Clinical Relevance of Antiphospholipid Antibodies Levels During the Course of Severe COVID-19

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To cite this article:
Soumia Nachate, Mahassine Moukaouim, Loubna Darfaoui, Zineb Nassiri, Imane Ibrahim, Houssam Rebahi, Hajar Chichou, Abdelhamid Hachimi, Mohamed-Abdenasser Semkaoui, Raja Hazime, Lamiae Essaadouni, Brahim Admou. Clinical Relevance of Antiphospholipid Antibodies Levels During the Course of Severe COVID-19. *International Journal of Immunology*. Vol. 9, No. 2, 2021, pp. 37-40. doi: 10.11648/j.iji.20210902.13

Abstract: The aim of this study was to determine the clinical significance of antiphospholipid antibodies (APLs) during the follow-up of nine severe COVID-19 patients admitted to the Intensive Care Unit of the University Hospital. The measurement of APLs (IgG and IgM anti-cardiolipin (aCL) and anti-β2-glycoprotein-1 (aB2GP1) was performed on the 1st day and after 15 days of admission, using the chemiluminescence assay (threshold =19 CU). The average age of patients was 64.7 ± 20, 44 years (ranges: 30-88 years), with a sex-ratio of 1.25. On day-1, APLs were positive in two cases, the first of which was positive for IgG aβ2GP1 (94.9 CU) and IgG aCL (24.8 CU), and the second was positive only for IgG aβ2GP1 (31.4 CU). On day-15, APLs showed negative results for both aβ2GP1 and aCL for the first case, and decreasing titers of aβ2GP1 for the second one. Interestingly, these two cases showed no thromboembolic events and had a good clinical outcome. Conversely, APL positivity occurred at day-15 in two cases, corresponding to IgG aB2GPI (49.3 CU) in one case, and IgG aCL (76 CU) in the other. Both cases presented with a prolonged activated-partial-thromboplastin-time, high levels of D-dimers and fibrinogen, associated with increased levels of ferritin and interleukin-6. Our series has shown that IgG aB2GPI or IgG aCL can be either transient or appear secondarily with significantly high titers. The latter condition was associated with a poor clinical outcome, which emphasizes the importance of APLs monitoring in severe COVID-19 as a potential prognostic factor.

Keywords: Severe COVID-19, Anti-phospholipid Antibodies, Monitoring, Prognosis

1. Introduction

Coronavirus disease 2019 (COVID-19) is associated with both severe systemic inflammation and a profound hypercoagulable state [1, 2]. Abnormal coagulation parameters are associated with poor prognosis and high mortality in critically ill COVID-19 patients [3]. The association of a prolonged activated partial-thromboplastin time (aPTT) and thrombosis in some patients is a reminder of antiphospholipid syndrome (APS) wich is an autoimmune disorder characterized by thrombotic events occurring in patients with persistent APLs positivity [4, 5]. These

auto-antibodies has been initially described in three patients presenting severe forms of COVID-19 associated with multiple cerebral infarctions raising the question of their role in the underlying mechanism of COVID-19-induced coagulopathy [6]. Since then, many reports have suggested a possible involvement of APLs in the pathophysiology and evolution of the disease [1]. The presence of APLs has been shown to be either associated with thrombotic complications or transient, resolving within a few weeks with no relevant clinical impact [6, 7, 8]. The aim of this study was to determine the clinical significance of APLs occurring during the follow-up of severe COVID-19 patients.

2. Patients and Methods

2.1. Settings and Patient Population

This study included severe COVID-19 patients admitted for up to 15 days in the Intensive Care Unit (ICU) for whom the SARS-COV-2 infection was confirmed by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) on nasopharyngeal swabs. No patient had prior medical history of APS or other related auto-immune disease. All patients received thromboprophylaxis following current guidelines. Patients were assessed for APLs on day 1 and 15 days after ICU admission.

2.2. Assessment of APLs Levels

Based on IgG and IgM anti-cardiolipin (aCL) and anti-β2-glycoprotein-1 (aB2GP1), APLs were measured using a chemiluminescence assay (BIO-FLASH®, Inova Diagnostics, cut-off value= 20 CU). Were simultaneously recorded the patient's clinical data as well as biological parameters including: Cell blood count, Lactate dehydrogenase, Cardiac troponin I, aPTT, Fibrinogen, D-dimers, Ferritin, C-reactive protein and Interleukin-6.

3. Results

Nine patients have been enrolled whose mean age was 64.7 ± 19.22 years (ranges: 30-88 years), with a sex-ratio of 1.25. The demographic characteristics, co-morbidities and clinical features of patients, as well as their laboratory results recorded on day 1 and day 15 are presented in table 1 and table 2.

On day-1, APLs were positive in two cases (patient 1 and 2): the first case showed positivity for IgG aβ2GP1 (94.9 CU) and IgG aCL (24.8 CU), and the second case was positive for only IgG aβ2GP1 (31.4 CU). On day-15, among the nine monitored patients, APLs became negative for both aβ2GP1 and aCL in the first case, and decreased in titer of aβ2GP1 in the second case. These two cases had a good clinical outcome and didn't manifest thromboembolic events.

A positivity for IgG aB2GPI occurred on day-15 in one case (patient 3) and for IgG aCL in another case (patient 4), with titers of 49.3 and 76 CU respectively. Both cases exhibited a prolonged aPTT, high levels of D-dimers and fibrinogen, associated with elevated levels of ferritin and interleukin-6, and finally died.

Table 1. Main Clinical and biological characteristics of 9 patients with severe COVID-19.

Demographic characteristics		
Mean age ± SD (year)	64,77 ± 20,44	
Gender (%)		
Male	66,7	
Female	33,3	
Medical history (%)		
Hypertension	44,5	
Diabetes	33,3	
Stroke	11	
Coronary artery disease	11	
Chronic obstructive pulmonary disease	11	
Asthma	11	
Symptoms (%)		
Fever	77,8	
Cough	88,9	
Dyspnea	77,8	
Chest pain	44,5	
Vomiting	22,3	
Abdominal pain	11	
Laboratory findings		
	1st day	15th day
White-cell count, median (IQR) (4000-10000 mm3)	7590 (2970-14570)	7000 (3620-99180)
Lymphocytes, median (IQR) (1000-4000 mm3)	800 (520-2650)	1920 (1110-4220)
Neutrophils, median (IQR) (2000-7500 mm3)	5970 (140-12070)	4290 (666-30540)
Platelet count, median (IQR) (150000-450000 mm3)	232 (110-550)	248 (162-345)
LDH, median (IQR) (0-250 U/liter)	345 (200-468)	230 (100-652)
Cardiac troponin I, median (IQR) (0-13 pg/ml)	11,8 (4,02-26,17)	116 (3,54-116)

Demographic characteristics		
aPTT, median (IQR) (30-35 sec)	27 (20,6-32,10)	22,5 (21-43,60)
Fibrinogen, median (IQR) (2-4 g/liter)	8 (2,20-8)	4 (1,90-5,90)
D-dimer, median (IQR) (0-0.5 mg/liter)	3,64 (0,57-28,47)	1,65 (0,78-21,27)
Ferritin, median (IQR) (15-150 µg/liter)	341 (8,5-1170,40)	409 (13-3153)
C-reactive protein, median (IQR) (0-5 mg/liter)	121 (4,64-282,88)	195 (1,51-195)
IL-6, median (IQR) (<7 pg/ml)	145 (1,50-201)	2592 (5,58-5000)

* SD: standard deviation, IQR: interquartile range, LDH: Lactate dehydrogenase, aPTT: Activated partial thromboplastin time.

4. Discussion

After the emergence of the new coronavirus, thromboembolic complications were frequently reported especially in severe covid-19 patients and found in up to 58% of deceased ones [9], This has caused a great deal of concern among medical researchers to investigate the pathophysiological mechanism of this life-threatening condition. One of the hypotheses that have arisen is the involvement of APLs in this process. Until then, the debate is still controversial; there are competing assumptions from those who plead the participation of APLs in the genesis of coagulopathy associated with COVID-19 and those who defend that they are not implicated.

By monitoring APLs in severe COVID-19 patients, we observed two principal statuses: transient positivity of APLs with no clinical impact, and a secondary occurrence of IgG aβ2GP1 and IgG aCL with high titers associated to a poor clinical course in two among nine monitored patients. This makes us suppose that APLs can be used as one of the markers to predict the prognosis in critically ill COVID-19 patients, whether or not they are involved in the occurrence of thromboembolic complications.

The first clinical condition characterized by the positivity of APLs since the initial admission (patients 1 and 2) may suggest pre-existing or concomitant APLs with the onset of COVID-19, which requires, on the one hand, an in-depth investigation of the medical history of the patients, and also suggests the potential role of SARS-CoV2 in inducing acute thromboembolic phenomena. A significant percentage of 57% and a large variety of APLs were detected in patients with severe COVID-19 [10]. These APLs may be associated with a hyperinflammatory state and thromboembolic events. The clinical and biological characteristics of the coagulopathy encountered in COVID-19 may resemble those of catastrophic antiphospholipid syndrome, but these two entities appear to be pathophysiologically different [10]. APLs in COVID-19 patients are mainly directed against β2GPI but display an epitope specificity different from antibodies in antiphospholipid syndrome [4]. Also, patients hospitalized in medicine wards for non-severe COVID-19 have been found to have APLs in 64% of cases, even with mild or transient levels, and these were significantly associated with the occurrence of thrombotic events [11].

Table 2. Antiphospholipid antibodies results at day-1 and day-15 of the monitoring.

Antiphospholipid antibodies																		
	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7		Patient 8		Patient 9	
	1st	15th	1st	15th	1st	15th	1st	15th	1st	15th	1st	15th	1st	15th	1st	15th	1st	15th
Anticardiolipin IgM (CU*)	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anticardiolipin IgG (CU)	24.8	-	-	-	-	-	-	76	-	-	-	-	-	-	-	-	-	-
anti-β2-glycoprotein I IgM (CU)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
anti-β2-glycoprotein I IgG (CU)	94.9	-	31.4	21.1	-	49.8	-	-	-	-	-	-	-	-	-	-	-	-
Clinical course	Favorable		Favorable		Death		Death		Favorable		Favorable		Favorable		Favorable		Favorable	

* CU: Chemiluminescence Unit, (-):< 20 CU.

In a recently published cohort of 172 COVID-19 patients, a positivity of APLs was observed in many patients since the first day of hospitalization, with variable titers [12]. The authors did not find a pre-existing APL syndrome, but demonstrated that the APLs associated with SARS-CoV2 are potentially pathogenic. Using mouse models, purified IgG APLs fractions from COVID-19 patients induced neutrophil activation similarly to those isolated from individuals with established APS [12]. Moreover, neutrophil mediators have been shown to promote inflammation and microvascular thrombosis during COVID-19 [13].

Interestingly, our first two cases with positive APLs at day-1 had no thrombotic complications, but had biological abnormalities as well as mild APLs levels, which turned to negative at day-15 in one case while the second case remained

slightly positive but around the cut-off. Then, it is legitimate to raise the question whether the anticoagulant treatment that had been administered to our patients prevented the occurrence of thrombotic events. Similarly, Devreese et al, reported two cases of triple positive APLs (IgG aCL, IgG aB2GP1 and Lupus anti-coagulant) among 31 COVID-19 patients. When retested after one month, the first patient whose IgG aCL were mildly positive became negative and his IgG aB2GP1 persisted with lower titers. In the second patient, repeated testing was negative. Both patients had no thromboembolic complications and showed good clinical outcome [14]. APLs show a low prevalence in COVID-19 patients and are not associated with major thrombotic events [4, 15]. Indeed, multiple acute infections, inflammation or thrombosis are known to trigger transient antiphospholipid antibodies [15, 16,

17], which must be taken into consideration to cautiously interpret the results of these auto-antibodies in general and establish their real clinical relevance [18].

In a Chinese study [7], Among three critical groups with similar clinical and laboratory features, the authors concluded that APLs may be helpful in predicting the poor clinical outcome if they are multiple and at least one APL is at moderate titers (>40 Units). Moderate and high titers of APLs correlate with various clinical and biological parameters [13]. Furthermore, the level of IgG aCL autoantibodies has been reported as an independent risk factor for COVID-19 severity, and could serve as a simple strategy to stratify COVID-19 patients according to disease severity and help in the therapeutic decision process [19].

Similarly, our two deceased patients had either IgG aB2GP1 or IgG aCL with moderate (49, 8 CU) and high (76 CU) titers respectively.

5. Conclusions

Our series revealed two main profiles of APL occurring in severe COVID-19: a favourable course in two cases of transient APL and a pejorative course for the two cases whose APL have risen significantly during the course of the disease. These findings consolidate the few data reported in literature. However, the clinical significance of these auto-antibodies remains poorly understood and requires more dedicated investigations on larger sample size with a long-term monitoring, including lupus anti-coagulant and even other APL specificities, in order to improve our knowledge about the potential pathogenic role of APL in COVID-19.

Disclosure Statement

The authors declare that they have no competing interests

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