PERSPECTIVES IN RHEUMATOLOGY

Are antiphospholipid antibodies just a common epiphenomenon or are they causative of immune-mediated coagulopathy in COVID-19?

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Abstract

The coronavirus disease 2019 (COVID-19) is the largest public health emergency in recent times. A significant number of patients develop a severe form of COVID-19 characterized by coagulopathy, organ failure, and elevated mortality. In addition, an unusually high frequency of antiphospholipid antibodies (aPLs) has been found in patients with COVID-19. These clinical and serological manifestations closely resemble those seen in the antiphospholipid syndrome (APS), especially in its catastrophic form, suggesting a role of aPLs in immune-associated coagulopathy. However, government bodies such as the American Society of Hematology have spoken out against the systematic search for aPLs in patients with COVID-19. In an attempt to bridge the gap on this hot topic, we conducted a comprehensive review of currently available cohort studies and case series systematically evaluating aPLs in COVID-19 patients. In this *Perspective*, we seek to identify both the frequency and the type of aPLs found in patients with COVID-19, as well as the potential association of these aPLs with vascular thrombosis and other distinctive characteristics of COVID-19. Furthermore, we investigated whether there is evidence that allows us to define the occurrence of aPLs in COVID-19 as an epiphenomenon, as has been observed in other systemic viral infections, or as antibodies against self-antigens bearing hallmarks that suggest a pathogenic role in immune-mediated thrombosis. Defining whether aPLs represent an epiphenomenon or they are actually involved in hemostatic abnormalities of COVID-19 is crucial both for uncovering novel mechanisms of immune-mediated thrombosis and for identifying potential prognostic biomarkers in this devastating disease.

Keywords Antiphospholipid antibodies · COVID-19 · Inflammation · Thrombosis

In December 2019, an outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection occurred in Wuhan, China, and rapidly spread worldwide. The World Health Organization (WHO) officially named the disease as coronavirus disease 2019 (COVID-19), which is now considered to be the largest public health emergency in the last century. Although most patients with COVID-19 are asymptomatic or develop only a mild condition, a significant proportion of patients develop a severe form of the disease, which is featured by pneumonia, coagulopathy, hyperinflammation, and organ failure [1].

On April 8, 2020, Zhang et al. described three severely ill COVID-19 patients with multiple cerebral infarctions who had circulating anticardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies, suggesting for the first time that coagulopathy associated with COVID-19 could be an acquired thrombophilia close to the spectrum of antiphospholipid syndrome (APS) [2]. However, a short time later, the American Society of Hematology (ASH) performed an antiphospholipid antibody (aPL) screen in 27 patients with COVID-19, in which they found that only four patients had

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lupus anticoagulant (LA) [3]. In contrast, no patient was positive for aCL or aβ2GPI antibodies. Based on the well-recognized fact that aPLs may transiently arise during acute infection, inflammation, or thrombosis, the ASH strongly recommended against routine aPL testing in COVID-19 patients, unless clinically indicated by the history or as part of a research protocol [3]. On the other hand, a higher-than-expected number of thrombotic episodes have been reported, both venous (pulmonary thromboembolism, venous sinus thrombosis, deep vein thrombosis) and arterial (myocardial infarction and stroke) in patients with COVID-19, despite the use of prophylactic or therapeutic anticoagulation [4]. As a growing body of evidence on the frequency and clinical associations of aPLs in COVID-19 is emerging, this issue has become a hot topic in clinical (rheumatology) practice.

A first question arises: do circulating levels of aPLs increase in COVID-19 patients? Currently, there are approximately 20 manuscripts with a suitable methodological design (case series and cohort studies) that address this issue. Table 1 summarizes the main data of these studies. Overall, the frequency of circulating aPL in COVID-19 patients has been consistently high, with figures around 54%, although the frequency between studies varies widely, ranging from 8 to 96% [5, 6]. This heterogenicity may reflect differences between the clinical phenotype of the patients studied (mild versus severe disease and early versus late disease), the type of aPL evaluated ("criteria" versus "non-criteria" antibodies) [7], or the presence of disease complications (i.e., venous and pulmonary thromboembolism or stroke). The role of ethnic, genetic, and geographic background cannot be ruled out.

The variety of aPLs described in COVID-19 is interesting. Most studies have focused on testing "criteria" antibodies. A positive test for LA is found in approximately one of every two patients with COVID-19, while the presence of aCL and aβ2GPI antibodies has been observed less frequently (around 10% for each) [8]. Concerning "non-criteria" antibodies, it should be noted that a wide variety of aPLs have been described in COVID-19 [7]. These include anti-phosphatidylserine (aPS), anti-prothrombin (aPT), and anti-annexin V (aAnnV) antibodies in both IgG and IgM isotypes, as well as aCL and aβ2GPI in IgA isotypes [6, 9–13]. Both the high frequency and diversity of aPLs strongly suggest that these antibodies are actively induced during acute SARS-CoV-2 infection. It is noteworthy that this prevalence is similar to that observed in several autoimmune diseases, although less than that found in patients with primary APS [14].

Once the elevated prevalence of aPL in COVID-19 has been established, the next issue to answer is: are these aPLs associated with the development of vascular thrombosis, or at least these antibodies are present in a specific clinical setting? Studies have consistently shown that aPLs occur in patients with COVID-19-associated hyperinflammation [15], a condition characterized by unusually high levels of C-reactive protein, ferritin, D-dimer, and interleukin-6, as well as increased activity of neutrophils [10, 16]. In parallel, aPLs are found more frequently in patients with severe or critical illness than in their counterparts with a milder disease [11, 17]. During acute SARS-CoV-2 infection, a consistent association has been observed between aPL positivity and the presence of other acquired thrombophilias, including protein C, protein S, antithrombin, and factor XII deficiency [12, 18, 19]. Therefore, low activity of natural anticoagulants and the presence of aPLs together may contribute to COVID-19 coagulopathy, although a pathogenic link remains to be demonstrated.

After the aforementioned association between circulating aPLs and the occurrence of stroke [2], other authors have explored the prothrombotic effects of these antibodies, reporting contradictory results. Several studies have consistently shown that aPLs are associated with the development of vascular thrombosis, particularly pulmonary thromboembolism and stroke [6, 9, 11, 12, 16, 20, 21]. A recent study has suggested that aPLs, even in weak or transient titers, are commonly found in patients hospitalized for COVID-19 [22]. This study identified that aPLs significantly associated with the occurrence of thrombotic events are aCL in IgG and IgM isotypes, aβ2GPI in IgA isotype, and positive LA. Furthermore, the triple positivity of aPLs seems to be of special relevance [22]. There are even some studies suggesting that the presence of aPLs may be a poor prognostic marker, as they are positive in COVID-19 patients who will eventually have a torpid clinical course [10, 11, 17, 23]. In contrast, other studies have failed to demonstrate an association between the presence of aPLs and the occurrence of thrombosis of any type [5, 13, 14, 17, 24, 25].

Infection-induced aPLs are typically transient and non-pathogenic. In SARS-CoV-2 infection, the concurrence of aPLs, large-vessel thromboses, thrombotic microangiopathy, and livedoid eruptions with complement activation over a short period of time strongly suggests an active role for these antibodies, leading to a condition that closely resembles catastrophic APS [16, 26]. Although the presence of LA in COVID-19 has been suggested to be spurious as most patients are on anticoagulant therapy, the use of diluted Russell's viper venom time (dRVVT) assays containing heparinase, which neutralizes any effect of heparins, virtually eliminates the possibility of false positive detection for LA [19]. Furthermore, the presence of aPS and aPT antibodies, which are associated with a prolonged activated partial thromboplastin time (aPTT), supports that LA positivity is due to circulating inhibitors in COVID-19 (i.e., autoantibodies against phospholipid-protein complexes) [9–11, 13, 24, 27]. Simultaneous elevations of anti-SARS-CoV-2 IgA, aCL IgA, and aβ2GPI IgA antibodies in patients with severe COVID-19, but not in those with mild disease, suggest that a vigorous antiviral IgA response, possibly triggered in the bronchial mucosa, may

Table 1 Summary of the main findings of studies on positive antiphospholipid antibodies in COVID-19

Ref. (country)	Cases (% male)	Age, years	aPL + (any)	aCL + IgM	aCL + IgG	aβ₂GPI + IgM	aβ₂GPI + IgG	LA +	Non-criteria aPL +
5 (Spain)	24 (58)	64 ± 14	8%	8.3%	0	8.3%	0	–	–
6 (France)	25 (68)	47 (35–64)	96%	52%	48%	0	4%	92%	aCL IgA=28% aβ₂GPI IgA=12% aPL IgM=56% aPL IgG=60%
8 (France)	56 (–)	–	~50%	10% of patients had aCL or aβ₂GPI IgG/IgM, no disaggregated information is reported.				45%	–
9 (Mexico)	21 (43)	m 62 (54–67)	57%	14%	10%	0	5%	–	aPT IgM=5% aPS IgM=14% aPS IgG=10% aAnnV IgM=19% aAnnV IgG=5%
10 (USA)	172 (–)	–	52%	23%	4.7%	5.2%	2.9%	–	aCL IgA=3.5% aβ₂GPI IgA=4.1% aPS/aPT IgM=18% aPS/aPT IgG=24%
11 (China)	79 (57)	60	47%	2.5%	5%	1.2%	15.1%	2.5%	aCL IgA=21.5% aβ₂GPI IgA=24% aPS/aPT IgM=8.8% aPS/aPT IgG=0%
12 (China)	19 (53)	m 65 (60–70)	52%	5.2%	10.5%	0	31.5%	5.2%	aCL IgA=31.5% aβ₂GPI IgA=36.8%
13 (Belgium)	31 (90)	m 63 (38–82)	74%	3%	19%	3%	9.6%	67.7%	aCL IgA=9.6% aβ₂GPI IgA=9.6% aPS/aPT IgG=6.4% aPS/aPT IgM=12.9%
14 (Italy)	122 (51)	54 ± 19	~50%	2.7%	13.4%	7.1%	6.3%	22.2%	aCL IgA=1.7% aβ₂GPI IgM=3.3%
16 (France)	150 (81)	m 63 (53–71)	–	–	–	–	–	87.4%	–
17 (Spain)	27 (44)	58 (20–90)	25%	0	0	0	0	22.2%	aβ₂GPI IgA=3.7%
18 (France)	89 (68)	m 68 (63–71)	72%	2.2%	5.6%	2.2%	5.6%	66.3%	–
19 (UK)	35 (69)	56 (18–83)	91%	–	–	–	–	91%	–
20 (USA)	68 (50)	~56	44%	1.4%	0	1.4%	0	44%	–
21 (China)	86 (62)	66 ± 11	37%	–	–	–	–	–	–
23 (Italy)	33 (51)	m 70 (22–90)	24%	15%	9%	6%	6%	–	–
24 (Italy)	122 (63)	68 ± 16	–	6.6%	5.7%	9%	15.6%	–	aPS/aPT IgG=2.5% aPS/aPT IgM=9.8% aβ2GPI IgA =6.6%
25 (France)	74 (–)	m ~64	88%	12% (any aCL or aβ2GPI in IgM/IgG isotypes) 85%					–
Total	1233 (60)	–	~54%	~10.4%	~10%	~3.3%	~7.8%	~52.5%	–

Age is presented as mean ± standard deviation unless otherwise specified; *m* denotes median (interquartile range)

aPL, antiphospholipid antibodies; *aCL*, anticardiolipin antibodies; *aβ₂GPI*, anti-β₂ glycoprotein-I antibodies; *LA*, lupus anticoagulant; *aPT*, anti-prothrombin antibodies; *aPS*, anti-phosphatidylserine antibodies; *aAnnV*, anti-annexin V antibodies

induce systemic autoimmunity [28]. Epitopic characterization of aβ2GPI antibodies recently showed that reactivity against the N-terminal domain 1 (anti-D1) or the C-terminal domains 4-5 (anti-D4-5) is found in approximately 5% (for each of the reactivities) of COVID-19 patients with positive aβ2GPI antibodies [24]. Although anti-D1 antibodies are characteristically associated with the development of thrombosis in APS patients, anti-D4-5 antibodies are often described in asymptomatic aPL carriers [29], suggesting the possibility of the simultaneous induction of both pathogenic and non-pathogenic aPLs in COVID-19, which would require additional prothrombotic stimuli to facilitate the development of thrombosis. This is in line with the "two-hit" hypothesis of vascular APS causality [30].

Finally, do these aPLs increase transiently, as in different inflammatory-mediated conditions, or do they remain high enough to meet current guidelines for classifying APS [31]? This is a pending issue that requires further investigation [32]. No study yet includes confirmatory tests for aPL at 12 weeks, which is the standardized time frame for classifying APS [31]. In patients with APS, aPL tests remain positive for long periods, whereas epiphenomenon-induced aPLs, on the other hand, may be transient. A single study reported retesting in ten patients with COVID-19 who initially had positive LA, showing that nine of them tested negative 1 month after their first positive result [13].

Defining whether aPLs represent an epiphenomenon or they are actually involved in hemostatic abnormalities of COVID-19 is crucial both for uncovering novel mechanisms of immune-mediated thrombosis and for identifying potential prognostic biomarkers in this devastating disease.

Data availability Not applicable.

Declaration

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Disclosures None.

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