Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Antiphospholipid antibodies are not elevated in patients with severe COVID-19 pneumonia and venous thromboembolism

1. Introduction

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are the main manifestations of venous thromboembolism (VTE). The causes for VTE are multifactorial and are not readily apparent in many cases. Hypercoagulable states (including antiphospholipid syndrome), are considered risk factors for the development of VTE [1]. Hemostatic abnormalities have been described in patients with novel coronavirus disease 2019 (COVID-19), including mild thrombocytopenia and increased D-dimer levels [2,3]. Thromboprophylaxis seems to be associated with lower mortality and is recommended for patients admitted with COVID-19 unless contraindicated [4]. An increased risk of VTE has recently been suggested in intensive care unit (ICU) patients with COVID-19 infection despite adequate thromboprophylaxis [5,6]. A Chinese center reported a case series of three patients with COVID-19 pneumonia and thrombosis associated with antiphospholipid antibodies represented by anticardiolipin (aCL) and anti–β2-glycoprotein I (aβ2GPI). No lupus anticoagulant was detected in any of these patients [7]. A French group published a brief letter studying 56 patients of which 25 patients (45%) were positive for lupus anticoagulant (LAC), while aCL or aβ2GPI were detected in only 5 out of 50 tested patients (10%, 3 associated to LAC) using IgG and IgM detection. However, the investigators did not report thrombotic complications in these patients [8]. These findings could suggest a role of antiphospholipid antibodies or lupus anticoagulant in the pathogenesis of thrombosis (either arterial or venous) in patients with COVID-19 pneumonia. The aim of our study was to evaluate the presence of antiphospholipid antibodies in hospitalized patients with COVID-19 pneumonia and confirmed VTE.

2. Material and method

This was a prospective observational study performed at a third-level hospital in Madrid. From March 26 to April 15, 2020, all consecutive patients hospitalized in Internal Medicine ward with diagnosis of COVID-19 pneumonia and diagnosed with symptomatic deep vein thrombosis (DVT) or pulmonary embolism (PE) confirmed by objective tests (compression ultrasonography for suspected DVT; helical computed tomography [CT] scan for suspected PE) were screened for antiphospholipid antibodies. Patients were included in the study if they were older than 18 years. COVID-19 diagnosis was defined by positive PCR in nasopharyngeal swab or by the presence of radiological and analytical findings highly suggestive of the disease. Patients were excluded if they had a previous diagnosis of antiphospholipid syndrome or previous assessment of antiphospholipid antibodies.

Patients included in the study were tested for antiphospholipid antibodies: anticardiolipin (aCL) and anti–β2-glycoprotein I (aβ2GPI). Antibodies were detected by indirect solid phase ELISA (ORG 515, Orgentec®) for anticardiolipin antibodies (normal range was: IgM aCL 0–7 U/mL, IgG aCL 0–10 U/mL) and by indirect solid phase ELISA (ORG 521, Orgentec®) for aβ2GPI antibodies (normal range was: IgM aβ2GPI 0–8 U/mL, IgG aβ2GPI 0–8 U/mL) and by indirect solid phase ELISA (ORG 521, Orgentec®) for anticardiolipin antibodies (normal range was: IgM aCL 0–7 U/mL, IgG aCL 0–10 U/mL) and by indirect solid phase ELISA (ORG 521, Orgentec®) for aβ2GPI antibodies (normal range was: IgM aβ2GPI 0–8 U/mL, IgG aβ2GPI 0–8 U/mL). Lupus anticoagulant was not assessed since testing is not recommended in acutely ill patients and under anticoagulant therapy. Oral informed consent was obtained in all patients prior to their participation in the study. The Institutional Ethics Committee approved the study. The authors received no specific funding for this work. Local protocol for thromboprophylaxis consisted in enoxaparin 40 mg per day or bemiparin 3500 UI per day.

3. Results

The study comprised 24 patients. During the study period, there were 785 patients admitted to Internal Medicine ward with diagnosis of COVID-19; thus, incidence of VTE in these population was 3.0% (95% IC 1.8–4.3%). Of these, 367 were discharged at the time of analysis; the incidence of VTE in these population was 6.5% (95% IC 4.2–9.6%). All but five patients received standard doses of thromboprophylaxis prior to VTE diagnosis. Mean age of the sample was 64.3 (SD 14.4) and 58.3% were male. The clinical and laboratory characteristics of the sample at the diagnosis of VTE are summarized in Table 1. None of them had known thrombophilia, recent long travel or pregnancy. Six patients (25%) were diagnosed with VTE on admission. For the rest of patients, median days from admission to VTE diagnosis were 14 (IQR 9.5–18). Eleven (45.8%) patients presented PE alone, nine (37.5%) patients presented DVT alone and four (16.6%) patients presented PE and DVT. Among patients with PE (n = 15), 6 (40%) patients had intermediate-risk PE and 9 (60%) patients had high risk PE, respectively. Location of the thrombosis is included in Table 1. No episodes of arterial thrombosis were observed. Two patients (8.3%) were weakly positive for anticardiolipin IgM (19.3 U/mL and 14.1 U/mL and 16.2 U/mL, respectively [normal range: 0–8 U/mL]) and anti–β2-glycoprotein I IgM (14.1 U/mL and 16.2 U/mL, respectively [normal range: 0–8 U/mL]). Anticardiolipin IgG and anti–β2-glycoprotein I IgG were negative in all patients.

4. Discussion

COVID-19, a viral respiratory illness caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), may predispose patients to thrombotic disease, both in the venous and arterial circulation, due to excessive inflammation, platelet activation, endothelial dysfunction, and stasis [2,3]. However, scarce data has been published about the incidence of VTE in these patients. A multicenter study of 184 patients with severe COVID-19 reported a VTE cumulative incidence of 27% (95% CI 17–37%) despite adequate thromboprophylaxis [1]. A single-center study from Italy found a cumulative incidence of 21% for thromboembolic events (both arterial and venous), with the majority of
patients receiving thromboprophylaxis [9]. Another single-center study from France also reported an incidence of PE of 20.6% [10]. These results suggested that VTE could remain underdiagnosed in patients with severe COVID-19.

Table 1
Clinical characteristics and laboratory tests of hospitalized patients with COVID-19 pneumonia and venous thromboembolism.

| Variable | N = 24 |
|----------|--------|
| Male, n (%) | 14 (58.3) |
| Ethnicity, n (%) | 21 (87.5) |
| Caucasian | 21 (87.5) |
| Latin American | 2 (8.3) |
| Other | 1 (4.1) |
| Previous VTE, n (%) | 2 (8.3) |
| Surgery (< 2 months), n (%) | 2 (8.3) |
| Active cancer, n (%) | 1 (4.3) |
| Body mass index, kg/m² (SD) | 29.8 (6.2) |

Clinical characteristics

| Variable | N = 24 |
|----------|--------|
| SARS-COV2 confirmed by PCR, n (%) | 22 (91.6) |
| Required ICU admission, n (%) | 3 (12.5) |
| Bilateral pulmonary infiltrates, n (%) | 21 (87.5) |
| Acute respiratory distress syndrome, n (%) | 18 (75) |
| PaO2/FiO2 200–300 mm Hg, n (%) | 1/18 (5.5) |
| PaO2/FiO2 ≥ 100 mm Hg, n (%) | 4/18 (22.2) |
| PaO2/FiO2 ≤ 100 mm Hg, n (%) | 13/18 (72.2) |
| SOFA (Sequential [Sepsis-Related] Organ Failure Assessment) points, n (%) | 4 (16.6) |
| < 2 | 2 (8.3) |
| ≥ 4 | 16 (66.6) |

VTE presentation

| Variable | N = 24 |
|----------|--------|
| Deep vein thrombosis | 13 (54.1) |
| Pulmonary embolism | 15 (62.5) |
| Principal arteries | 2 (8.3) |
| Lobar arteries | 7 (28.8) |
| Segmental arteries | 13 (86.6) |
| Subsegmental arteries | 7 (28.8) |

Laboratory tests and outcome

| Variable | N = 24 |
|----------|--------|
| Lymphopenia < 1.3 (×10⁹/L), n (%) | 21 (87.5) |
| Thrombocytopenia < 140 (×10⁹/L), n (%) | 3 (12.5) |
| Fibrinogen (mg/dL) > 400 mg/dL, n (%) | 21 (87.5) |
| INR > 1.2, n (%) | 3 (12.5) |
| aPTT (s), > 38 s, n (%) | 1 (4.1) |
| D-dimer, mg/mL median (IQR) | 2549.5 (2376-8899.5) |
| < 1000 mg/mL, n (%) | 1 (4.1) |
| > 1000 mg/mL, n (%) | 23 (95.9) |
| Interleukin-6, > 4 pg/mL, n (%) | 15 (93.7) |
| Ferritin, > 274 ug/L, n (%) | 23 (95.9) |
| LDH, > 225 U/L, n (%) | 21 (87.5) |
| C-reactive protein, mg/L, median (IQR) | 71 (31.7-143.7) |
| Days of hospitalization, median (IQR) | 24 (15–27.2) |
| Clinical outcome, n (%) | 15 (62.5) |
| Death | 0 |
| Hospital discharge | 15 (62.5) |
| Hospitalized at the time of the study period | 9 (37.5) |

aPTT: activated Partial Thromboplastin Time; INR: international normalized ratio; VTE: Venous thromboembolism.

a ARDS defined as the combination of arterial oxygen tension/fractional inspired oxygen (PaO2/FiO2 < 300 mm Hg) and bilateral pulmonary infiltrates.

b More than one location can be affected in the same patient.

c Laboratory tests were measured at the diagnosis of VTE episode. The laboratory parameters has been categorized according to the normal laboratory ranges.

d Available data from 16 patients.

The sample of our study consisted in 24 patients with COVID-19 pneumonia and venous thromboembolism. Among them, 72.2% had severe acute respiratory distress syndrome (ARDS) and 66.6% had > 4 points according SOFA, denoting the markedly high severity of the sample. However, at the time of the study period no patient had died and 62.5% had been discharged. Despite the severity of the clinical presentation, thrombocytopenia was not frequent. On the other hand, markedly high D-dimer levels were found in the majority of the sample. The two previously mentioned publications [7,8] had suggested a role of antiphospholipid antibodies or lupus anticoagulant in patients with COVID-19 pneumonia and thrombotic complications. The first article was a case series of 3 patients from a Chinese center. In this publication, titration of antibodies was not reported and 2/3 patients had a high cardiovascular risk and previous history of thrombosis [7]. The second article was a brief letter studying 56 patients from two French centers. Among them, aCL or aβ2GPI were detected in only 5 out of 50 tested patients (10%, 3 of them associated to lupus anticoagulant) using IgG and IgM detection. Again, the investigators did not report the titres of the antibodies and their sample did not comprise patients with thrombotic events [8]. Antiphospholipid antibodies abnormally target phospholipid proteins, and the presence of these antibodies is central to the diagnosis of the antiphospholipid syndrome. However, these antibodies can also arise transiently in patients with critical illness and various infections [7]. Our study only found 2 (8.3%) patients with anticardiolipin IgM and anti-β2-glycoprotein I IgM weakly positive, suggesting that the presence of antiphospholipid antibodies is not frequent among patients with COVID-19 pneumonia who suffer venous thromboembolism.

This study has several limitations: First, the study was limited to acutely ill patients in non-ICU setting and may not be representative of the whole COVID-19 population. Second, it did not include a serologic confirmation 3 months after the first positive measurement of antiphospholipid antibodies. Third, the restricted sample size could have limited the clinical significance of our findings. Fourth, the prevalence of antiphospholipid antibodies in non-VTE patients is unknown.

In conclusion, the prevalence of antiphospholipid antibodies among COVID-19 patients with VTE in our cohort was low, suggesting that antiphospholipid antibodies might not be involved in the pathogenesis of venous thromboembolism in patients with severe COVID-19 pneumonia.

Declaration of conflict of interest

The authors state that they have no conflict of interest.

Addendum

F. Galeano-Valle, P. Demelo-Rodríguez and J. del-Toro-Cervera designed the study. P. Demelo-Rodríguez, C.M. Oblitas, M.M. Ferreiro-Mazón and J. Alonso-Muñoz collected data. F. Galeano-Valle and P. Demelo-Rodríguez performed statistical analysis and wrote the manuscript. F. Galeano-Valle, P. Demelo-Rodríguez, C.M. Oblitas, M.M. Ferreiro-Mazón, J. Alonso-Muñoz and J. del-Toro-Cervera critically reviewed the manuscript.

References

[1] K.R. Bruni-Fitzgerald, Venous thromboembolism: an overview, J Vasc Nurs. 33 (3) (2015) 95–99.
[2] G. Lippi, M. Plebani, Henry B. Michael, Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis, Clin Chim Acta 506 (2020) 145–148.
[3] G. Lippi, E.J. Favaloro, D-dimer is associated with severity of coronavirus disease, (COVID-19): A Pooled Analysis, Thromb Haemost In press, 2019.
[4] N. Tang, H. Bai, X. Chen, J. Gong, D. Li, Z. Sun, Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy, J Thromb Haemost. 18 (5) (2020) 1094-1099.
[5] F.A. Klok, M.J.H.A. Kruip, N.J.M. van der Meer, M.S. Arbous, D.A.M.P.J. Gommers, J Thromb Haemost. 18 (5) (2020) 1094–1099.
K.M. Kant, et al., Incidence of thrombotic complications in critically ill ICU patients with COVID-19, Thromb Res. (2020) [epub ahead of print].

[6] J.F. Llitjos, M. Leclerc, C. Chochois, J.M. Monsallier, M. Ramakers, M. Auvray, K. Merouani, High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients, J Thromb Haemost. (2020) [Epub ahead of print].

[7] Y. Zhang, M. Xiao, S. Zhang, P. Xia, W. Cao, W. Jiang, et al., Coagulopathy and antiphospholipid antibodies in patients with covid-19, N Engl J Med. 382 (17) (2020) e38.

[8] I. Harzallah, A. Debléquis, B. Dréonou, Lupus anticoagulant is frequent in patients with Covid-19, J Thromb Haemost. (2020) [Epub ahead of print].

[9] C. Lodigiani, G. Iapichino, L. Carenzo, M. Cecconi, P. Ferrazzi, T. Sebastian, et al., Venous and Arterial Thromboembolic Complications in COVID-19 patients. Admitted to an Academic Hospital in Milan, Italy, Thromb Res, 2020 [epub ahead of print].

[10] J. Poissy, J. Goutay, M. Caplan, E. Parmentier, T. Duburcq, F. Lassalle, et al., Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence, Circulation. (2020) [epub ahead of print].