Supplemental Materials and Methods

Extracellular vesicle enumerations by flow cytometry

The flow cytometry method was reported according to the MIFlowCyt-EV guideline[1].

Annexin-V (AnnV)-Fluorescein/FITC was from Tau Technologies BV (Kattendijke, The Netherlands). PBS without calcium was used as a control for AnnV-FITC The following antibodies were purchased from Beckman Coulter (Villepinte, France): CD31-PE, clone 1F11; CD146-PE-Cyanin 5/PC5, clone TEA 1/34; CD41-PE-Cyanin 7/PC7, clone P2; CD15-Allophycocyanin/APC, clone 80H5; as well as their respective isotype controls: IgG1-PE, -PC5, -PC7, -APC, clone 679.1 Mc7. CD235a-BV650, clone GA-R2 and its isotype control antibody IgG2b-BV650, clone 27-35 was from Becton Dickinson (San Jose, CA, US). The antibodies were used at their optimal final concentrations, between 0.8 and 3 µg/ml. All the isotype controls were matched with their relevant antibody conjugates in terms of fluorescence backgrounds.

Extracellular vesicles (EVs) were enumerated by fluo-sensitive flow cytometry (CytoFLEX S, Beckman Coulter) with the side scatter (SSC) on the 405 nm laser (VSSC) at low flow rate (10µL/min) following size-related standardization with Megamix-Plus SSC beads (BioCytex, Marseille, France). The instrument performances were daily checked, using Cytoflex Daily QC Fluorospheres (Beckman Coulter), Megamix-Plus SSC and FSC for scatter parameters and SPHERO Rainbow 8-peak (Beckman Coulter) for fluorescence parameters. Ten microliters of citrated platelet-free plasma was incubated with the antibodies during 20 min at room temperature. Samples were diluted in 250µL of Binding Buffer (Tau Technologies BV) supplemented by hirudine at 2ATU/mL (Cryopep, Montpellier, France). The absolute EV counts (events per µl) were determined using MP Count Beads (Biocytex, Marseille, France). To confirm the vesicular origin of the preparation, sample was depleted from EV by sample

lysis with 0.5% Triton buffer (Triton X-100, Sigma-Aldrich, St Louis, MO, US) or nanofiltration (0.1µm).

Flow cytometry data (FCS files) were analyzed with Kaluza analysis software. Platelet-EVs, erythrocyte-EVs, granulocyte-EV, and endothelial-EVs were defined as AnnV+/CD41+, AnnV+/CD235a+, AnnV+/CD15+ and AnnV+/CD31+/CD41- events, respectively.

Results are included in table 1. While increased compared to the upper reference values of the laboratory (x3-4 for platelet-EVs; ref 500-3000 EVs/µl, x1.3 for erythrocyte-EVs, ref 0-400 EVs/µl; x3-4 for granulocyte-EVs; ref 0-50 EV/µl; x1.2 for endothelial EVs, ref 0-150 EVs/µl), no significant difference was found between moderate and severe forms of COVID-19, except a significant decrease of platelet-EVs in severe forms. This may be related to a consumption of EVs in intravascular microthrombi.

1. Welsh JA, Van Der Pol E, Arkesteijn GJA, Bremer M, Brisson A, Coumans F et al. MIFlowCyt-EV: a framework for standardized reporting of extracellular vesicle flow cytometry experiments. J Extracell Vesicles. 2020; 9(1):1713526.

Supplemental table 1, Septic shock and severe COVID-19 patient's clinical characteristics

	Septic shock	Severe COVID-19	p-value
Demographic information			
Total No.	218	38	
Age*	66.5 [57-73]	56 [52–70]	0.005
Sex M/F	137/81	23/15	0.856
Cardiovascular risk factors			
Obesity, n (%)	45 (23)	11 (34)	0.187
Chronic Kidney Disease, n (%)	20 (9.8)	8 (22)	0.050
Hypertension, n (%)	81 (39.5)	14 (37)	0.857
Diabetes, n (%)	53 (25.9)	6 (16)	0.220
Cardiovascular disease, n (%)	32 (15.6)	9 (24)	0.240
Oxygen Saturation*, %	97 [94.9-98.5]	94 [79–96]	0.052
Invasive mechanical ventilation, n (%)	128 (63.1)	18 (47)	<0.0001
Anticoagulation Therapy	138 (68)	32 (84)	0.019
Outcomes			
28-day mortality, n (%)	40 (18.3)	4 (11)	0.173
Microorganism			
GRAM-, n (%)	109 (50)	NA	
GRAM+, n (%)	67 (31)	NA	
Fungi, n (%)	20 (9)	NA	

* median [25th–75th percentile]; aPTT, activated partial thromboplastin time; F, female; M, male; NA, not applicable; PAI-1, plasminogen activator inhibitor 1