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Letter to the Editors-in-Chief

The pathogenesis of thromboembolic disease in covid-19 patients: Could be a catastrophic antiphospholipid syndrome?

In December 2019 an epidemic of pneumonia caused by SARS-CoV-2, a novel coronavirus, developed from Wuhan, China [1]. The outbreak rapidly spread all over the world and the World Health Organization (WHO) declared the SARS-CoV-2 disease (named COVID-19) a pandemic on 11th March 2020.

About 15–20% of infected patients can develop interstitial pneumonia with alveolar damage and severe Acute Respiratory Distress Syndrome (ARDS), leading in 5% of cases to death [1]. The most severe patients need intensive care and show an increased risk of thromboembolic events, caused by an hyperinflammatory response targeting endothelium and leading to a pro-coagulation state, worsened by hypoxia and prolonged immobilization. These patients can develop pulmonary thrombosis and/or multi-organ failure due to thrombotic microangiopathy [2].

Although some patients meet the diagnostic criteria for the Disseminated Intravascular Coagulation (DIC) [3], there is a group of COVID-19 severely ill patients in which the pathogenesis of the diffuse thrombotic status remains unclear.

The Catastrophic Antiphospholipid Syndrome (CAPS) is a rare, life-threatening condition characterized by multiple thrombosis, affecting mainly small vessels and involving three or more organs, developing in less than a week, and associated with persistent antiphospholipid antibodies (aPL) positivity [4]. Peculiarly CAPS is triggered by precipitating factors and infections, mainly in respiratory tract, are the most frequent events [5].

The clinical course and the autopsy findings of 75 patients died for COVID-19 at Papa Giovanni XXIII Hospital in Bergamo between 19th March and 09th April 2020 suggest that some patients may have developed CAPS and we evaluate the presence of serum aPL to confirm the diagnosis.

Serum samples, collected 24 h before death and frozen at −20 °C, were available only for 35 patients out of 75 autopsies performed (26 male and nine female, ratio 2.88:1; age range 57–92 years, mean and median age 73 years). All the clinical records were evaluated postmortem to collect the available medical history, comorbidities, therapies, and laboratory and autopsy findings.

IgA, IgG and IgM anti cardiolipin antibodies (ACA) and anti β2 glycoprotein 1 (aβ2GP1) antibodies were tested on the BIO-FLASH®

platform (Inova Diagnostics, San Diego, CA, USA) with chemiluminescent methods; to assess positive result the manufacturer's cut off 20 CU (Chemiluminescent Units) was used. IgG and IgM anti phosphatidylserine/prothrombin (aPS/PT) antibodies were measured with a commercial ELISA kit (QUANTA Lite® aPS/PT, Inova Diagnostics, San Diego, CA, USA) on QUANTA-Lyser® 3000 (Inova Diagnostics, San Diego, CA, USA), using the manufacturer's cut off (30 Units). Biochemistry assays were tested on Atellica® Solution (Siemens Healthcare GmbH, Germany) and coagulation profile was assessed on CS-5100 System (Sysmex, Japan), using the manufacturer's cut-offs.

The study was conducted in accordance with the Helsinki Declaration and under the terms of all relevant local legislations. Study approval was obtained from the local ethical committee.

The demographic characteristics of enrolled patients are summarized in Table 1.

The clinical and historical data were available for 29 patients. 24/29 (82.8%) had one or more comorbidities. Three patients (10.3%) were previously diagnosed for autoimmune diseases (two Rheumatoid Arthritis and one Hashimoto Thyroiditis); only one patient was previously treated with oral anticoagulant and seven patients with antiplatelet therapy. All the patients, but one, were treated for COVID-19 infection with a standard therapy composed by antibiotics, hydroxychloroquine and anti-retroviral drugs; in addition, six patients also received prednisone; 13 patients undergone a treatment with low molecular weight heparin (LMWH). A patient died before starting of therapy.

Laboratory features and aPL testing are showed in Table 2

Lactate dehydrogenase, C reactive protein and interleukin-6 were markedly increased in all patients, except for one with slightly increased value for interleukin-6 alone, indicating a hyperinflammatory state.

The coagulation tests showed that all but one patient had increased value for D-Dimer, and in 12/32 patients (38.7%) the value was over 10,000 ng/mL; prothrombin time (PT-INR) and activate thromboplastin time were generally normal or slightly prolonged. Fibrinogen test was performed only in 17/35 patients, with increased values in 9/17 (52.9%) but reduced in one.

32/35 (91.4%) patients had evidence of kidney and/or hearth and/

Table 1
General characteristics of selected patients.

Male/female (ratio)	26/9 (2.9:1)
Age (y) mean (range)	73 (52–82)
Days of hospitalization –median (range)	7 (2–28)
Comorbidity (n°/total patients)	
Information not available	6/35 (17.1%)
Hypertension	17/29 (58.6%)
Cardiovascular Disease (different than hypertension)	10/29 (34.5%)
Diabetes	8/29 (27.6%)
Obesity	4/29 (13.8%)
Kidney disease	5/29 (17.3%)
Liver disease	3/29 (10.3%)
Autoimmune disease	2/29 (6.9%)
Hematological disease	2/29 (6.9%)
Pulmonary disease	1/29 (3.4%)
Gastrointestinal disease	1/29 (3.4%)
No comorbidity	6/29 (20.7%)
Autopsy evidence	35/35 (100%)
(n° of patients with evidence of thrombosis of the small vessels ≥3 organs/total)	

Table 2
Main laboratory findings and antiphospholipid antibodies (aPL) testing.

Test	N° samples (%)	Range	Median (95%CI)
CRP >1 mg/dL	34/35(97.1)	1.8–41.4	15.1 (11.9 to 21.2)
LDH >300 U/L	32/35(91.4)	338–17,109	527 (448 to 687)
IL6 >3.4 pg/mL	35/35(100)	4.7–10,000	129 (73 to 169)
D-Dimer >1000 ng/mL	30/32(93.7)	1367–35,000	8508 (3531 to 23,287)
PT (INR) >1.5	6/35(17.1)	1.53–2.86	1.90 (1.53 to 2.86)
aPTT (Ratio) >1.25	17/33(51)	1.29–2.32	1.49 (1.35 to 1.84)
PLT count <100 × 10⁹/L	2/35(5.7)	36–67	52[a] (NA)
Fibrinogen >450 mg/dL	9/17(52.9)	493–1193	748[a] (584 to 912)
s-Creatinine >1.30 mg/dL	20/35(57.1)	1.35–7.22	2.76 (2.10 to 3.45)
AST >40 U/L	25/34(73.5)	41–7081	78 (65 to 102)
ALT >40 U/L	20/35(57.1)	41–2265	73 (57 to 232)
hsTnI >53 ng/L	9/13(69.2)	174–11,828	305 (174–11,828)
PCT >10 ng/mL	3/19(15.8)	11.8–55.10	23.60[a] (NA)
Ferritin >250 ng/mL	29/34(85.3)	296–16,500	1489 (1116 to2420)
ACA IgG (CU)	35/35(100)	0.8–46.7	3.7 (2.9 to 4.4)
ACA IgM (CU)	35/35(100)	0.7–38.4	2.9 (1.9 to 4.0)
ACA IgA (CU)	35/35(100)	0.8–17.8	4.0 (2.5 to 5.2)
aβ2GP1 IgG (CU)	35/35(100)	1.6–13.9	6.25 a (5.24 to 7.26)
aβ2GP1IgM (CU)	35/35(100)	0.1–9.7	1.2 (0.8 to 1.6)
aβ2GP1 IgA (CU)	35/35(100)	0.0–10.3	0.3 (0.0 to 1.6)
aPS/PT IgG (Units)	35/35(100)	5.5–34.4	8.5 (7.3 to 12.1)
aPS/PT IgM (Units)	35/35(100)	1.2–50.4	7.6 (4.10 to 11.90)

(CRP: C-Reactive Protein; LDH: Lactate Dehydrogenase PT: Prothrombin Time; INR: International Normalized Ratio; aPTT: activated Prothrombin Time; IL6: interleukin-6; PLT: platelet; AST: Aspartate transaminase; ALT: Alanine transaminase; hsTnI: High-sensitivity cardiac troponin I; PCT: Procalcitonin; ACA: Anti-cardiolipin; aβ2GP1: anti β2 glycoprotein 1; aPS/PT: anti phosphatidylserine/prothrombin; NA: not applicable; CU: chemiluminescent units).
[a] Mean value.

or liver damage.

10/35 patients (28.6%) were diagnosed for thromboembolic event with imaging techniques: four pulmonary embolism and two cerebral ischemia.

The autopsy and the following microscopic evaluation on tissues samples demonstrated vascular involvement characterized by the presence of multiple recent microvascular and macrovascular thrombosis and the absence of microangiopathy with neutrophilic endothelial infiltrate in three or more organs in 35/35 (100%) patients, mainly lung, heart, liver and kidney.

We found 3/35 (8.6%) aPL positive patients: one for ACA IgG and two for ACA IgM but all the values were low (< 3× the cut off). No

patients tested positive for ACA IgA neither for aβ2GP1 isotypes. 3/35 (8.6%) patients were positive for aPS/PT, one for IgG and two for IgM, but values were < 2× the cut off. No patients showed simultaneous positivity for ACA and aPS/PT.

Venous and arterial thromboembolic phenomena are well described in a high percentage of COVID-19 patients, showing high levels of D-Dimer and, in some cases, prolonged coagulation tests, especially PT-INR [2].

The thromboembolic disease in COVID-19 could have a multifactorial pathogenesis, [6]. Hypoxia and prolonged immobilization are important factors, but the diffuse inflammatory state and the resulting "cytokine storm" seems to be the main triggers. The autopsies that have been conducted at Papa Giovanni XXIII Hospital in Bergamo, highlighted thrombotic damages in several organs. Several diseases such as DIC, sepsis, macrophage activated syndrome (MAS) and CAPS could be the underlying causes of such damages [4].

Recently some authors have been evidenced that the most COVID-19 patients meet the diagnostic criteria for DIC [7]; in our cohort, the appropriate assays and information were available for 17 (48.6%) patients and only six (17.1%) meet the DIC criteria: platelet count and PT-INR were almost normal and fibrinogen values, when tested, were increased, possibly reflecting the hyperinflammation state; none of our patients except one showed hemorrhages and the histological findings consisting with both microvascular and macrovascular thrombosis did not fit with classical morphological features of DIC.

Likewise, none of our patients fulfilled sepsis clinical criteria and the histological post-mortem findings were not consistent with this assumption.

For macrophage activated syndrome, Carsana et al. [8] reported the presence of mainly monocyte/macrophage infiltration in lung biopsies of COVID-19 patients but normal platelet count, high level of fibrinogen and absence of hepato- and splenomegaly in our cohort of patients, exclude also this hypothesis.

To the best of our knowledge, the presence of antiphospholipid antibodies in literature are reported only in three Chinese patients with multiple brain infarcts revealed with imaging techniques [9]. Our patients fulfilled the main clinical diagnostic criteria for CAPS [4]: evidence of involvement in three or more organs; development of manifestations simultaneously or in less than a week, confirmation by histopathology of small vessel occlusion in at least one organ.

Based on these findings, to meet all the diagnostic criteria of CAPS, it was necessary to demonstrate the presence of aPL [5]. Unfortunately, in our cohort almost all the patients were negative for the aPL profile tested. Only 6/35 (17.1%) patients showed very low and not relevant antibodies levels but it is reported that slightly and transient increase of aPLs could be a common finding during any kind of infectious, whereas CAPS is always characterized by very high levels of autoantibodies [4]. Three conventional aPL markers are described in literature and used to diagnose CAPS: ACA, aβ2GPI and Lupus Anticoagulant (LAC). The main limit of our work it is that our patients were not tested for LAC due to the lack of plasma samples. It is reported in literature a very high concordance between LAC and aPS/PT testing and Litvinova et al. [10] reported that all CAPS patients are positive for both IgG and IgM aPS/PT but only three of our patients were slightly positive for these tests. In particular, one out of two patients, found positive for aPS/PT IgM was previously diagnosed with Hashimoto thyroiditis and was also positive for myeloperoxidase antibodies, thus suggesting a general activation of the immune system in an autoimmune-predisposed patient.

Another limit of our study is that some patients were not tested for the complete biochemistry panel assays, mainly fibrinogen, which is an essential parameter for DIC scoring criteria or to contextualize other causes of hypercoagulation; this was due to incomplete knowledge of thromboembolic events and the hypercoagulability state of patients in the very first weeks of the pandemic.

In conclusion, the presence of systemic thromboembolic events in COVID-19 patients became quickly a well-known and established

conditions, sometimes life-threatening. However, it is difficult to categorize those vascular events into a conventional disease: in our experience we did not find any significant association with anti-phospholipid antibodies. It is most likely that several factors contribute to trigger the hypercoagulability status and the thromboembolism but, on the basis of our results, CAPS is probably not involved into the pathogenesis of these phenomena.

Ethical approval

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the local Ethics Committee of Bergamo (100/20; 27/04/2020).

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Authors' contributions

G.P., M.S., A.S., M.G.A. contribute to the study design, data collection, data analysis and interpretation, draft of the manuscript and its critical revision; V.M., R.M., L.C., R.R., A.G., G.G. contribute to data collection and analysis, critical revision, paper final approval.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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