



Antiphospholipid antibodies in critically ill COVID-19 patients with thromboembolism: cause of disease or epiphenomenon?

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Abstract

Coronavirus 2019 disease (COVID-19) is associated with coagulation dysfunction that predisposes patients to an increased risk for both arterial (ATE) and venous thromboembolism (VTE) and consequent poor prognosis; in particular, the incidence of ATE and VTE in critically ill COVID-19 patients can reach 5% and 31%, respectively. The mechanism of thrombosis in COVID-19 patients is complex and still not completely clear. Recent literature suggests a link between the presence of antiphospholipid antibodies (aPLs) and thromboembolism in COVID-19 patients. However, it remains uncertain whether aPLs are an epiphenomenon or are involved in the pathogenesis of the disease.

Keywords COVID-19 · Critically ill patients · Venous thromboembolism · Arterial thromboembolism · Antiphospholipid antibodies · Antiphospholipid antibodies syndrome

Highlights

- Critically ill COVID-19 patients suffer from hypercoagulable and hypofibrinolytic states which lead to both ATE and VTE
- The presence of aPLs has been hypothesized as a cause of COVID-19 associated coagulopathy
- The methodology for detecting aPLs is complicated and suffers from many pitfalls
- The data on the occurrence of aPLs in critically ill COVID-19 patients and their association with thrombotic events are still limited and contradictory

Introduction

Patients with coronavirus disease 2019 (COVID-19) suffer from hypercoagulable and hypofibrinolytic states which lead to both arterial (ATE) and venous thromboembolism (VTE) [1, 2]. A variety of potential risk factors for thrombosis have been proposed including inflammation, hypoxemia, endothelial dysfunction, platelet activation and dysregulation of the complement system [3–5]. Moreover, the presence of antiphospholipid antibodies (aPLs) has been hypothesized as a cause of COVID-19 associated coagulopathy (CAC) [6].

Evidence in the literature supports an overall increased risk of developing aPLs due to various infections. The earliest known example dates back to 1906 when a complement fixation test was used for the diagnosis of syphilis in human serum [7]; a phospholipid, called cardiolipin, was the tissue extract utilized to perform this test. In 1983 cardiolipin was used for the first time as the antigen in solid-phase aPL specific assays [8]. Since then many viral and bacterial infections have been found to be associated with aPL positivity [9–12]. According to a recent systematic meta-analysis [13], thrombotic events have been shown to be prevalent in patients with high aPLs who had hepatitis C virus (HCV) and hepatitis B virus (HBV). The current understanding of the relationship between coronavirus 2019, thrombogenesis and aPLs, however, is complex and still unclear.

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The purpose of this narrative review is to analyze the literature on the possible role of aPLs on hypercoagulable and hypofibrinolytic states of critically ill COVID-19 patients.

Thromboembolic events in COVID-19 patients

In COVID-19 patients, both ATE and VTE have been reported due to the strong thrombotic tendency [14, 15]. In particular, arterial thrombosis includes cerebral infarction, myocardial infarction, and limb arterial thrombosis, while venous thrombosis includes deep vein thrombosis (DVT) and pulmonary embolism (PE).

A recent meta-analysis [16] of 42 studies involving 8271 COVID-19 patients has shown that the overall VTE incidence was 21%, with a DVT rate of 20% and PE rate of 13%, while the ATE rate was 2%. Among critically ill patients the VTE rate was 31%, DVT rate was 28%, PE rate was 19% and ATE rate was 5%, despite pharmacological thromboprophylaxis. An analysis restricted to studies in intensive care unit (ICU) patients [17], in which computed tomography pulmonary angiography (CTPA) or compression ultrasound (CUS) as diagnostic tests were carried out on clinical suspicion, found the incidence of VTE at 24%, PE with or without DVT 19% and DVT alone 7%. Moreover, in both severe and mild COVID-19 patients, a high incidence (from 14.7 to 25%) of asymptomatic DVT has been recorded [1, 18].

Tang and colleagues first suggested that thrombosis in COVID-19 patients was associated with a poorer prognosis [19]. Thromboembolism in COVID-19 significantly increased the odds of mortality by as high as 74% (OR 1.74; 95% CI 1.01–2.98; $p=0.04$) [16].

Several mechanisms contributing to this high thromboembolic risk have been proposed. The excessive proinflammatory cytokines, including interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , complement system proteins, tissue factor expression on monocytes/macrophages, neutrophil activation and neutrophil extracellular traps, produce activation of coagulopathy [20, 21]. Moreover, the thromboinflammation causes endothelial damage that increases thrombin generation [22, 23]. In the postmortem evaluation of COVID-19 pulmonary tissues, the arterial vessels demonstrated neutrophilic and mononuclear cellular infiltration and apoptosis of endothelial cells. A distinctive factor for COVID-19 was a marked presence of diffuse thrombosis of the peripheral small vessels [24].

The direct infection of vascular endothelial cells is the unique characteristic of COVID-19; this activation and dysfunction seems to be a major forerunner to thrombosis [25]. Moreover, the presence of thrombotic stroke, reported even in young patients, provides some clinical evidence supporting the possible involvement of aPLs in COVID-19 on endothelial dysfunction [26, 27].

Antiphospholipid antibodies following infections and their influence on hemostasis

The aPLs are a group of autoantibodies including lupus anticoagulant (LA), IgG/IgM anticardiolipin (aCL) and IgG/IgM anti- β 2-glycoprotein I (a β 2GPI) antibodies that have as primary targets phospholipid binding proteins.

β 2GPI and cardiolipin are ubiquitous molecules. In genetically susceptible individuals, viral and bacterial agents may induce autoimmune disease with generation of pathogenic antibodies. Pathogens can contain chemical structures that mimic normal host self-proteins, resulting in a mechanism known as “molecular mimicry”. This process has been demonstrated in mice that developed aPLs after immunization with synthetic peptides of viral origin similar to the phospholipid binding site of β 2GPI [28]. The results included significant thrombocytopenia, prolonged activated partial thromboplastin time (aPTT) and increased fetal loss. It has been postulated that a β 2GPI antibodies exert a direct pathogenic effect by interfering with homeostasis reactions occurring on the surface of platelets, vascular endothelial cells or the placenta [29].

An association between infections and aPLs has been reported in several studies [9–12, 30–32]. A high number of infectious diseases are characterized by increases in aPLs. Systemic reviews and meta-analysis have shown that human immunodeficiency virus (HIV), HCV, HBV, human T-lymphotropic virus type 1 (HTLV-1), Epstein-Barr virus (EBV), varicella virus, cytomegalovirus (CMV), parvovirus B19, streptococcal and staphylococcal infections and gram-negative bacteria are significantly associated with aPL positivity. Although IgM isotypes of the aCL antibodies seem to be mainly produced during infections, IgG has also been detected.

Many infections are characterized by an appearance of aPLs, however their presence does not necessarily lead to the development of thrombotic events and, consequently, of the antiphospholipid syndrome (APS) [33].

APS is a systemic autoimmune disease characterized by venous (DVT or PE), arterial (ischemic stroke), microvascular thrombosis or obstetrical events (pregnancy loss); it was first observed in some patients with Systemic Lupus Erythematosus (SLE) who developed recurrent thrombosis, recurrent abortions in pregnant women or neurological disorders [34].

A small number of patients (<1%) develop catastrophic aPL syndrome (CAPS) defined as small vessel thrombosis affecting three or more organs in less than one week in the presence of aPLs [35]. CAPS, which is often triggered by an event such as infection, is associated with high mortality (50%) [36].

According to the revised Sapporo criteria, diagnosis of APS requires the presence of one clinical (thrombosis or pregnancy) and one persistently positive laboratory test among aPLs (i.e., LA, IgG/IgM aCL, IgG/IgM a β 2GPI) [37]. APLs are a *conditio sine qua non* for diagnosis of APS. The latest update from the Subcommittee for the Standardization (SCC) of the International Society of Thrombosis and Haemostasis (ISTH) for LA and aPLs recommends performing all three tests (LA, aCL and a β 2GPI) to diagnose APS. Moreover, positive laboratory tests should be confirmed 12 weeks after the initial testing [38]. Re-testing after 3 months to ensure the reliability is recommended particularly in cases of an initial triple-positive test [39].

Evidence in the literature has shown that patients with more than one positive test, and particularly those with triple positivity (LA, aCL and a β 2GPI), have an increased risk of thrombotic APS [39]. Double positivity (mostly LA negative) is generally at lower thrombotic risk [40]. The presence of antibodies of the same isotype reinforces the reliability of the results [41]. Patients with isolated positive LA, but negative aCL and a β 2GPI, have a low risk of a thromboembolic event [42]. No association with thromboembolic events was shown in isolated aCL [43] or a β 2GPI positivity [44]. However, IgA a β 2GPI antibodies were independently associated with arterial thrombosis in patients with SLE and APS [45]. The presence of IgA a β 2GPI antibodies has been identified as an independent risk factor for acute myocardial infarction [46] and cerebral ischemia [47].

Recently, new tests [i.e. antiphosphatidylserine/prothrombin antibodies (aPS/PT)] have been investigated in addition to the current aPL panel in APS for the risk of thromboembolic events when the aPL profile consists of double positivity (aCL and a β GPI, same isotype with LA negative) [48]. Positive aPS/PT may highlight a false negative or borderline LA [49]. However, it is imperative that tests are repeated after an initial positive result on a second occasion after 12 weeks [50].

According to the “two-hit” theory, infectious agents can act as the initial trigger of the production of antibodies cross-reacting with β 2GPI and infectious peptides, and also induce an inflammatory response which is necessary for thrombosis to occur [51]. A recent meta-analysis [13] analyzed sixty observational studies reporting on patients with acute or chronic viral infections and showed a higher prevalence of thromboembolic events among patients who developed elevated aPLs in HCV and HBV infections; however, the only statistically significant increased thromboembolic risk was observed in patients with HCV.

Inhibition of natural anticoagulant activity, particularly the protein C system, was the first prothrombotic mechanism identified in aPLs. APLs impair the activation of protein C, as well as the ability of activated protein C to inactivate factors V and VIII [52, 53]. APLs also inhibit the activity of

tissue factor pathway inhibitor [54] and neutralize the ability of β 2GPI to stimulate the activity of tissue-type plasminogen activator, which inhibits fibrinolysis [55]. Moreover, aPLs, particularly a β 2GPI, activate endothelial cells, monocytes, neutrophils, and platelets [56–59]. Endothelial activation leads to transformation from the anticoagulant endothelial surface to a procoagulant phenotype [60].

In conclusion, evidence in the literature supports an increased risk of developing aPLs following various infections. Although aPLs are capable of modifying the hemostatic mechanisms towards thrombotic phenomena, their presence is not always accompanied by thrombotic manifestations of APS.

Antiphospholipid antibodies following COVID-19 infection: a pathogenic mechanism of thrombotic complications

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection profoundly impacts the immune system of the host. A growing body of literature has provided understanding of how immune cell dysfunction contributes to the inflammatory response in COVID-19 patients [61]. Clinical manifestations of COVID-19 depend on a balance between SARS-CoV-2 virulence and host characteristics. In particular, a recent study [62] has described the profound immune dysregulation in COVID-19 patients with severe illness compared with those with moderate symptoms. Vlachoyiannopoulos et al. [63] found the presence of several systemic autoimmune reactivities [i.e. antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), antibodies to extractable nuclear antigens (ENA), aCL antibodies, a β 2GPI antibodies and anti-cyclic citrullinated peptide] in almost 70% of critically ill COVID-19 patients tested. In the same line, van der Linden et al. [64] reported that greater than 80% of ICU-treated COVID-19 patients had detectable aPLs, especially IgA antibodies. Moreover, the high presence of IgA-aPLs was associated with increased severity of illness [65].

Additionally, hyper-activation of the immune system may trigger autoimmunity [66] in predisposed individuals; immune-mediated manifestations, such as hemolytic anemia, myositis, Guillain-Barré syndrome, have been described in COVID-19 patients [67–69].

The mechanism of interaction between the immune system and coagulation could be mediated by pulmonary surfactant, as it is rich in phospholipid-binding protein [70]. Surfactant is produced by Type II pneumocytes which express high levels of angiotensin-converting enzyme 2 receptors that are a target of SARS-CoV-2. Pneumocyte necrosis leads to surfactant leakage, exposing phospholipid proteins to the immune system

[71]. Moreover, aPLs engage cell surfaces where they activate endothelial cells, platelets and neutrophils [72].

The role of aPLs in activating endothelial cells in COVID-19 has been demonstrated in vitro. Zhou et al. [73] found that COVID-19 IgG fractions enriched for aPLs potentiate thrombosis when injected into mice.

The immune-mediated mechanism seems to be more evident in critically ill patients. Xiao et al. [74] found that aPLs were not present among COVID-19 patients who were not in critical condition.

Several studies have assessed aPLs in critically ill COVID-19 patients and some have found an association between thrombotic events, both arterial and venous, and aPLs detection (Table 1). Regarding arterial thrombosis, Zhang et al. [26] described three critically ill patients with SARS-CoV-2 infection that developed multiple cerebral infarcts associated with positivity for aCL IgA, β 2GPI IgA and IgG. Similarly, Hossri et al. [75] reported two cases selected from patients requiring critical care that revealed significant APS manifesting as multiple cerebral and splenic infarcts in the first case, and peripheral arterial disease in the second. Likewise, Fan et al. [76] observed a higher prevalence of aPLs in patients with ischemic stroke than in those without stroke (83.3 vs 26.9%, $p < 0.05$). Xiao et al. [74] confirmed that patients with multiple aPLs had a significantly higher incidence of cerebral infarction compared to patients who were negative for aPLs; moreover, the authors hypothesized that in genetically predisposed patients, COVID-19 may trigger the development of “COVID-19 induced APS like syndrome”. Finally, Zhang et al. [77] found four cerebral infarction events in COVID-19 patients admitted to the ICU with aPLs of multiple isotypes.

Additionally, Pineton de Chambrun et al. [78] also confirmed the presence of aPLs in COVID-19 patients with venous thrombotic events. The authors described the profile of aPL positivity in a series of 25 critically ill patients with severe COVID-19 and found a high frequency of aPLs; most patients had positive LA (92%). Considering LA, aCL (IgG/IgM/IgA) and β 2GPI (IgG/IgM/IgA) antibodies, 8 patients (32%) had single aPL positivity, 13 (52%) had double positivity and 3 (12%) had triple positivity. All six patients who experienced PE were aPL positive and three of these had double positivity.

In conclusion, while based on data from limited studies the high prevalence of aPLs in critically ill COVID-19 patients could contribute to thrombotic events both arterial and venous, exacerbating the immune response in predisposed individuals.

Antiphospholipid antibodies following COVID-19 infection: an epiphenomenon

Many studies looking at the causes of aPTT prolongation in critically ill COVID-19 patients have shown that LA

can be detected in a wide percentage, ranging from 3 [73] to 92% [78]. The first case series [27] found the presence of LA in 31 of the 35 investigated patients; the positivity for LA was significantly higher among COVID-19 patients than in a historical control cohort (91% vs 26%, $p < 0.001$). Helms et al. [14] in a large study of COVID-19 acute distress syndrome patients reported an 88% of prevalence of LA.

However, questions about the role of aPLs in CAC have arisen in the literature. First, Zhang et al. [26] cautioned that the presence of aPLs in COVID-19 patients might be a coincidence. Indeed, aPLs may lead to thrombotic events that are difficult to differentiate from other causes of multifocal thrombosis in critically ill patients such as disseminated intravascular coagulation, heparin-induced thrombocytopenia and thrombotic microangiopathy. Likewise, Amezcua-Guerra et al. [79] reported a high frequency (12 to 21 patients) of aPLs in patients with severe and critical COVID-19, but only 2 of these patients developed thromboembolic events. Along similar lines, Frapard et al. [80] observed no significant association with thrombosis in critically ill COVID-19 patients with aPLs positivity; in particular, the rate of thromboembolic events was in the same range in patients with aPLs compared to patients without aPLs [23 (48%) vs 9 (45), $p = 0.83$]. Similarly, Borghi et al. [81] showed no association between aPLs positivity in ICU-COVID-19 patients and major thrombotic events. The positivity for aCL and β 2GPI antibodies in COVID-19 patients was at medium/low titers with reactivity against epitopes different and in contrast with those at medium/high titers associated with vascular events in primary APS. This may explain the lack of association between aPLs and thrombotic events in critically ill COVID-19 patients. Moreover, approximately 57% of patients have prolonged aPTT and only a small proportion of them carry aCL and β 2GPI antibodies. Other factors could likely be responsible for the prolonged aPTT phenomenon.

Finally, Siguret et al. [82] reported 26 DVT and 4 PE events in 28 critically ill COVID-19 patients. LA, based on dilute Russell's viper venom time (dRVVT) system, was positive in 82% of patients with thrombotic complications and in 87% of patients without ($p = 0.7$). Patients with positive aCL IgG/IgM and β 2GPI IgG had no significantly increased thrombosis risk during ICU stay ($p = 0.3$). The authors concluded that despite high prevalence, the presence of aPLs in COVID-19 patients was not associated with thrombotic events.

Another point of discussion is that most of these studies assessed aPLs in COVID-19 patients at one point in time and did not repeat tests at least 12 weeks after. Devreese et al. [83] retested the LA-positive patients after 1 month and found a negative test in 9 of 10 retested patients, suggesting that aPLs in COVID-19 patients could be transient.

Table 1 Summary of studies published that have assessed aPL antibodies in critically ill COVID-19 patients

Reference	N. patients	Thrombotic events N (%)	aPL tests	Positive aPL tests N (%)	Positive aPL tests in thrombotic events N (%)	Negative aPL tests in thrombotic events N (%)	Positive aPL tests in no thrombotic events N (%)
Helms [14]	150	64 (42.7) 25 (16.7) PE 3 (27.3) ECMO thrombosis 3 (2) DVT 2 (1.3) cerebral ischemic attack 1 (0.7) limb ischemia 1 (0.7) mesenteric ischemia	LA	50 (87.7)	N/A	N/A	N/A
Zhang [26]	3	3 (100) (multiple cerebral infarctions)	aCL IgA + aβ2GPI IgA + aβ2GPI IgG	3 (100)	3 (100)	N/A	N/A
Vlachoyiannopoulos [63]	29	N/A	aCL aCL IgG aCL IgM + IgG aβ2GPI aβ2GPI IgG aβ2GPI IgM aβ2GPI IgG + aβ2GPI IgM	7 (24.1) 4 3 10 (34.5) 2 5 3	N/A	N/A	N/A
Xiao [74]	66 critical patients 13 non-critical patients	25 (37.9) in critical patients 17 (25.8) (DVT distal) 2 (3) (DVT proximal) 5 (7.6) (cerebral thrombosis) 1 (1.5) (myocardial infarction) 0 (0) in non-critical patients	aPL LA aβ2GPI IgG aβ2GPI IgA aβ2GPI IgM aCL IgA aCL IgG aCL IgM aβ2GPI IgA + aCL IgA aβ2GPI IgA + aCL IgA + aβ2GPI IgG aβ2GPI IgA + aCL IgA + aCL IgG + aβ2GPI IgG LA + aβ2GPI IgA + aCL IgA + aCL IgG + aβ2GPI IgG aPS/PT IgG aPS/PT IgM	31 (47) 2 (3) 12 (18.2) 19 (28.8) 1 (1.5) 17 (25.8) 12 (18.2) 1 (1.5) 15 (22.7) 10 (15.2) 4 (6.1) 1 (1.5) 7 (10.6) 0 (0)	15 (48.4) N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A	10 (28.5) N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A	16 (51.6) N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A N/A
Hossri [75]	2	1 (cerebral infarcts) 1 (limb arteries occlusions)	aCL IgG + aCL IgM	2 (100)	2 (100)	N/A	N/A
Fan [76]	86	6 acute ischemic stroke	APS panel [(aβ2GPI (IgG + IgM + IgA) + aCL (IgG, IgM, IgA)]	12 (37.5)	5 (83.3)	1 (16.7)	7 (26.9)

Table 1 (continued)

Reference	N. patients	Thrombotic events N (%)	aPL tests	Positive aPL tests N (%)	Positive aPL tests in thrombotic events N (%)	Negative aPL tests in thrombotic events N (%)	Positive aPL tests in no thrombotic events N (%)
Zhang [77]	19	12 (63.1) 7 (36.8) (acroschemia) 4 (21) (cerebral infarction) 1 (5.3) (jugular thrombosis)	aPL LA a β 2GPI IgM a β 2GPI IgG a β 2GPI IgA aCL IgA aCL IgG aCL IgM	10 (52.6) 1 (5.3) 0 (0) 6 (31.6) 7 (36.8) 6 (31.6) 2 (10.5) 1 (5.3)	4 (21)	N/A	N/A
Pineton de Chambrun [78]	25	6 (24) PE	LA aCL a β 2GPI aPS + aPE + aCL + a β 2GPI	23 (92) 13 (52) 3 (12) 18 (72)	LA 2 (33.3) LA + aCL 4 (66.7)	0 (0)	LA 6 (24) LA + aCL 8 (32) aCL 1 (4) LA + a β 2GPI 1 (4) LA + aCL + a β 2GPI 3 (15.7)
Amezcu-Guerra [79]	21	2 (9.5) PE	aCL IgG aCL IgM a β 2GPI IgM a β 2GPI IgG aPS (IgG) aPS (IgM) PT (IgM) Antiannexin V IgM Antiannexin V IgG	2 (17) 3 (25) 0 (0) 1 (8) 2 (17) 3 (25) 1 (18) 4 (33) 1 (8)	1 (50)	0	10 (83.3)
Frapard [80]	37	24 (65) 9 (24) PE 12 (32) PE or DVT 11 (55) RRT thrombosis	aPL aCL or a β 2GPI IgA aCL or a β 2GPI IgM or IgG	11 (30) 7 (19) 6 (16)	23 (48)	9 (45)	N/A
Borghini [81]	122	16 (13.1) 8 venous thrombosis 8 arterial thrombosis	aCL IgG (ELISA) aCL IgM (ELISA) a β 2GPI IgM (ELISA) a β 2GPI IgG (ELISA) a β 2GPI IgA (ELISA) aPS/PT (ELISA)	7 (5.7) 8 (6.6) 9 (11) 19 (15.6) 8 (6.6) 15 (12.3)	0 (0)	16 (13.1)	N/A
Siguret [82]	74	26 DVT 4 PE 1 stroke 1 CVC thrombosis	LA aCL IgG/IgM/a β 2GPI IgG	63 (85) 9 (12.2)	23 (82) 5 (18)	N/A	40 (87) 4 (9)

Table 1 (continued)

Reference	N. patients	Thrombotic events N (%)	aPL tests	Positive aPL tests N (%)	Positive aPL tests in thrombotic events N (%)	Negative aPL tests in thrombotic events N (%)	Positive aPL tests in no thrombotic events N (%)
Devreese [83]	31	12 (38.7)	aPL	23 (74.2)			
		4 (12.9) CVC thrombosis	LA	21 (67.7)	10 (83.3)	2 (16.7)	14 (73.7)
			aCL IgG	6 (19.3)	5 (41.7)	7 (58.3)	3 (15.8)
		2 (6.5) clotting circuit dialysis	aCL IgM	1 (3.2)	0 (0)		0 (0)
			aCL IgA	2 (6.4)	1 (8.3)	11 (91.6)	2 (10.5)
		3 (9.7) clotting circuit ECMO	a β 2GPI IgA	4 (12.9)	1 (8.3)	11 (91.6)	2 (10.5)
			a β 2GPI IgM	1 (3.2)	0 (0)	11 (91.6)	3 (15.8)
		2 (6.5) DVT	a β 2GPI IgG	3 (9.7)	0 (0)	11 (91.6)	2 (10.5)
		1 (3.2) stroke	aPS/PT (IgG)	3 (9.7)	1 (8.3)	11 (91.6)	2 (10.5)
			aPS/PT (IgM)	3 (9.7)	1 (8.3)	11 (91.6)	3 (15.8)

CVC central venous catheter, ECMO extracorporeal membrane oxygenation, DVT deep vein thrombosis, ICU intensive care unit, PE pulmonary embolism, RRT renal replacement therapy, aPTT activated partial thromboplastin time, aPL antiphospholipid, LA lupus anticoagulant, aCL anti-cardiolipin, a β 2GPI anti- β 2-glycoprotein I, aPS antiphosphatidylserine, PT antiprotease, N/A not available

In conclusion, aPLs in critically ill COVID-19 patients seem to not be predictive of vascular events; however, a possible prolonged aPTT linked to their presence must be carefully evaluated and should not be a barrier to the use of anticoagulation therapy in prevention or treatment of thrombosis in CAC.

Laboratory diagnosis of APS in critically ill COVID-19 patients: obstacles to overcome

The methodology for detecting aPLs is complicated and suffers from many pitfalls [84]. Not every positive test has diagnostic importance and several variables affect the results.

LA is a laboratory phenomenon characterized by prolongation of phospholipid-dependent coagulation tests. In vitro, LA bound to phospholipid binding proteins compete with coagulation factors for phospholipid binding sites, thereby reducing the coagulation process [85]. The presence of LA is confirmed when an excess of phospholipids added to the reagent mixture normalizes the clotting times [40].

Due to antibody heterogeneity and reagent variability [40], the ISTH guidelines [41] recommend performing two different tests, based on different principles, usually dRVVT and LA-responsive aPTT; dRVVT is recommended for its specificity and aPTT with low phospholipid concentration for its sensitivity.

Numerous laboratory variables can affect assays used for LA detection including the type and the content of phospholipids in the reagent mixture, activator, plasma preparation, expression of results and cut-off values. Since there is high variability in the performance of clinical laboratories, the rate of false-positive and false-negative tests is also relatively high [86].

False positive LA results can occur in patients treated with heparin. However, a recent study [87] showed that enoxaparin caused false-positive aPTT-based LA detection only at supra-therapeutic anti-Xa activity levels. Several reagents, such as dRVVT and some LA-specific aPTT reagents, contain heparin neutralizers and therefore LA screening is not possible if the content of heparin in the test plasma exceeds the reagent neutralization capacity [37]. Checking anti-Xa activity before LA testing ensures reliable results if anti-Xa activity levels are within the therapeutic range. Furthermore, there are commercial dRVVTs and aPTTs containing neutralizers that quench heparin up to 0.8 U mL⁻¹. Therefore, samples should be taken at least 12 h after the last dose of low molecular weight heparin (LMWH) is administered [37, 41].

Testing during the acute phase should be interpreted with caution. A raised level of FVIII causes a shortening of aPTT that could confuse the interpretation of LA test. Moreover, C-reactive protein (CRP) interferes with phospholipid in the reagent, prolonging phospholipid dependent clotting tests

[86, 88]. One drawback, reported within the use of coagulation assays in LA testing, is their sensitivity to elevated CRP levels. Data on LA should be interpreted with care for the possibility of false positive results when CRP-sensitive reagents are used and when tests are conducted on patients with elevated CRP-values. In daily practice, laboratory staff interpreting LA testing should be aware of this interference in critically ill COVID-19 patients in which the CRP-values could be high because of systemic inflammation [89].

On the other side, the detection of aCL and β 2GPI antibodies is too tedious. There are numerous commercial assays; even, for the same assays, the inter-laboratory variability is high [90].

One of the drawbacks of the aCL ELISA is low specificity. Defining cut-off reference values of aPLs is one of the factors that determines the classification of a patient as APS or not. Test results of aCL and β 2GPI detected by ELISA should be considered positive if they are above the cut-off value, calculated as greater than the 99th percentile [91].

Of note, it is important to detect cofactor β 2GPI to eliminate the antibodies associated with drugs. Additionally, differentiation between autoimmune or pathogenic and non-immune or non-pathogenic aPLs by laboratory technique is of paramount importance.

In conclusion, the diagnostic value of the aPLs tests in critically ill COVID-19 patients is currently under debate because of methodological problems related to the use of anticoagulants and due to the interference with inflammatory proteins.

Conclusions

Critically ill COVID-19 patients are at risk of thrombosis, both arterial and venous, despite heparin treatment. The contribution of aPLs to COVID-19 thrombosis does not seem clear yet. Due to the severe condition of COVID-19 patients admitted to the ICU, a large number of patients are likely not being screened by CUS or CTPA and many thrombotic events may be underestimated. Microvascular thromboses are difficult to evaluate and often impossible to differentiate from other causes of organ dysfunction without autopsies. Moreover, isolated LA positivity may depend on the complicated methodology of coagulation tests that are prone to interference; anticoagulant therapy with heparin as well as inflammatory proteins (i.e. CRP) can influence the laboratory results.

Currently, the data on the occurrence of aPLs in critically ill COVID-19 patients and their association with thrombotic events are limited and contradictory. Further studies are needed with long term follow-up to determine whether aPLs represent a simple and transient epiphenomena or are causally involved in the pathogenesis of thrombosis in CAC.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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