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Early View

Research letter

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Microparticles in COVID-19 as a Link Between Lung Injury Extension and Thrombosis

***Running Title*: Procoagulant Microparticles and COVID-19**

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Authors' contributions

Olivier Morel: study conception and design, data collection and interpretation, drafting of the manuscript, and critical revision for important intellectual content
Benjamin Marchandot: drafting of the manuscript and editing
Laurence Jesel: Study conception and design, drafting of the manuscript and critical revision for important intellectual content
Laurent Sattler: Data collection and interpretation
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Among the distinctive features of COVID-19, numerous reports have stressed the importance of vascular damages associated with coagulopathy onset [1]. Histologic analysis of pulmonary vessels in patients with Covid-19 revealed severe endothelial injury associated with intracellular SARS-CoV-2 virus and disrupted endothelial cell membranes together with widespread thrombosis and occlusion of alveolar capillaries. Microparticles (MPs) shed by apoptotic/stimulated cells of various cellular lineages including platelets, leukocytes, macrophages, lung endothelium or endothelial cells are reliable markers of vascular damage[2]released upon pro-inflammatory conditions and behave as active participants in the early steps of clot formation[3]. Circulating MPs promote pro-coagulant responses due to the exposure of tissue factor, the physiological activator of the coagulation cascade, and of negatively-charged phospholipids such as phosphatidylserine required for the assembly of the tenase and prothrombinase coagulation complexes ultimately leading to thrombin generation, through which they can precisely be quantified [4]. MPs carry Angiotensin converting enzyme (ACE)1 and up-regulate ACE1 expression in neighbouring endothelial cells [5]. By contrast, exosomes were recently reported to convey ACE2, the cell-entry receptor for SARS-Cov2 [4] in the vasculature[6]. ACE2 converts angiotensin II (Ang II) into angiotensin 1–7 (Ang 1–7) which, by virtue of its actions on the Mas receptor, limits the noxious effects of Ang II. Pioneering data have demonstrated that the renin angiotensin system has a crucial role in severe acute injury and the ACE2 has a protective role in acute lung injury mediated by SARS-CoV [7]. According to this paradigm, the loss of ACE2 function following binding by SARS-CoV-2 may contribute to unopposed angiotensin II accumulation that further exacerbate tissue injury and promote inflammation MPs release and thrombosis. During SARS-COv2 infection, we hypothesized that various factors including inflammatory burden, Ang II, altered shear stress, hypoxic vasoconstriction, could enhance MPs shedding

by various cell lineages including the alveolar vascular endothelium and contribute to clot formation

We identified consecutive COVID-19 patients admitted to non-ICU and ICU units at Strasbourg University Hospital from February 25th to April 19th of 2020. Blood samples were obtained in the 48h following admission. Patients were eligible if lupus anticoagulant (LA) was positively detected and remaining plasma available. To ascertain their contribution in COVID-19 lung injuries and related coagulopathy: procoagulant MPs levels, pro-inflammatory cytokines (Interleukin-6 (IL-6), Monocyte Chemotactic Protein-1 (MCP-1)), and cyto-adhesins (Intracellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule 1 (VCAM-1)), as markers of endothelium damage were measured in 52 patients with COVID-19 infection and positive LA. In addition, leukocytes, platelet, fibrinogen, aPTT, vWF ag and D-dimers levels were determined. Control groups were age-matched and consisted of 40 non-COVID-19 patients with positive LA screened at the Hematology department for acquired coagulopathy and 20 healthy volunteers (Blood donor) without any cardiovascular risk factors or significant comorbidities.

Measurement of MPs procoagulant activity was performed with the ZYMUPHEN MP-Activity ELISA kit (Hyphen Biomed, Neuville-sur-Oise, France). MPs were captured onto insolubilized annexin V, their phosphatidylserine content measured by prothrombinase assay using a microtitration plate reader equipped with kinetics software. In this assay, blood clotting factors (FXa, FVa, FII) and calcium concentrations were determined to ensure that phosphatidylserine is the rate-limiting parameter in the generation of soluble thrombin from prothrombin. Results were expressed as nanomolar PhtdSer equivalent (nmol L^{-1} PhtdSerEq) by reference to a standard curve constructed with liposomes of known composition and concentration. With respect to characterization of MPs by flow cytometry, this functional method allows the determination of MPs prothrombotic properties [4,8-10].

Haemostasis assays (fibrinogen, vWF:Ag, D-dimer, LA detection) were analysed on STA-R® Evolution (Diagnostica Stago ®, Asnières-sur-Seine, France) with standard commercial reagents and protocols.

Lupus anticoagulant (LA) detection.

Two screening tests were performed, respectively a Diluted Russel Viper Venom Time (dRVVT screen) made with the STA®-Staclot dRVV Screen reagent (Stago), and an activated partial prothromboplastin time (APPT) performed with the STA®-PPT A reagent (Stago). Positivity of one or both screening tests induced a mixing test at 1:1 proportion with a commercial frozen PNP (Cryocheck™ Pooled Normal Plasma, Cryocheck, Montpellier, France). Moreover, a positive dRVVT screen induced a confirmatory test with an increased concentration of phospholipids (dRVVT confirm), performed with the STA®-Staclot dRVV Confirm reagent (Stago). dRVVT screen, DRVVT confirm and APTT results were expressed as a ratio of patient-to-PNP. Mixing tests results were expressed as an index of circulating anticoagulant (ICA). LA was considered as positive only if the normalized dRVVT ratio (screen ratio/confirm ratio) was > 1.2 and all causes of false positive were excluded (i.e. anticoagulation conditions).

MCP-1, IL-6, VCAM-1 and ICAM-1 concentrations were determined with Quantikine ELISA kit (Research and Diagnostic Systems, Minneapolis, United States).

The extent of COVID-19 disease on chest computed tomography (CT) was assessed as pulmonary injuries extension in percentage of the total lung parenchyma and classified according to the ESR/ESTI guidelines as minimal (stage 1 <10%), moderate (stage 2 = 10 to 25%), severe (stage 3 = 25 to 50%) and critical (stage 4 >50%). Patients with suspected acute pulmonary embolism, based on their clinical, echocardiographic or laboratory parameters had a CT pulmonary angiography performed, either at the admission or during their stay. The present study was approved by the research ethics committee of Strasbourg Hospital and written informed consent was obtained from all patients.

Among the 52 patients with COVID-19 infection and positive lupus anticoagulant (mean age 54±20 years; male sex 78.8%), twelve patients experienced acute pulmonary embolism (APE) and twenty showed severe lung injury severity (>50%) assessed by chest CT. No baseline differences between COVID-19 patients and non COVID-19 patients with positive LA could be evidence apart from obesity (18 (34.6%) vs. 3 (7.5%), p=0.002) and smoking habits (2 (3.8%) vs. 7 (17.5%), p=0.029).

A stepwise increase in pro-inflammatory cytokines and cyto-adhesins levels could be evidenced among the three groups, with highest values measured in COVID-19 patients (Figure, panel A). Procoagulant MPs were 2·5 times higher in patients with LA as compared to healthy volunteers 5.7 [3.3-8.7] and with no further increase between COVID-19 and non–COVID-19 patients (13.2 [6.7-21.4] vs. 13.0 [7.5-20.3](Figure, panel B). Index of circulating anticoagulant were equivalent between non-Covid 19 and Covid 19 patients (1.41 [1.35-1.58] vs 1.38 [1.27-1.49]; p=0.233). No impact of obesity on MPs release or cytokines and cytoadhesins levels could be established. Highest levels of procoagulant MPs could be evidenced in patients with pulmonary embolism (20.6 [12.1-27.1] vs. 10.1 [5.6-18.8] p=0.003)(Figure, panel C) or those with severe lung injuries (19.8 [11.0-27.4] vs. 8.1 [4.6-17.8]; p=0.004) (Figure, panel D). By multivariable analysis, MPs were the sole independent predictor of critical lung injury (4.06 95% CI [1.10-14.93]; p =0.035).

In the past, several reports have emphasized that circulating endothelial MPs are a reliable marker of early lung destruction in cigarette smokers [11], pulmonary hypertension [12] and various lung diseases [13]. More recently, a study performed in microvascular lung endothelial cells and in patients with acute respiratory distress syndrome has emphasized the importance of ACE-bearing MPs, as an important marker of disease severity [14]. Consistent with this paradigm, we report that procoagulant MPs are associated with the extent of lung injuries in COVID-19 and pulmonary thrombosis. Additional work is needed to confirm that

MPs behave as bioactive shuttles spreading the thrombotic burden within the vasculature in SARS-CoV-2 infection.

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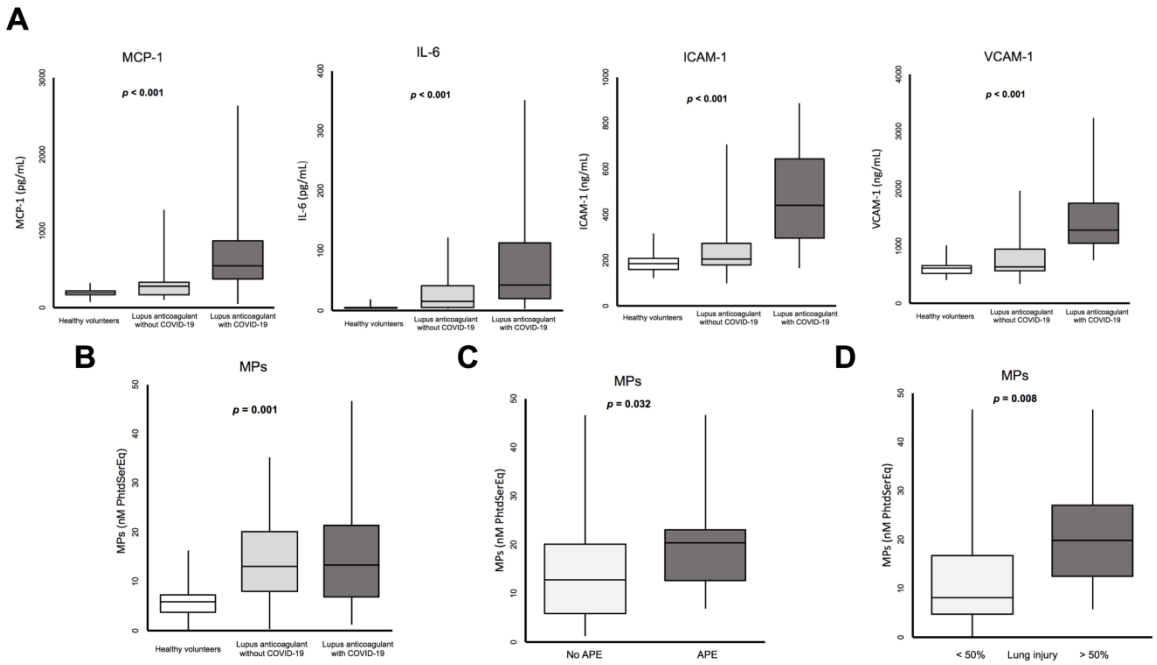

Figure. Panel A. Pro-inflammatory cytokines (MCP-1 and IL-6) and cytoadhesins (ICAM-1 and VCAM-1) levels in the study population. Panel B. Procoagulant MPs levels in the study population. Panel C. Procoagulant MPs levels in Covid-19 patients stratified by concomitant in-hospital acute pulmonary embolism. Panel D. Procoagulants MPs levels in Covid-19 patients stratified by lung injury severity (critical > 50%) assessed by computed tomography. Results are expressed as median [25-75] th interquartile range. Bars represent min and max values. Comparisons between groups were made using non-parametric tests. Abbreviations: APE, acute pulmonary embolism; Covid-19, coronavirus disease 2019; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MPs, microparticles; VCAM-1,vascular cell adhesion molecule-1