Anti- β 2GPI: anti-beta-2 glycoprotein 1; LAC: lupus anticoagulant.

Table 4. Thromboembolism cases of patients in APAs positive COVID and non-COVID groups.

	COVID Group		Non-COVID Group	
	APAs Positive	APAs Negative	APAs Positive	APAs Negative
Thromb. Positive	2	2	0	2
Thromb. Negative	7	20	7	19

APAs: antiphospholipid antibodies; COVID: coronavirus disease; Thromb.: thromboembolism.

Discussion

The main finding of this study is that, while presence of conventional APAs was similar between the COVID-19 and non-COVID-19 group, positivity of LAC test was higher in the COVID-19 group than in the non-COVID-19 group. Additionally, it was not associated with thrombosis.

The complex relationship of COVID-19 pneumonia and thrombosis and abnormal coagulation was noticed firstly by Tang et al., when they retrospectively analyzed 183 patients with COVID-19 pneumonia. Those non-survivor patients had significantly higher D-dimer, fibrin degradation product levels, longer prothrombin time (PT), and activated partial thromboplastin time (aPTT) compared to survivors on admission [7]. Then Klok et al. [8] reported a high rate of arterial or venous thrombosis, which was predicted by prolongation of the PT or aPTT, in almost one third of ICU patients with COVID-19. At nearly the same time, Helms et al. reported that there were more thrombotic complications in COVID-19 adult respiratory distress syndrome (ARDS) patients than in patients with non-COVID-19 ARDS. Although PT, antithrombin, and fibrinogen were higher in COVID-19 ARDS patients than in non-COVID-19 ARDS patients, aPTT and D-dimer were lower in COVID-19 patients [9]. A report by Zhang et al. was one of the first studies that brought to mind the question of whether APAs play a role in the pathogenesis of thrombosis susceptibility in COVID-19 patients. In this report, three cases of COVID-19 patients, with arterial thrombosis and positive

test results for ACA IgA, Anti- β 2GPI IgA, and IgG, but negative LAC test results were reported [10]. The titer levels of positive antibodies and total number of ICU patients with COVID-19 were unmentioned [10]. They drew attention to thrombosis in ICU patients with COVID-19 may be related to APAs. Then Harzallah et al. [11] studied the LAC test in a total of 56 patients with COVID-19. In those 25 cases (45%) were LAC positive, whereas ACA or Anti- β 2GPI were positive in only five of 50 tested patients (10%) and only three of them were positive with LAC together. In this study, there was no information about the clinical severity of patients and no control group was included. Then, Bowles et al. [12] studied aPTT in 216 patients with COVID-19 and one fifth of the patients had prolonged aPTT. Most of those patients were positive for LAC. There was also no control group in this study. Recently, Hossri et al. [13] reported two COVID-19 cases with arterial thrombosis, both were ACA IgM and IgG positive and Anti- β 2GPI negative. These studies brought up the relationship between COVID-19 and APS.

APS, associated with the presence of APAs, is an autoimmune thrombotic condition characterized by arterial and/or venous thrombosis and/or obstetric complications [14]. APAs, sometimes detected during infections, are a heterogeneous group of antibodies and only some of them may be detected by laboratory tests. There is a lack of standardization and harmonization of these tests. APAs, of which Anti- β 2GPI IgM and IgG and ACA IgM and IgG were included in revised classification criteria for APS [15]. The LAC test was also included in this criterion. LAC test positivity has a stronger relationship with thrombosis than positivity of anti- β 2GPI or ACA tests. Chambrun et al. [16] studied conventional APAs in 25 ICU patients with COVID-19 and only one did not have APAs, while the others had at least one APA. The majority of patients (92%) had LAC positivity, which may associated with patients' usage of non-fractionated heparin. ACA and anti- β 2GPI antibody positivity rate were 52% and 12%, respectively. That is a relatively rational but still high rate of positivity of APAs in ICU

patients with COVID-19. Additionally, only six of them were associated with venous thrombosis. The major limitation of the study is that no control group was included [16]. Devreese et al. studied the presence of APAs in critically ill patients with COVID-19 to investigate the incidence of APAs and its relation with thrombosis. They found frequent rate of single LAC positivity (67.7%) in ICU patients with COVID-19. However, it was not related clearly to thrombosis. Triple APAs positivity and high ACA/Anti- β 2GPI titers were rare in their study, as in the present study [17]. Borghi et al. studied conventional APAs other than LAC in 122 severe COVID-19 patients. The highest rate of APAs, Anti- β 2GPI IgG, was 15.6% and presence of any APAs was not associated with thrombosis in their study. They also found APAs against β 2GPI in patients with COVID-19 were not targeted in the same domains of β 2GPI in classical APS [18]. Xiao et al. categorized COVID-19 patients as critical and noncritical ill patients, when investigating conventional APAs other than LAC. They detected at least one APAs positivity rate of 47.0% in critically ill patients, but no APAs in noncritical patients. They also found an association between multiple positivity of APAs and cerebral infarcts [19]. In the present study, where we assessed ICU patients with COVID-19 in terms of conventional APAs, only two patients with COVID-19 had positive results for ACA IgM and anti- β 2GPI IgA and it was similar with non-COVID-19 ICU patients. Therefore, this positivity was not associated with thrombosis in COVID-19 patients, as in most other studies. Six patients with COVID-19 had positive results for LAC and it was higher in the COVID group than in the non-COVID group. Two of the LAC positive COVID-19 patients had arterial thrombotic events in 28 followed days. This result brings to mind that potentially clinically important antibodies against non- β 2GPI dependent cofactor/phospholipid complexes such as anti-phosphatidylserine and anti-phosphatidylserine/prothrombin complex may be the reason of thrombosis in our LAC positive patients with COVID-19.

Typically, LAC assays can detect all antiphospholipid antibodies regardless of their isotypes and anti- β 2GPI and ACA are reported positive only when present in medium or high titers. However, in the non-COVID-19 group of our study, only 3% from the 25% APA positive patients were also LAC positive. We think, antiphospholipids other than anti- β 2GPI and ACA, such as antibodies against phosphatidylserine, phosphatidyl ethanolamine, annexin II, annexin A5, and vimentin/cardiolipin complexes may cause this positivity. Also, in the diagnosis of APS, The clinical significance of ACA and anti- β 2GPI antibodies of the IgA isotype in APS is still a debatable issue and they are recommended in cases where IgG and IgM are negative. IgA anti- β 2GPI seems to be the most prevalent isotype in patients with SLE, with a significant association with thrombotic events. There has been a growing interest against IgA anti- β 2GPI domain 4/5 as a novel subgroup of clinically relevant APA [20]. For this reason, we deemed it appropriate to measure IgA anti- β 2GPI in our study, and as a result, none of the patients in both groups were found to be IgG and IgM anti- β 2GPI

positive, but although it is not statistically significant, only IgA isotype was found as positive in two of COVID group and four of non-COVID group.

The strengths of the present study were, a non-COVID-19 ICU patient group included in the study and conventional APAs were studied. In addition, of the patients, who underwent LAC testing, those who used unfractionated or low molecular weight heparin or vitamin K antagonists were excluded in this study. The most important limitations of our study were that it was studied in a small population and was a monocentric study. Another limitation of our study is that, although it is tried to be prevented by using mixing test and dRVVT reagent, tests may be interfered due to elevated CRP and other inflammatory markers and clotting factor inhibitors etc. On the other hand, detecting of APAs during acute inflammatory and thrombotic state and not repeating the relevant tests of the patients after 12 weeks may be other limitations.

Conclusion

In conclusion, clinical significance of APAs in patients with COVID-19 pneumonia remains to be determined. Additionally, it is not yet clear how long these APAs persist and their contribution to thrombotic events in patients. Because the presence of classical solid phase APAs are similar in ICU patients with and without COVID-19 and positivity of the LAC test is more common in ICU patients with COVID-19 than without COVID-19, antibodies against non- β 2GP dependent cofactor/phospholipid complexes may be associated with thrombosis in ICU patients with COVID-19.

Author contributions

Samet Karahan: concepts, design, definition of intellectual content, literature search, data acquisition, manuscript preparation, manuscript editing; Kemal Erol: definition of intellectual content, manuscript editing, manuscript review; Recep Civan Yuksel: patient treatment and follow-up; Cem Artan: laboratory evaluations; İlhami Celik: patient treatment and follow-up, laboratory evaluations, manuscript editing, manuscript review.

Conflict of interest

None.

References

1. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (London, England). 2020;395:507–13.
2. Han X, Cao Y, Jiang N, et al. Novel coronavirus pneumonia (COVID-19) progression course in 17 discharged patients: comparison of clinical and thin-section CT features during recovery. *Clin Infect Dis*. 2020;71:723–31.
3. Yin S, Huang M, Li D, Tang N. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. *J Thromb Thrombolysis*. 2020.

4. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost.* 2020;18:1094–9.
5. Martirosyan A, Aminov R, Manukyan G. Environmental triggers of autoreactive responses: induction of antiphospholipid antibody formation. *Front Immunol.* 2019;10:1609.
6. Simpson S, Kay FU, Abbara S, et al. Radiological Society of North America Expert Consensus Statement on Reporting Chest CT Findings Related to COVID-19. Endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA. *J Thorac Imaging.* 2020;2:2.
7. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost.* 2020;18:844–7.
8. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* 2020;191:145–7.
9. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al., CRICS TRIGGERSEP Group (Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* 2020;46:1089–98.
10. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, Jiang W, et al. Coagulopathy and antiphospholipid antibodies in patients with Covid-19. *N Engl J Med.* 2020;382:e38.
11. Harzallah I, Debliguis A, Drénou B. Frequency of lupus anticoagulant in Covid-19 patients. *J Thromb Haemost.* 2020;18:2778.
12. Bowles L, Platten S, Yartey N, Dave M, Lee K, Hart DP, et al. Lupus anticoagulant and abnormal coagulation tests in patients with Covid-19. *N Engl J Med.* 2020;383:288–290.
13. Hossri S, Shadi M, Hamarsha Z, Schneider R, El-Sayegh D. Clinically significant anticardiolipin antibodies associated with COVID-19. *J Crit Care.* 2020;59:32–4.
14. Favaloro EJ, Wong RCW. Antiphospholipid antibody testing for the antiphospholipid syndrome: a comprehensive practical review including a synopsis of challenges and recent guidelines. *Pathology.* 2014;46:481–95.
15. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost.* 2006;4:295–306.
16. Pineton de Chambrun M, Frere C, Miyara M, et al. High frequency of antiphospholipid antibodies in critically-ill COVID-19 patients: a link with hypercoagulability? *J Intern Med.* 2020.
17. Devreese KMJ, Linskens EA, Benoit D, Peperstraete H. Antiphospholipid antibodies in patients with COVID-19: a relevant observation? *J Thromb Haemost.* 2020;18:2191–2201.
18. Borghi MO, Beltagy A, Garrafa E, et al. Prevalence, specificity, and clinical association of anti-phospholipid antibodies in COVID-19 patients: are the antibodies really guilty? *medRxiv.* 2020.
19. Xiao M, Zhang Y, Zhang S, et al. Brief Report: Anti-phospholipid antibodies in critically ill patients with Coronavirus Disease 2019 (COVID-19). *Arthritis Rheumatol.* 2020.
20. Andreoli L, Fredi M, Nalli C, Piantoni S, Reggia R, Dall'Ara F, et al. Clinical significance of IgA anti-cardiolipin and IgA anti- β 2glycoprotein I antibodies. *Curr Rheumatol Rep.* 2013;15:343.