Letter to the Editor

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Evidence for increased circulating procoagulant phospholipids in patients with COVID-19 pneumonia and their prognostic role

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To the Editor,

Patients with SARS-CoV-2 infection are prone to thrombotic complications associated with increased mortality [1, 2]. Disseminated intravascular coagulation and thrombotic microangiopathy are important pathogenetic factors but the detailed pathophysiology has not been fully elucidated [3, 4]. Microparticles (MP) are small phospholipid vesicles originated normally from a variety of cells like leukocytes and endothelial cells but mostly platelets, exerting an important role in blood coagulation and inflammation by expressing procoagulant activity [5].

We prospectively studied 19 consecutive patients hospitalized at the University Hospital of Patras from March 3 to May 3, 2020 diagnosed with COVID-19 pneumonia according to established criteria [6]. Blood was sampled within 24 h of admission, before any institution of anticoagulant treatment. Pneumonia severity classification was based on the American Thoracic Society guidelines [7]. Patients were enrolled in the context of an infectious diseases and sepsis protocol approved by Regional Research Ethical Committee (9632/17-05-2016). Prophylactic low molecular weight heparin (LMWH) was administered according to COVID-19 treatment guidelines [6]. Patients were followed for 14 days for development of severe respiratory failure (SRF) (PO_2/FiO_2 <200) requiring mechanical ventilation (MV). Patients with SRF underwent computed tomographic pulmonary angiography (CTPA) for exclusion of thromboembolic disease. Nineteen age and sex-matched healthy persons were included as the control group.

A full blood count, biochemical profile, CRP, ferritin, and coagulation factors were measured. Antithrombin III (ATIII), protein C (PC), protein S (PS) and the procoagulant phospholipid (PPL) activities were measured on a STA-R Instrument (Diagnostica Stago, Asnières, France) using the same batches of reagents (Diagnostica Stago). The activities of ATIII and PC were assayed using a chromogenic substrate method (STA-Stachrom ATIII and STA-Stachrom Protein C, respectively). PS was assayed by a clotting method (STA-Staclot Protein S). The PPL activity was measured using the STA Procoag-PPL test (Diagnostica Stago, Asnières, France). A shortening clotting time of the sample indicate an increase in PPL. Data were analyzed by non-parametric Mann–Whitney U test (SPSS statistical package, version 24.0 SPSS Inc., Chicago, IL, USA).

All subjects enrolled presented normal levels of natural coagulation inhibitors ATIII, PC and PS. As compared to controls, patients with COVID-19 pneumonia presented significantly increased PT, D-Dimers and fibrinogen ($p<0.001$, respectively) and significantly decreased *STA*-procoag-PPL clotting time ($p<0.001$) indicative of higher PPL activity (Table 1). There were no differences of PPL activity between COVID-19 patients with severe (n=6) and non-severe (n=13) pneumonia. Patients who developed SRF with need for MV presented significantly higher levels of PPL activity at admission ($p=0.045$) and higher ferritin values ($p=0.038$). The *STA*-procoag-PPL clotting time was positively correlated with D-dimers and fibrinogen levels

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Table 1: Measured inflammatory and coagulation parameters in COVID-19 pneumonia patients and healthy controls.

Parameters (at admission)	Reference values	Healthy controls (n=19)	COVID-19 pneumonia (n=19)	p-Value	COVID-19 pneumonia Non-severe (n=13)	COVID-19 pneumonia Severe (n=6)	p-Value	No need for MV (n=15)	Need for MV (n=4)	p-Value
Gender		9M/10F	8M/11F	NS	4M/9F	4M/2F	NS	5M/10F	3M/1F	NS
Age, years		59 (55–64)	63 (55–67)	NS	63 (56–67)	65 (53–72)	NS	64.5 (55.7–67.2)	62 (48–70)	NS
CRP, mg/dL	<0.5	0.2 (0.1–0.3)	4.8 (2.6–10.6)	<0.001	3.1 (2.2–5.8)	12.1 (7.2–21.5)	0.007	3.7 (2.4–10.6)	7.2 (3.9–18.1)	NS
Ferritin, ng/mL	16–323	55 (40–80)	276 (180–690)	<0.001	195 (148–288)	932 (358–2,496)	0.003	224 (169–381)	1,250 (314–3,200)	0.038
Coagulation factors										
Platelets, $\times 10^3/\mu L$	150–400	260 (190–300)	291 (179–411)	NS	291 (203–388)	243 (108–564)	NS	291 (172–375)	359 (185–657)	NS
PT, s	13.5–17.0	13.5 (13.2–13.9)	16.5 (15.0–17.6)	<0.001	16.1 (14.6–17.2)	17.5 (15.6–18.6)	NS	16.1 (15.0–17.8)	17.0 (15.2–17.7)	NS
aPTT, s	24–35	31 (29–33)	30 (27–32)	NS	28 (26–31)	31 (29–35)	NS	29 (26–32)	30 (27–33)	NS
D-dimers, µg/mL	0–0.5	0.0 (0.0–0.1)	1.6 (0.8–3.0)	<0.001	1.6 (0.8–3.4)	1.9 (1.1–6.5)	NS	1.4 (0.8–2.7)	2.7 (1.6–11.9)	NS
Fibrinogen, mg/dL	200–400	300 (287–365)	642 (537–691)	<0.001	618 (509–679)	667 (591–709)	NS	653 (525–703)	623 (594–677)	NS
Protein C, %	70–130	116 (110–123)	117 (110–120)	NS	115 (109–120)	118 (114–123)	NS	115 (109–120)	119 (118–126)	NS
Protein S, %	55–140	85 (81–95)	87 (79–92)	NS	84 (78–90)	91 (80–93)	NS	84 (78–90)	90 (82–95)	NS
Antithrombin III, %	80–120	102 (92–112)	104 (95–111)	NS	106 (89–110)	102 (97–112)	NS	102 (91–110)	108 (101–112)	NS
STA-procoag- PPL clotting time, s	61.0–83.0	70.6 (68.5–76.8)	34.6 (29.5–46.5)	<0.001	34.6 (26.6–46.7)	36.0 (29.9–55.6)	NS	39.8 (29.7–56.7)	29.9 (24.3–30.9)	0.045

Results are presented as median (interquartile range). M, male; F, female; MV, mechanical ventilation; CRP, C-reactive-protein; PPL, procoagulant phospholipids; PT, prothrombin time; aPTT, activated partial thromboplastin time; NS, non-significant.

($p<0.05$, respectively). No overt clinical or CTPA-detected thrombotic events were observed in our study cohort during hospitalization.

In our patients, increased D-dimer levels and PT prolongation could be suggestive of DIC but were not accompanied by thrombocytopenia or low fibrinogen levels. This is in accordance with previous reports demonstrating that most patients with COVID-19 would not be classified as having DIC according to the DIC score of the International Society on Thrombosis and Haemostasis [1, 3]. The novel finding of the present study is the increased activity of PPL in patients with COVID-19 pneumonia on admission, irrespectively of pneumonia severity. Moreover, higher PPL activity on admission was associated with progression of COVID-19 pneumonia to SRF. Consequently, according to the presented results, PPL activity measured on admission in patients with COVID-19 pneumonia is not an index of pneumonia severity but might exert a prognostic role for progression to SRF. MPs exert an important pathophysiological role in coagulation and inflammation [5]. Regarding the role of PPL in inflammation, higher PPL activity and ferritin values on admission were associated with progression of COVID-19 pneumonia to SRF. In addition, in all COVID-19 pneumonia patients, PPL activity was correlated with the inflammatory indices of D-Dimers and fibrinogen. Despite the potential prothrombotic role of PPL, no overt clinical or CTPA-detected thrombotic events were observed, most likely because all our patients were administered prophylactic LMWH. The positive correlation of PPL activity with D-Dimers indicates their possible role in the COVID-associated thrombotic microangiopathy. Platelet-derived MP provides a catalytic surface for the prothrombinase enzyme complex with subsequent thrombin formation and promotion of expression of endothelial adhesion molecules [5]. Nevertheless, in the deteriorated patients, pulmonary microvascular thromboses could not be excluded by the negative results of CTPA [3].

In conclusion, the preliminary results of this study demonstrate for the first time that PPL activity is increased in patients with COVID-19 pneumonia, possibly related to their hyperinflammatory and hypercoagulable state. Our results indicate circulating PPLs as a potential inflammatory marker of progression of COVID-19 pneumonia to SRF, while its role as a prothrombotic index needs to be further elucidated.

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