Chapter

Antiphospholipid Antibodies in Patients with COVID-19

Cristiana Isabel Sieiro Santos

Abstract

Patients infected with COVID-19 are at higher risk of thrombosis, suggesting an important role of COVID-19 induced coagulopathy. Abnormal coagulation parameters such as elevation in D-dimer are found in patients, with frequent thrombotic events ranging from peripheral ischemia, pulmonary thromboembolism to disseminated intravascular coagulation. Recently, the role of antiphospholipid antibodies (aPL) in the pathophysiology of COVID-19 have been questioned but it remains unclear whether they contribute to coagulopathy. We aim to evaluate the presence of aPL, including LAC, aCL (IgG, IgM), aβ2GPI (IgG, IgM) in a cohort of patients with SARS-CoV-2, study clinical associations and discuss the relevance. The relevance of aPLs in patients with COVID-19 is yet to be determined. Inflammation is closely associated to thrombosis and the presence of inflammatory mediators in COVID-19 infection can lead to thrombosis. Further studies are needed before to determine the role of aPL in COVID-19 patients and their relationship with thrombosis. The presence of aPL should be carefully interpreted as it is important to evaluate the persistence of aPL positivity in patients infected with COVID-19.

Keywords: antiphospholipid antibodies, covid-19, thrombotic, cardiolipin, lupus anticoagulant

1. Introduction

COVID-19 has swept through the world in the last 6 months with 28,584,158 confirmed cases, including 916,955 deaths, being reported to the World Health Organization (WHO), on September 13th 2020 [1].

COVID-19 is caused by SARS-CoV2-virus, a member of coronaviridae that includes MERS-CoV2 which is responsible for severe respiratory illness and causes acute respiratory distress syndrome [2]. Many patients infected with COVID-19 develop a hyperinflammatory response due to cytokine storm syndrome which associates with high mortality [3]. Recent studies demonstrated an association between COVID-19 severity and inflammatory biomarkers such as C-reactive protein, procalcitonin, IL-6 and ferritin. In addition, a high incidence of thrombotic events suggests an important role of COVID-19-induced coagulopathy, despite the use of prophylactic doses of low molecular weight heparin [4]. Advanced age and comorbidities are predictors of increased mortality in COVID-19, which may facilitate thrombosis in these individuals [5].

Several hemostasis laboratory parameters are altered in patients with COVID-19 which constitutes an argument in favor of coagulopathy [6–10]. Abnormal

coagulation parameters such as prolonged aPTT coagulation times, D-dimer elevation and fibrinogen degradation products correlate with COVID-19 severity and are risk factors for higher mortality [10–12].

There are increasing cases of thrombotic events ranging from venous thromboembolic disease, pulmonary thromboembolism to disseminated intravascular coagulation, as well as reports of arterial thrombosis including strokes and myocardial infarctions [13–15]. The activation of leukocytes, endothelium and plaquettes due to cytokine storm, as well as hypoxic vaso-occlusion and direct activation of cells by viral transduction are several mechanisms by which COVID-19 infection may lead to thrombosis [16].

Recently studies have been published about the role of antiphospholipid antibodies (aPL) in SARSCoV-2 patients, leading investigators to start measuring aPL in these patients because of the hypercoagulable state [17–21].

Antiphospholipid syndrome is an autoimmune condition that leads to autoantibodies creation. These autoantibodies react against phospholipids and phospholipid-binding proteins such as beta-2-glycoprotein I (β2GPI) and activate endothelial cells, platelets, and neutrophils, leading to thrombosis [22, 23]. The ability to promote thrombosis in arterial and venous circuits is a defining characteristic of antiphospholipid syndrome. The catastrophic variant of antiphospholipid syndrome is sometimes fatal and resembles to diffuse coagulopathy seen in patients with COVID-19 [24]. Classification criteria for APS includes lupus anticoagulant (LAC), anticardiolipin (aCL) and antibeta2-glycoprotein I antibodies (aβ2GPI) IgG or IgM, if persistently present [25].

The theory involving aPL in COVID-19 infected patients is intriguing however, most studies published so far only include one point of measurement, usually during the acute phase, without confirmation after at least three months, as defined in laboratory criteria of antiphospholipid syndrome [26].

Lupus anticoagulant (LA) is a well-known case of aPTT prolongation, which can be detected in a significant percentage of COVID-19 infected patients. Several biological causes such as high fibrinogen, factor VIII levels and biomarkers such as elevated C-reactive protein (CRP) and the presence of antiphospholipid antibodies may affect the aPTT [27].

We should also keep in mind that aPL can arise transiently in patients with critical illness and various infections [28]. The presence of these antibodies can lead to thrombotic events, making it difficult to differentiate from other types of thrombosis. Viral infections, such as VIH, hepatitis C and parvovirus B19 are triggers of transient aPL, due to a mechanism of molecular mimicry mechanism [29].

To investigate the role of aPL in COVID-19, it is important to evaluate all criteria of aPL, including LAC, aCL and aβ2GPI antibodies with their isotype and to obtain confirmation after at least 3 months, in concordance with the laboratory criteria of APS.

In this report, we illustrate the presence of aPL, including LAC, aCL (IgG, IgM) and aβ2GPI (IgG, IgM) in a cohort of patients with SARS-CoV-2 and discuss the relevance.

2. Materials and methods

We performed a single-centre, cross-sectional study between March 15th and September 15th 2020. The data from all patients who had tested positive for COVID-19 and had presented with thromboembolic complications were collected in a prospectively maintained database and compared to the data from patients without thromboembolic complications. Inclusion criteria: (a) aged >18 years old; (b) no

previous diagnosis of APS (c) positive for COVID-19 (d) patients that required hospitalization. Registry data included the following co-variables: (1) sociodemographic baseline characteristics, such as sex and age; (2) baseline comorbidities including hypertension, dyslipidaemia, hyperuricemia, diabetes mellitus, heart disease, lung disease; (3) need for anticoagulation prior to hospitalization (4) thromboembolic complications during admission (5) mean time of hospitalization (days) (6) laboratorial work (7) aPL positity and isotype determination during hospitalization and confirmed after a 3 month period.

2.1 COVID-19 detection

The diagnosis of SARS-CoV-2 infection was confirmed in all the patients by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay or serologic testing.

2.2 Analytical parameters

Screening blood tests included hemogram, D-dimer, C-reactive protein (CRP), procalcitonin, transaminases (serum alanine aminotransferase, serum aspartate aminotransferase), serum lactate dehydrogenase, ferritin, fibrinogen, coagulation times (PTT and aPTT).

2.3 Thrombosis diagnosis

Computerized tomography was performed to identify COVID-19-thromboembolic related complications. Duplex ultrasound was systematically performed to diagnose proximal and distal lower extremity deep vein thrombosis. Thoracic computer-tomography/angiography was performed if pulmonary embolism was suspected and brain computer-tomography in the case of stroke.

All patients received prophylactic or therapeutic dose low molecular weight heparin (LMWH) (enoxaparin) or unfractionated heparin (UFH), accordingly to thrombotic risk evaluated at admission.

2.4 Detection of aPL

Determination Serum aCL and aβ2GP1 (IgG, IgM) were determined by the chemiluminescence assay (CIA) (INOVA) and IgG/IgM aPS/PT were determined by ELISA (INOVA).

The detection of LA in human citrated plasma was performed by the HemosIL dRVVT Screen and HemosIL dRVVT confirm assays, as recommended by the International Society on Thrombosis and Hemostasis (ISTH).

aPL positivity was determined at admission and confirmed after 3 months.

2.5 Statistical analysis

Mann–Whitney U test, χ^2 test, or Fisher's exact test were utilized to compare differences between group A (with thromboembolic complications) and group B (without thromboembolic complications). A two-sided α of less than 0.05 was considered statistically significant. Statistical analyses were performed on SPSS 20.0 package (SPSS Inc.).

Patient dRVVT screen (low phospholipid concentration) and confirm (high phospholipid concentration) results were normalized. Cut-off value was 1.20 for both screen ratio and screen ratio/confirm ratio, demonstrating the

phospholipo-dependence; Anticardiolipin IgG/IgM and/or anti-β2-glycoprotein-I IgG antibodies was defined as elevated if the titer was >20 CU (99th percentile).

3. Results

Median age in the patient population was 63.2 (range 36-83) years, with a female predominance (66%). The median stay at the hospital was 9 (range 2-19) days. Sociodemographic baseline characteristics, medical history, and comorbidities, as well as thrombotic complications are shown in **Table 1**.

On admission, they received prophylactic (45%) or therapeutic (55%) dose low molecular heparin. None had received any anticoagulant drug nor had thromboembolic events prior to admission. Thrombotic events were reported in 18 patients and included 7 pulmonary embolisms, 1 aortic thrombosis, 1 brachial vein thrombosis, 1 stroke, 1 heart attack, and 7 deep vein thrombosis.

Patients with thrombosis were older (64.8 (IQR 36-83) vs. 60.4 (43-79), p 0.04), more likely to have diabetes (9(50%) vs. 2 (12.5%), p 0.03), dyslipidaemia (12 (66%) vs. 5 (36%), p 0.04) and higher levels of D-dimer (3797 (IQR 671-6407) vs. 480 (362-944), p 0.03), serum lactate dehydrogenase (451.3 (IQR 286.3-637.5) vs. 277.7 (205.8-317.5), p 0.02) and a higher mean hospitalization time (9 (6-11) vs. 5.7 (2.5-9.5), p 0.04).

Overall, 6 out of 18 patients with thrombotic complications were negative for all criteria aPL (LAC, aCL and aβ2GPI IgG and IgM), 10 patients had at least one aPL positive, 8 of which were LAC positive. 2 out of 8 positive LAC patients were positive but that this is not a false positive result since CRP was elevated up to 20-40 mg/L and routine aPTT was more prolonged then expected according to the CRP level and aCL IgG was positive. Two patients with negative for LAC were single positive for aCL IgG. 2 patients out of the 16 patients without thrombotic complications was positive for LAC, and aCL and 1 was positive for LAC alone.

Repeat positive aPL results three months after the first occasion could be performed in all patients except for 3, since two patients died from thrombotic complications associated with COVID-19 and the other patient could not be reached. aPL was repeated in 13 patients that were positive during the first period of testing. 6 out of 13 patients were LAC negative on the second occasion. Out of the 10 with positive aPL and thrombotic complications during the first period of testing, 1 was triple positive for LAC, aCL and aβ2GPI and another was positive for LAC and aCL. As for patients without thrombotic complications, one patient was positive for LAC and aCL and another was positive for LAC alone.

The group with thrombotic complications had a higher aTPP time than the group without thrombotic complications however, the difference between the two was not significative. Patients with positive LAC had a more prolonged aTPP time than those with negative LAC. 5 out of 8 patients with thrombotic complications that had positive LAC had a mean aTPP of 46.2 s. Out of the 5, 3 had positive LAC after 3 months.

When exploring the effect of hyperinflammation and thrombosis, we found that C-reactive protein (CRP) levels significantly associated with D-dimer levels (p 0.004) and aPL elevation (p 0.02) in the group with thrombotic events during the first testing period however, no association was found between CPR and aPL (p 0.5) and D-dimer elevation (0.8) during the second testing period.

We found no differences between both groups in aPL positivity, aTTP and TP times (s), fibrinogen, lymphocytes, C-reactive protein, serum creatine, serum ferritin and procalcitonin.

Demographics and past medical history	Patients with thrombotic complications (n = 18)	Patients without thrombotic complications (n = 16)	OR (Cl 95%)	P value
Male	7 (39%)	9 (64%)	0.35 (0.08-1.50)	0.16
Age (years)	64.8 (IQR 36-83)	60.4 (43-79)	—	0.04
Smokers (%)	10 (55%)	4 (25%)	3.75 (0.89-16.2)	0.08
Hypertension (%)	9 (50%)	7 (43%)	1.28 (0.33-4.97)	0.74
Diabetes (%)	9 (50%)	2 (12.5%)	7 (1.22-40.1)	0.03
Dyslipidaemia (%)	12 (66%)	5 (36%)	4.4 (1.05-18.6)	0.04
Hyperuricemia (%)	2 (11%)	1 (0.06%)	1.85 (0.15-22.9)	0.62
Cardiovascular disease (%)	3 (17%)	2 (12.5%)	1.2 (0.17-8.4)	0.85
Pulmonary disease (%)	2 (11%)	0 (0%)	5 (0.22-112.3)	0.31
Coagulation tests Prothrombin time (s)	13.5 (12-34)	12.6 (10.8-13.4)	—	0.47
Activated partial thromboplastin time (s)	36.3 (30.2-46.7)	33.5 (31.3-36.7)	—	0.86
Fibrinogen (g/L)	536 (393-947)	556 (569-962)	—	0.31
D-dimer (ng/mL)	3797 (671-6407)	480 (362-944)	—	0.03
aPL during hospitalization Positive dRVVT	8 (44%)	3 (19%)	3.47 (0.72-16.5)	0.12
Elevated cardiolipin (IgG/IgM)	5 (27%)	2 (13%)	2.70 (0.44-16.3)	0.28
Elevated anti-β2-glycoprotein-I IgG antibodies	5 (27%)	0 (0%)	13.40 (0.68-265.5)	0.08
aPL confirmed after 3 months Positive dRVVT	3 (20%)	2 (19%)	1.08 (0.13-8.8)	0.95
Elvated cardiolipin (IgG/IgM)	2 (13%)	1 (5%)	1.87 (0.15-22.8)	0.62
Elevated anti-β2-glycoprotein-I (IgM/IgG)	1 (7%)	0 (0%)	2.82 (0.10-74.5)	0.53
Other laboratory parameters Blood leucocytes (mm3)	111,063 (6550-12,000)	7264 (6500-8650)	—	0.28
Lymphocytes (mm3)	1415 (1050-1740)	1575 (740-1710)	—	0.81
Platelets (mm3)	258,266 (201000-317,000)	254.294 (202.000-313.000)	—	0.86
Serum creatinine (μmol/L)	1.04 (0.7-1.21)	0.87 (0.715-1.03)	—	0,87
Serum alanine aminotransferase (IU/L)	33 (17.3-58.8)	38 (22.5-53.5)	—	0.60
Serum aspartate aminotransferase (U/L)	27 (18.8-101.8)	32 (21-50)	—	0.39
Serum lactate dehydrogenase (IU/L)	451 (286.3-637.5)	277.7 (205.8-317.5)	—	0.02

Demographics and past medical history	Patients with thrombotic complications (n = 18)	Patients without thrombotic complications (n = 16)	OR (CI 95%)	P value
C reactive protein (mg/L)	100 (16.5-195.9)	30.6 (1.7-85.2)	—	0.39
Procalcitonin (μg/L)	0.18 (0.047-0.23)	0.067 (0.022-0.096)	—	0.18
Serum ferritin (ng/ml)	539.4 (140-792)	559.5 (70-897.5)	—	0.60
Mean hospitalization	9 (6-11) days	5.7 (2.5-9.5) days	—	0.04
Outcome Outcome (death/ discharged), N (%)	2/18 (11%)	1/16 (6.25%)	— —	0.70

Table 1.
Data are presented as median (25th-75th percentile) or numbers (%) as appropriate. Abbreviations: COVID-19: coronavirus disease 2019. aPL: antiphospholipid antibodies. Patient dRVVT screen (low phospholipid concentration) and confirm (high phospholipid concentration) results were normalized, i.e. expressed as ratios versus reference plasma results. Results are expressed as screen ratio/ confirm ratio. Cut-off value was 1.20 for both screen ratio and screen ratio/confirm ratio; Anticardiolipin IgG/IgM and/or anti-β2-glycoprotein-I IgG antibodies was defined as elevated if the titer was >20 CU (99th percentile), a cut-off provided by the manufacturer.

To control for possible confounding variables, sequential multivariate regression analyses were performed. In the multivariate analysis, the following risk factors were associated with thrombosis: age (p = 0.02) and D-dimer (p = 0.03). Diabetes and dyslipidaemia were significant predictors in univariate but not in multivariate analysis.

4. Discussion

The incidence of arterial and venous thrombosis associated with COVID-19 and laboratorial parameters has raised questions about a possible COVID-19 related coagulopathy. aPL has been considered as one of the mechanisms leading to a proinflammatory and hypercoagulable state. Hemostatic changes observed in COVID-19 patients have been previously associated with other coronavirus, which can activate the coagulation system and lead to thrombotic events [30].

Our study evaluates the incidence of aPL in a cohort of patients with COVID-19 and compares the incidence of aPL between two groups: the first group with thrombotic complications and the second group without thrombotic complications because of SARS-CoV2.

Several studies have suggested that aPL may be associated with thrombotic complications in COVID-19 patients and discussed the relevance of measuring aPL titers.

In most studies, the aPL confirmation after 12 weeks is often missing. Measuring LAC, aCL and aβ2GPI is useful for identifying patients at risk. Current criteria recommend increased levels of IgG and IgM aCL and aβ2GPI to confirm APS. The role of IgM aPL has been discussed based on a less strong association with thrombosis compared to IgG [31].

Zhang et al. has recently described the case of three patients with multiple cerebral infarctions that tested positive for aCL IgA and aβ2GPI IgG and IgA, without referring the titer or confirmation [28].

Harzallah et al. tested 56 patients for aPL and discovered that 45% had LAC positive, 10% aCL or aβ2GPI IgG or IgM positive. Titers of aCL or aβ2GPI were not reported and no association with thrombosis was mentioned [32].

In our study, patients with thrombosis 56% showed positivity for aPL, in the patients without thrombosis 19% tested positive for at least one aPL during admission. We should also take into consideration that most patients were treated with heparins to prevent thrombotic complications.

The majority of aPL measuring studies in the break of COVID-19 lack confirmed positivity of aPL after three months. Positive results of LAC, aCL or aβ2GPI need to be confirmed after 12 week-period to confirm persistent positivity. In our study, we had the opportunity to retest most patients at a second time point. In the group with thrombotic complications, out of 8 patients who had positive LAC on a first occasion, 3 had persistent LAC after 12 weeks and 5 turned into negative. Out of the 5 patients with positive aCL during admission, 2 had persistent aCL and out of the 5 patients with positive aβ2GPI, 1 had persistent aβ2GPI after 12 weeks, with one patient being triple positive for LAC, aCL and aβ2GPI.

Transient antibodies have been described in viral diseases or drugs; therefore, re-testing is crucial to avoid overdiagnosis of APS patients that were not persistently positive [25].

Several studies have demonstrated that viral and bacterial infections, due to molecular mimicry between viral and bacterial products and β2GPI- derived amino acid sequences can induce autoantibodies such as aPL. In most cases, these infection induced-aPL are transient and can associate with thrombosis. It has been mentioned that only with the appropriate genetic background can these antibodies become pathogenic and induce thrombosis [33].

The most common laboratory abnormalities identified in patients with COVID-19 include decreased albumin and lymphocyte count and elevated C-reactive protein (CRP), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), aspartate transaminase (AST), alanine transaminase (ALT), and D-dimer [34, 35]. These abnormalities are associated with worse outcome. In our study, we have found that most patients with COVID-19 had hemostatic abnormalities, such as decreased lymphocyte count, elevated lactate dehydrogenase and elevated D-dimer and found significant differences between patients with thrombotic events and patients without thrombotic events.

Many of the laboratory abnormalities represent a balance between an acute phase reaction to the infection, including high CRP, high fibrinogen, high factor VIII, and high von Willebrand factor (VWF) and consumption of coagulation factors due to systemic or localized thrombosis and increase in D-dimer and fibrinogen [36, 37].

aPL analyses was performed during the acute phase in our study, which is mostly discouraged by current guidelines since, elevated levels of CRP may result in false positive LAC. In our cohort, we have found a significant association between CRP and aPL elevation and D-Dimer levels during the first testing period which could be interpreted as an argument in favor of these theory.

Comparing our research to previous studies that highlight the association of aPL and thrombosis, it remains unclear whether all these patients were prophylactically anticoagulated, as it was the case in our cohort.

Our study has some limitations: (1) In the analysis, for some patients, we only had one time-point (2) small sample size may influence statistical analysis (3) determination of aPL did not included aCL and aβ2GPI IgA.

5. Conclusion

The relevance of aPLs in patients with COVID-19 is yet to be determined. Inflammation is closely associated to thrombosis and the presence of inflammatory

mediators in COVID-19 infection can lead to thrombosis. Further studies are needed before to determine the role of aPL in COVID-19 patients and their relationship with thrombosis. The presence of aPL should be carefully interpreted as it is important to evaluate the persistence of aPL positivity in patients infected with COVID-19.

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Conflict of interest

The authors have declared no conflicts of interest.

Author details

Cristiana Isabel Sieiro Santos
Complejo Asistencial Universitario de León, León, Spain

*Address all correspondence to: cristysieirosantos@gmail.com

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