Original Article

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TANAFFOS

Lupus Anticoagulant Is Associated with Critical Cases and High Mortality in COVID-19: A Literature Review

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Background: In severe COVID-19 cases, a hypercoagulable state may occur. Antiphospholipid syndrome-related auto-antibodies (APSRAs) contribute to coagulopathy, but their role in COVID-19 remains unclear. We aimed to investigate the prevalence of positive APSRAs and their effect on clinical outcomes in confirmed COVID-19 patients.

Materials and Methods: In this cross-sectional study, severe hospitalized COVID-19 cases were enrolled. Demographic and clinical data were obtained from the day of admission. APSRAs including IgG and/or IgM anticardiolipin (aCL) and anti-β2-glycoprotein1 (anti-β2GP1) as well as lupus anticoagulant (LAC) were measured.

Results: In this study, 54 severe COVID-19 cases with positive RT-PCR and chest CT scans were recruited. Positive APSRAs were found in 7 (12.9%) patients. Positive LAC was a more prevalent marker as compared to other tests (11.1%). The prevalence of positive aCL (IgM or IgG) and anti-ß2 GPI (IgM or IgG) was 1.8% (in an elderly woman). Lower oxygen saturation was found in the positive APSRAs group as opposed to the negative APSRAs group (70.3±9 vs. 84.8±9.7%). The mortality rate in the positive APSRAs group was significantly higher relative to the negative APSRAs group (83.3% vs. 27.1%; P-value: 0.01). Likewise, the mechanical ventilation requirement in the positive group was also higher (50% vs. 27.1%, P-value: 0.28).

Conclusion: This study indicated that LAC might be associated with critical cases and high mortality of COVID-19. Nonetheless, the mortality was not related to macrothrombotic incidence.

Keywords: COVID-19; SARS-COV-2; antiphospholipid syndrome; lupus anticoagulant

INTRODUCTION

Since December 2019, the vast spreading of a novel coronavirus known as SARS-CoV-2 has been the main culprit in the recent outbreak of acute respiratory syndrome (SARS) (1). The majority of cases with COVID-19 represent mild symptoms or even remain asymptomatic. On the other hand, rare cases with COVID-19 experience critical illness and subsequent organ failure (2).

In severe cases of COVID-19, the presence of a hypercoagulable state is a menace, leading to several unfavorable clinical outcomes (3,4). SARS-CoV-2 leads to macrovascular and microvascular thrombosis, including dysregulated cytokine release as well as activated leukocytes, endothelium, and platelet using several pathways (5,6). Neutrophil extracellular traps can also contribute to prothrombotic milieu (7).

Antiphospholipid syndrome (APS) as an acquired thrombophilia affects 2-3 in 100.000 individuals (8). APS includes venous and arterial thrombosis with expressing myriads of clinical manifestations (9). APS-related autoantibodies (aPL) are IgG and/or IgM anticardiolipin (aCL) and anti-β2-glycoprotein1 (anti-β2GP1) as well as lupus anticoagulant (LAC) (9,10). However, aPL is not unique to APS and can be detected in other clinical conditions, including autoimmune disorders, medications, or infectious diseases (11). aPL was also detected during viral infection, but the exact pathogenicity is still controversial. Some studies have shown that there is an association between aPL and thrombosis in COVID-19 (6,12). On the other hand, the American Society of Hematology recently stated: "Currently, the data on aPL in COVID-19 are very limited, and it is not clear if they represent an epiphenomenon or if they are really involved in any hemostatic abnormality in the COVID-19 disease" (13).

It has not been definitely proven whether COVID-19 is associated with aPL and the clinical outcome, as there is no control group of COVID-19 patients with negative aPL available. Therefore, we decided to investigate the prevalence of positive aPL and their impact on the clinical outcome of confirmed COVID-19 patients.

MATERIALS AND METHODS

In this cross-sectional study, severe hospitalized COVID-19 cases based on WHO criteria and definitions were enrolled. All the participants were above 18 years old. COVID-19 infection was confirmed by RT-PCR, CT-scan, and clinical manifestation.

Exclusion Criteria were: 1) Presence of any suspicious history of autoimmune disorders or any thromboembolic events 2) Previous history of COVID-19 3) Receiving any medications interfering with detection of aPL including corticosteroids, heparin, and interferon (α and β) prior to the sampling procedure.

Specimen preparation

Prior to prescription of anticoagulant drugs, blood samples were collected into light blue cap vacutainer tubes (nine parts of freshly collected blood with one part of 0.11 mol/L 3.2% sodium citrate) filled to the proper level and into clot activator red top tubes.

For plasma preparation, the light blue cap vacutainer tubes were inverted gently 3-4 times immediately after venipuncture to ensure proper mixing of blood and anticoagulant. Citrate concentration was adjusted in patients who had hematocrit values above 55%. The tubes were centrifuged for 15 minutes at 2500 x g. Plasma was removed, and it was re-centrifuged for 10 minutes at 3000 rpm. Supernatant platelet free plasma was harvested into 1 mL aliquots for Dilute Russell's Viper Venom Time (dRVVT) and mixing tests.

For serum separation, red top tubes were allowed to remain at room temperature for 30 minutes, after which samples were centrifuged at 3000rpm for 15 minutes. For aCL and anti-β2GP1 measurements, 1cc of supernatants were transferred into microtubes and stored at -20 C.

Laboratory experiments

Serum samples were used to measure aCL and anti-β2GP1 IgM and IgG levels. For this purpose, serum samples were allowed to thaw at room temperature. Thereafter, experiments were conducted within less than an hour. In order to obtain reliable results, icteric, lipemic, hemolyzed or bacterially contaminated samples were not used. ELISA method was used for quantitative measurements of aCL IgG and IgM (AESKU, Germany, Lot number: 21040) as well as anti-β2GP1 IgG and IgM (AESKU, Germany, Lot number: 21080). Moreover, freezing-thaw cycles were avoided and all the experiments were conducted according to manufacturer instructions.

Detection of LAC was performed according to the International Society of Thrombosis and Haemostasis (ISTH) Scientific Standardization Committee (SSC) guideline (14). dRVVT test was performed using lupus anticoagulant kit (SEKISUI, Japan) according to manufacturer protocols. Briefly, the kit employed reagent 1

(low phospholipids concentration and Dilute Russell's viper venom) and reagent 2 (high phospholipids concentration and Dilute Russell's viper venom). Plasma sample (75µL) was mixed with sample dilution (15 µL) at a 37 C Ben Mari. Thereafter, Reagent 1 was added, and clotting time was measured. This process was repeated with reagent 2. To obtain clotting time ratio, the clotting time with reagent 1 was divided by the clotting time with reagent 2. Results equal or more than 1.3 were considered as positive LAC.

We also performed mixing PTT tests for the patients to confirm the presence of LAC. One part of patient's plasma was mixed with one part of normal pooled plasma (NPP). In the first step, clotting times were measured immediately for each patient's plasma, NPP and 1:1 mixed plasma. If immediate mixing study was normal based on Rosner index, incubation for 2 hours was performed at 37 C. After incubation step, patient's plasma and NPP was mixed and clotting times were calculated for patient's plasma, NPP, and 1:1 mixed plasma.

Rosner index was used to interpret the immediate and incubated mixing studies. The Rosner index was calculated using the following formula (15):

Rosner index = (Clotting time of 1:1 mix - clotting time of NPP)/ (Clotting time of the patient's sample) ×100

Rosner index>15 was considered as the presence of an inhibitor such as LAC.

The primary outcome was oxygen saturation, and the secondary outcomes were the mortality rate and the need for mechanical ventilation. The study was approved by the Ethics in Medical Research Committee (ID: IR.SBMU.RETECH.REC.1399.1362).

RESULTS

In this study, 54 severe COVID-19 cases with positive RT-PCR and chest CT scans were recruited. The mean ± SD of the participants' age was 61.8±17.7. The gender distribution of men and women was 57.4% and 42.6%, respectively. The mean ± SD of SPO_2 at admission was 83.2±10.7%. Percentages for ICU admission, ventilation requirement, and mortality were 38.9%, 26.9%, and 33.33, respectively. The symptoms and laboratory information at admission have shown in Table 1.

Table 1. Characteristics of the patients at baseline

Symptoms/Marker	% or Mean ± SD	Symptoms/Marker	% or Mean ± SD
Fever	57.4%	**CRP**	63±79 mg/dl
Cough	83.3%	**ALT**	39±33 mg/dl
Headache	11.1%	**LDH**	526±312 mg/dl
Dyspnea	79.6%	**Hemoglobin**	12.8±2.8 mg/dl
Diarrhea	7.4%	**ESR**	44±28 mg/dl
WBC	7398±6260/ µL	**BUN**	45.7±42 mg/dl
Lymphocyte	14%	**Lactate**	22±15 mg/dl
Platelet	170000±67400/ µL	**PT**	10.8±3.3 second

Seven patients (12.9%) had positive aPL. The frequency of positive LAC was higher as compared to the other aPL tests. Positive aCL (IgM or IgG) and anti-ß2 GPI (IgM or IgG) rate was 1.8% (in an elderly woman who had no past medical history except hypertension). Oxygen saturation was lower in patients with positive aPL as compared to those with negative aPL (70.3±9 vs 84.8±9.7%). In patients with positive aPL, the mortality rate was higher than that in patients with negative aPL (83.3% vs 27.1%; P-value: 0.01). Mechanical ventilation requirement was observed in 50% of aPL-positive cases and 27.1% of aPL-negative cases (P-value: 0.28). The mortality rate was significantly higher in the patients with lower oxygen saturation at admission (P: 0.001), higher respiratory rate (P:0.032), lower lymphocyte count (P:0.03), higher LDH (P:0.001), prolonged INR (P:0.04), higher urea and creatinine (P:0.002), higher ESR (P:0.03), and higher serum lactate (P:0.028). However, there was no association between the levels of CRP, ferritin, and D-dimer with mortality rate.

Purpuric and ecchymotic rashes were observed in ten patients in our study. The prevalence of non-specific maculopapular rash was significantly higher in the aPL-positive patients compared to the aPL-negative patients (66.7% vs. 12.5%, P: 0.008)

DISCUSSION

In this study, we observed a high positive LAC rate in patients with critical COVID-19. In contrast to aPL-positive patients, aPL-negative results were associated with better outcomes including adequate oxygen saturation, lower mechanical ventilation requirements, and better survival rates. Positive LAC can be found in a variety of clinical conditions including infections, malignancies, autoimmunity, and any inflammatory stimuli due to cellular damage (16). Some investigations have researched the association between aPL and viral infections. A study reported a high prevalence of positive aCL in some viral infections including HIV (56%), Epstein-Barr virus (EBV) (50%), and hepatitis C virus (HCV) (21%) (17). However, other studies have shown that in the same population with HIV, HCV, and hepatitis B virus (HBV) infections, the LAC-positive rates were 2%, <1%, and 1%, respectively (18).

Interestingly, our results showed that when compared to some viral infections, the prevalence of aCL and anti-β2GP1 positivity was lower in COVID-19 patients. On the other hand, positive LAC was more common in COVID-19 cases. The high prevalence of positive aPL in COVID-19 patients may be due to the significant or massive cell destruction in COVID-19. According to a retrospective study, LAC was found to be positive in 37% of COVID-19 patients. All participants in this study were negative for aCL and anti-β2 glycoprotein (19). Also, only one patient in our study was positive for aCL and anti-β2 glycoprotein. Previous studies have mentioned that positive aPL was common in critically ill patients (20). This can be explained by extensive cell damage in critically ill patients, which in turn leads to aPL production (21,22).

One study examined the association between positive LAC and thrombotic complications in lung cancer cases. Positive LAC in lung cancer patients was related to shortened survival time. Also, it correlated with the progression of hypercoagulable state and venous thrombotic complications (23). In our study, the mortality was not related to macro-thrombotic incidence. In agreement with our report, some studies showed that the positive aPL was not associated with major thrombotic events (12,14,24-32). Studies have also confirmed that positive LAC cannot predict the incidence of thrombosis, nor can it help determine anticoagulant therapy (32,33). However, in another study, higher titers of aPL were associated with neutrophil hyperactivity including the release of neutrophil extracellular traps (NETs), higher platelet counts, severity of COVID-19, and the acceleration of venous thrombosis (6).

Moreover, in some studies, there was an association between incidence of thrombosis and positive aPL (34, 35). In a study, patients with aPLs had a significantly higher incidence of cerebral infraction (20). A case series that analyzed critically ill patients with COVID-19, showed a significantly higher prevalence of antiphospholipid antibodies observed in patients with ischemic stroke (35).

In our study, the incidence of macrovascular events in LAC-positive patients was not high. This may be underdiagnosed in critically ill patients. Microvascular thrombosis may occur and cause organ dysfunction, but without a tissue biopsy, clinical evaluation and detection of microvascular events are usually impossible. A recent study has shown that patients with positive aPL have a poorer prognosis but the difference was not significantly proven (36). In a cohort study based on RT-PCR, the positive LAC rate in COVID-19 cases was higher than in COVID-19 negative patients. The study also showed that positive LAC was independently associated with thrombosis. In terms of mortality and the need for mechanical ventilation, no differences were found between LAC-positive and negative COVID-19 cases (34). Some studies indicated there is no association between positive aPL and severe COVID-19 (26, 28-31, 34). However, in a study the death due to COVID-19 complications was higher in aPL-positive patients (37) and in another study, antibodies were significantly associated with severe COVID-19 (38). In our investigation, LAC-positive patients showed higher mortality and lower oxygenation. We also provided some studies about the association between aPL antibodies and COVID-19 (Table 2).

Table 2. Summary of studies about aPLs antibodies in COVID-19

Study	Number of covid-19 patients	Number of aPL positive	Link to covid-19 severity	Link to thrombosis events
Lerma et al. 2020 (26)	64	9.4%	not related	not related
Galeano-Valle et al. 2020(25)	24	8.3%	–	not related
Gatto et al. 2020 (27)	122	18%	–	not related
Siguret et al. 2020 (28)	74	12%	not related	not related
Fan et al. 2020 (35)	86	37.5%	A significantly higher prevalence of antiphospholipid antibodies was observed in patients with ischemic stroke than in those without stroke.	Related
Rothstein et al. 2020 (29)	844	aPL present in 7/9 (78%) tested patients with ischemic stroke	Not related	Not related
Devreese et al. 2020 (44)	31	74.2%	–	Not related
Reyes et al. 2020 (34)	68	44%	Not related	LA was found to be independently associated with thrombosis
de Ocáriz et al. 2020 (32)	27	22.2%	–	Not related
Bertin et al. 2020 (12)	31	51.6%	–	Not related
Ferrari et al. 2020 (31)	89	71.9%	Not related	Not related
Hasan Ali et al. 2020 (38)	64	–	Antibodies significantly associated with severe COVID-19	–
Xiao et al. 2020 (20)	79	47%	–	Patients with multiple aPLs had a significantly higher incidence of cerebral infarction compared to patients
Borghi et al. 2020 (24)	123	IgG/IgM aCL =5.7/6.6% anti-b2GPI IgG/IgA/IgM 15.6/6.6/9.0% of patients	–	Not related
Pascolini et al. 2021 (37)	33	45%	Related Patients with poor prognosis (death due to COVID-19 complications) had higher frequency of auto-antibodies.	–
Espinosa G, 2022 (30)	158	Patients were positive for at least one classification criteria aPL = 23.4% patients were positive for at least one non-criteria apL= 19%	Not related	Not related

It is currently believed that aPL affects endothelial and circulating monocytes to produce cytokines including interleukin 6 (IL6) and tumor necrosis factor (TNF) (39,40). Consequently, the production of cytokines and inflammation makes COVID-19 worse.

As mentioned in the guidelines, the use of heparin will produce false positive results for LAC; therefore, LAC testing should be performed at least 12 hours after administration of heparin (41, 42). We took this into account for all patients and excluded patients whom had been treated before sampling. A new meta-analysis reported a greater prevalence of the positive LAC test than ours (18). One of the reasons for this difference is neglecting this fact by many studies in meta-analysis.

According to the guidelines of the British Hematology Standards Committee, the preventive dose of heparin has little effect on the LAC test results (42). In any case, the risk of false positive results in connection with the administration of heparin or other anticoagulants was not included in our study.

In addition, as C-reactive protein (CRP) has a high affinity for phospholipids, elevated CRP can lead to false positive LAC in an infection episode (43). The CRP levels in our study were not significantly different between LAC-positive and negative patients. Therefore, the risk of false positive LAC due to elevated CRP was reduced. Moreover, the difference in mortality rate and severity of COVID-19 between LAC-positive and LAC-negative patients was not associated with CRP. In the study of Devreese et al., ten LAC-positive COVID-19 patients were retested one month later, and 9 of them turned negative. This study shows that aPL is mostly temporary and may be an artifact caused by the acute phase of the infection and increased levels of CRP and fibrinogen (44).

The small sample size was the most important limitation of our study. On the other hand, it is better to compare all patients with and without autoimmune disorders and other exclusion criteria belonging to our study to detect the true positivity and also the nature and trend of changes in serology of well-known patients with positive aPL. Also, retesting is not used for persistence of aPL/LA.

CONCLUSION

Although there was no evidence that positive LAC directly leads to decreased survival time, the study indicated that positive LAC may be associated with critical cases and high mortality of COVID-19. It is a long way to determine the distinct pathophysiologic effects of wandering aPL in critical patients: true effect or innocent bystander.

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Conflict of interests

We declare no competing interests.

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