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ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH COVID-19: TREND OVER TIME

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ABSTRACT

Purpose Aim of the study was to investigate whether aPL positivity correlated with thrombosis in COVID-19 patients and whether it was transient or persistent.

Methods We enrolled COVID-19 patients who underwent aPL tests: Lupus Anticoagulant (LA); IgM, IgG, IgA anticardiolipin antibodies (aCL); and IgM, IgG anti- β 2-Glycoprotein-I antibodies (a β 2GPI).

Results Twenty-eight out of 73 (38.4%) patients resulted positive for at least one aPL assay: 32.8% for IgA aCL, 6.8% for IgM aCL and 4.1% for IgM a β 2GPI. No patients tested positive for IgG aPL or LA at the first determination. Seven (9.6%) patients developed thrombotic events during hospitalization, with 4 of them resulting positive for aPL.

In patients with thrombotic events during hospitalization the risk of death was increased 9-fold (LR+8.9, $p=0.003$). Patients with double positivity for aCL and a β 2GPI IgM had a LR+ of 6.3 to have thrombotic events ($p=0.012$) and a LR+ of 4.9 to have elevated D-dimer levels ($p=0.027$). In 10 out of 28 positive patients, aPL was detected in a second occasion at least 12-weeks apart and two patients confirmed the positivity.

Conclusion Results suggest that double positivity for aCL and a β 2GPI IgM increases the risk of thrombosis in COVID-19, unlike IgA aCL positivity. APL positivity may be persistent, and it is advisable to monitor it over time.

Keywords: antiphospholipid antibodies, COVID-19, thrombosis, persistent positivity

INTRODUCTION

Since the beginning of the SARS-CoV-2 outbreak, antiphospholipid antibodies (aPL), a known thrombotic risk factor, have been studied in COVID-19 patients, in whom thromboembolic events have been associated with poor prognosis [1].

The association between infections and aPL is historically known, causing transient positivity for aPL or representing the so-called "second hit" in causing thrombosis in APS patients [2].

The prevalence of aPL in COVID-19 patients is variable, ranging from 8.3% to 74% [3-6]. Furthermore, these studies are heterogeneous and difficult to compare: in some studies, aPL has been searched in critical COVID-19 patients, a condition associated with a greater thrombotic risk [5], in others aPL has been tested in patients with varying degrees of COVID-19 severity [7]. In addition, aPL levels are not reported in all studies and LA was sometimes performed in patients on anticoagulant therapy, with the risk of false positivity [8,9]. To date, the pathogenetic role of aPL and the antibodies trend over time are still unknown.

Aim of the present study was to investigate whether aPL positivity correlated with thrombosis in COVID-19 patients and whether it was transient or persistent.

PATIENTS AND METHODS

PATIENTS

We included all consecutive COVID-19 patients hospitalized in one of the COVID-19 Units of the Policlinico Umberto I, Sapienza University of Rome from April 1, 2020 to June 7, 2020 in this prospective study. The diagnosis of SARS-CoV-2 infection was confirmed in all the patients by nasopharyngeal swab for SARS-CoV-2 on real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR). Patients' exclusion criteria were: non-COVID-19 patients and patients affected by autoimmune inflammatory rheumatic diseases (AIIRD).

METHODS

IgG and IgM aCL and a β 2-GPI were detected by chemiluminescence assay using Zenit RA Immunoanalyzer (A. Menarini Diagnostics, Florence, Italy), IgA aCL by EliA detection kit by ThermoFisher Scientific (Waltham, USA). LA was analyzed using two coagulation systems, a dilute sensitized activated partial thromboplastin time (aPTT) and a dilute Russell's viper venom time (dRVVT), and then performing a confirm test with reagents and instrumentation by Hemoliance Instrumentation Laboratory, Lexington, MA, USA.

STATISTICAL ANALYSIS

Data are expressed as mean \pm standard deviation (S.D.) or median (interquartile range) according to the distribution of the variables. χ^2 -test or Fisher exact test was used for comparison of categorical variables. P values < 0.05 were considered statistically significant. SPSS 20.0 statistical software package (SPSS Inc., Chicago, IL, USA) was utilized for all statistical tests.

RESULTS

We enrolled 73 hospitalized patients affected by COVID-19 (40 females and 33 males, all Caucasian) with a mean age of 65 years (S.D. 17.6). Clinical and demographic characteristics of COVID-19 are reported in Table 1. The most common symptoms at disease onset were fever (82%), dyspnea (60%) and cough (31.5%); the median time from appearance of the first symptoms to hospital admission and positive nasopharyngeal swab for SARS-CoV-2 on RT-PCR assay was 5 days (IQR 9.25). In 64 out of 73 patients (87.7%) bilateral distribution of patchy shadows or ground-glass opacity were found in high resolution computed tomography (HRCT) of the chest, the remaining patients did not show any sign of pneumonia. Almost all patients (75%) reported at least one comorbidity; in addition, 8 (10.9%) patients presented a medical history of arterial thrombosis and one (1.4%) patient reported an history of venous thrombosis.

Twenty-eight out of 73 (38.4%) patients were found positive for at least one aPL assay at low-medium titer. Twenty-four out of 73 (32.8%) patients showed positivity for IgA aCL, 5/73 (6.8%) for IgM aCL and 3/73 (4.1%) for IgM a β 2GPI. Among the positive patients 24 out 28 (85.7%) patients showed positivity for IgA aCL, 5/28 (17.8%) were found positive for IgM aCL and 3/28 (10.7%) for IgM a β 2GPI. IgG aCl and IgG a β 2GPI were negative in all patients. At the time of hospitalization, LA test was performed only in 12 patients who were not on anticoagulant therapy, no patients tested positive.

Seven out 73 (9.6%) patients developed thrombotic events during hospitalization, among them 4 (57%) tested positive for aPL (Table 2).

We observed that the risk of death was 9-fold higher in patients with thrombotic events during hospitalization [likelihood positive ratio (LR+) 8.9, $p=0.003$] and that patients with double positivity for IgM aCL and IgM $\alpha 2$ GPI had a LR+ of 6.3 to have thrombotic events ($p=0.012$) and a LR+ of 4.9 to have an increase in D-dimer levels ($p=0.027$). IgA aCL, the most prevalent aPL, did not result associated with thrombosis.

At follow-up, 10 out of the 28 aPL positive patients died, 8/28 were lost to follow-up. Ten aPL positive patients, 3 men and 7 women [mean age of 55.9 years (S.D. 20.8)], were tested for aPL on a second occasion at least 12 weeks apart from the first determination. None of them had a previous medical history of thrombosis, one patient reported an obstetrical history of 2 fetal deaths.

Overall, only 2/10 (20%) patients confirmed the positivity for at least one aPL assay. These patients tested positive for LA while at the first determination were positive for IgA aCL; at baseline LA test was not performed as they were on anticoagulant therapy. Both 2 patients had no previous or recent history of arterial and/or venous thrombosis, pregnancy morbidities or extra-criteria manifestations suggestive of APS.

Among the 4 patients who developed at least one thromboembolic event during hospitalization and who tested positive for aPL at the first determination, it was possible to repeat the aPL tests after 12 weeks only in one patient (a 74 years-old female) who had experienced three thrombotic events (myocardial infarction, peripheral arterial thrombosis, peripheral venous thrombosis) and she was found negative. The other 3 patients died during hospitalization due to complications of SARS-CoV-2 infection.

DISCUSSION

The results of the present study confirm that thrombotic events increase the risk of death in COVID-19 patients. In addition, we showed that, in COVID-19 patients, double positivity for IgM aCL and IgM $\alpha 2$ GPI increased the risk of thrombotic events. For the first time, in this study, we evaluated the persistence of aPL after 12 weeks since the first determination.

It's well known that infections may lead to transient aPL positivity without clinical manifestations or trigger aPL-related features. To explain such phenomena various possible mechanisms have been proposed, such as molecular mimicry, post-translational modifications, exposition of self-antigens on the cell surface [10]. A wide variety of

infectious agents have been linked to aPL positivity, such as CMV, EBV, HIV, HBV, HCV and parvovirus B19 [11,12]. In a systematic review, aPL have been detected in 293 patients during 50 different infections. Among these patients, 72 patients fulfilled APS criteria, 128 patients reported a thrombotic event in association with a transient aPL positivity and 93 patients did not show any association between aPL and APS-related features [13]. In patients with SARS-CoV-2 infection, aPL has been detected in a variable range of subjects (8.3-74%) [3-6]. In our study, 38.4% of COVID-19 patients showed positivity for at least one aPL assay at low-medium titer. In agreement with a previous chinese study, the IgA isotype was found to be the most prevalent aPL in our cohort; those authors suggested that SARS-CoV-2 may preferentially induce the IgA isotype of aPL due to SARS-CoV-2 mucosal tropism. [4].

The possible pathogenetic role of aPL in COVID-19 is still far from being clear; we observed that COVID-19 patients with double positivity for IgM aCL and IgM $\alpha\beta$ 2GPI have a 6-fold increased risk of thrombotic events and a 5-fold increased risk of higher D-dimer plasma levels. Besides, thrombotic events appeared to increase the risk of death (LR+=8.9, p=0.003).

Xiao and colleagues evaluated the dynamic changes in aPL levels in 6 COVID-19 patients over a period of 60 to 77 days from disease onset; the results obtained suggest that aPL levels are fluctuating and they encouraged further studies with a long-term follow-up on COVID-19 patients positive for aPL [4]. Indeed, to date, only 2 case reports have tested aPL on a second occasion after at least three months in aPL positive COVID-19 patients [14,15].

At the best of our knowledge, this is the first study in which aPL-positive COVID-19 patients were re-tested after at least 12 weeks apart. The results of our study suggest that the 20% of COVID-19 patients may show persistent positivity for aPL. Given that aPL is a thrombotic risk factor, it may be appropriate to monitor antibodies trend over time and minimize other thrombotic risk factors in patients with SARS-CoV-2 infection.

The main limitations of our study are represented by the small sample size and the one-center design. Our results should be validated in larger and multicenter studies.

CONCLUSIONS

In conclusion, the results of our study confirm that thrombotic events increase the risk of death in COVID-19 patients and indicate that double positivity for aCL and a β 2GPI IgM increases the risk of thrombosis in patients, unlike positivity for IgA aCL.

Antiphospholipid antibodies positivity resulted to be persistent in a small percentage of COVID-19 patients, given that aPL is a thrombotic risk factor, it may be appropriate to monitor antibodies trend over time and minimize other thrombotic risk factors in patients with SARS-CoV-2 infection.

Table 1. Clinical and demographic features of the 73 Covid-19 patients

Features	73 Covid-19 patients
Age – yr mean (S.D.)	65 (17.6)
Female	40
Comorbidities	55
Hypertension	24
Diabetes	15
Cardiovascular disease	9
Chronic obstructive pulmonary disease or asthma	6
Malignancy	6
History of arterial thrombosis	8
History of venous thrombosis	1
Signs and symptoms, median (IQR)	
Fever	60
Dyspnea	44
Cough	23
Diarrhea	4
Chest pain	1
Ageusia/Anosmia	4
HRCT chest:	
Bilateral ground glass opacity	64
Baseline laboratory values, median (IQR)	
Lymphocyte count, cells/L	1350 (1010)
Lactate dehydrogenase, U/L	226 (98.5)
Ferritin ug/L	332.5 (445.5)
D-dimer mcg/L	734 (846)

PaO₂:FIO₂, mmHg	376 (132)
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Table 2. Clinical and demographic features of the 7 Covid-19 patients that developed thrombotic events

Features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age - yr	67	78	83	43	70	74	95
Sex	female	female	female	male	male	female	male
Medical History	Malignancy, Hypertension	Stroke	Chronic obstructive pulmonary disease	No medical history	Chronic obstructive pulmonary disease, Hypertension	Malignancy,	Hypertension
Initial findings							
Signs and symptoms	Dyspnea	Dyspnea	Dyspnea	Fever, ageusia/anosmia, chest pain	Fever, cough	Dyspnea	Dyspnea
HRCT chest: Bilateral ground glass opacity	yes	yes	yes	yes	yes	yes	yes
Baseline laboratory values							
Lymphocyte count, cells x 10 ⁶ /L	220	210	2330	1580	1680	600	1390
Lactate dehydrogenase, U/L	223	321	199	227	226	349	199
Ferritin mcg/L	614	317	213	387	462	2455	197
D-dimer mcg/L	730	1213	2912	282	688	1298	1097
PaO₂:FIO₂, mm Hg	132	120	442	534	348	493	314
Anticoagulant therapy at the time of the thrombotic event	Therapeutic dosage	Prophylactic dosage	Prophylactic dosage	Not administered	Therapeutic dosage	Therapeutic dosage	Therapeutic dosage
Thrombotic events	Stroke	Pulmonary embolism	Peripheral venous thrombosis	Myocardial infarction	Pulmonary embolism	Myocardial infarction, peripheral arterial thrombosis, peripheral venous thrombosis	Peripheral venous thrombosis
Antiphospholipid antibodies	IgA aCL	IgM aCl 24 MPL, anti-IgM β2glycoprotein I 25 U/mL	negative	negative	negative	IgM aCl (23 MPL), anti-IgM β2glycoprotein I 22 U/mL	IgA aCL
Outcome	Exitus	Exitus	Suicide	Discharged	Discharged	Discharged	Exitus
Antiphospholipid antibodies tested after at least 12 weeks	NP	NP	NP	NP	NP	Negative	NP

Declarations Not applicable

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Conflicts of interest ‘None declared’

Availability of data and material Data that support the findings of this study are available on request from the corresponding author.

Code availability Research code are available on request from the corresponding author.

Authors' contributions *Simona Truglia and Silvia Mancuso*: Conceptualization, Methodology, Validation. *Maurizio Sorice*: Methodology, Validation, Writing- Reviewing and Editing. *Cristiano Alessandri, Claudio Maria Mastroianni, Roberta Misasi, Valeria Ricciari*: Data curation, Writing- Original draft preparation.

Roberta Misasi, Flavia Pasquali: Formal analysis. **Antonella Capozzi, Gloria Riitano:** Investigation. **Fabrizio Conti:** Supervision, Validation.

Ethics approval This study was approved by the local ethic committees.

Consent to participate All participants gave written informed consent.

Consent for publication All participants gave written consent for publication.

REFERENCES

- [1] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3. Epub 2020 Mar 11. Erratum in: *Lancet* 2020;395:1054-62.
- [2] Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. *Semin Arthritis Rheum* 2002;31:256-63.
- [3] Galeano-Valle F, Oblitas CM, Ferreiro-Mazón MM, Alonso-Muñoz J, Del Toro-Cervera J, di Natale M, et al. Antiphospholipid antibodies are not elevated in patients with severe COVID-19 pneumonia and venous thromboembolism. *Thromb Res* 2020;192:113-5.
- [4] Xiao M, Zhang Y, Zhang S, Qin X, Xia P, Cao W, et al. Antiphospholipid Antibodies in Critically Ill Patients With COVID-19. *Arthritis Rheumatol* 2020;72:1998-2004.
- [5] Pineton de Chambrun M, Frere C, Miyara M, Amoura Z, Martin-Toutain I, Mathian A, et al. High frequency of Antiphospholipid Antibodies in Critically-ill COVID-19 Patients: a Link with Hypercoagulability? *J Intern Med* 2021;289:422-4

- [6] Devreese KMJ, Linskens EA, Benoit D, Peperstraete H. Antiphospholipid antibodies in patients with COVID-19: A relevant observation? *J Thromb Haemost* 2020;18:2191-201.
- [7] Borghi MO, Beltagy A, Garrafa E, Curreli D, Cecchini G, Bodio C, et al. Anti-Phospholipid Antibodies in COVID-19 Are Different From Those Detectable in the Anti-Phospholipid Syndrome. *Front Immunol* 2020;11:584241.
- [8] Bowles L, Platton S, Yartey N, Dave M, Lee K, Hart DP, et al. Lupus Anticoagulant and Abnormal Coagulation Tests in Patients with Covid-19. *N Engl J Med* 2020;383:288-90
- [9] Harzallah I, Debliquis A, Drénou B. Lupus anticoagulant is frequent in patients with Covid-19. *J Thromb Haemost* 2020;18:2064–5.
- [10] Radic M, Pattanaik D. Cellular and Molecular Mechanisms of Anti-Phospholipid Syndrome. *Front Immunol* 2018;9:969.
- [11] Cervera R, Asherson RA. Antiphospholipid syndrome associated with infections: clinical and microbiological characteristics. *Immunobiology* 2005;210:735-41.
- [12] Sene D, Piette JC, Cacoub P. Antiphospholipid antibodies, antiphospholipid syndrome and viral infections. *Rev Med Interne* 2009;30:135-41
- [13] Abdel-Wahab N, Lopez-Olivo MA, Pinto-Patarroyo GP, Suarez-Almazor ME. Systematic review of case reports of antiphospholipid syndrome following infection. *Lupus* 2016;25:1520-31.

- [14] Loos CMJ, Yperzeele L, Jadoul C, Baar I, Jorens PG. Deep cerebral venous sinus thrombosis with transient antiphospholipid antibodies in COVID-19 disease. *Acta Neurologica Belgica* 2021;2:1-3.
- [15] Balanchivadze N, Xie P, Kuriakose P, Barthel B, Dabak V. Transient Anti-Phospholipid Antibodies in Two Patients With COVID-19. *Cureus*. 2021;13:e13026.