SUPPLEMENTAL MATERIALS

Platelets promote thrombo-inflammation in SARS-CoV-2 pneumonia

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Major Resources Table

Antibodies

Target antigen	Fluorochrome	Clone	Vendor or Source
PAC1	FITC	PAC1	BD Biosciences
CD14	PE	MϕP9	BD Biosciences
CD41	FITC	HIP8	BD Biosciences
CD41	PE	HIP8	BD Biosciences
CD45	PerCP-Cy5 .5	2D1	Biolegend
CD66b	BV421	G10F5	Biolegend
CD62P	FITC	CLBThromb/6	Beckman coulter

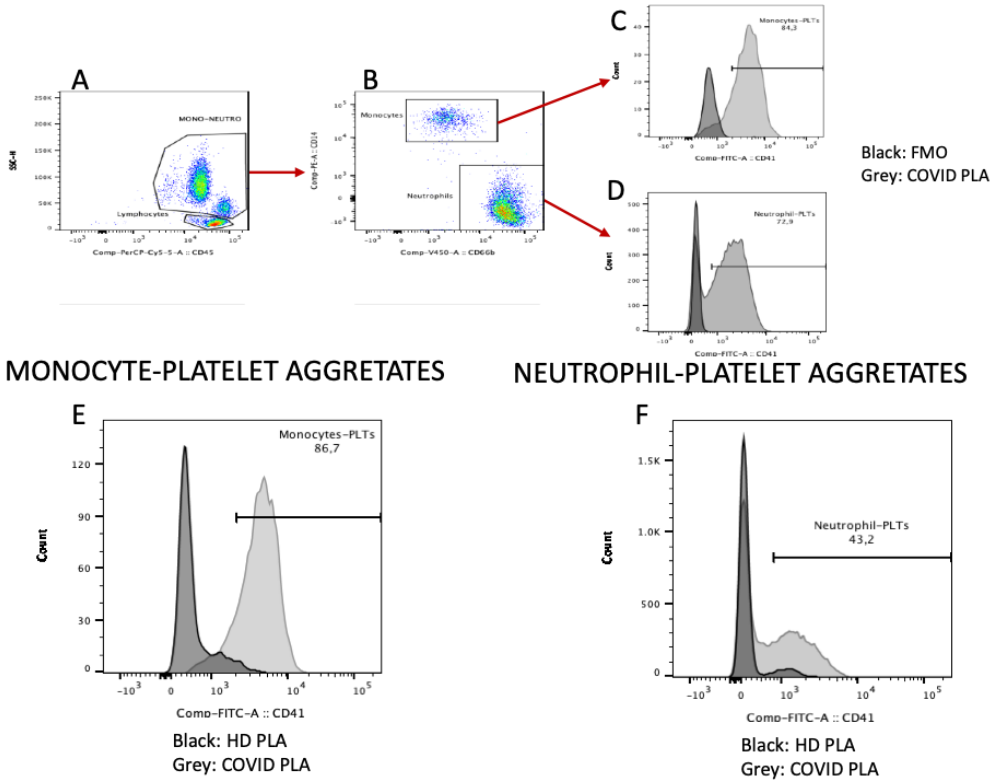
Supplemental Materials

Table I

Material	Vendor or Source
Collagen Equine tendon collagen (Collagen reagent Horm®) used to evaluate platelet activation markers	Takeda (Austria)
Cal-LyseTM Lysing solution	Invitrogen
Flow cytometer FACS- Canto II	BD (Franklin Lakes, NJ, USA)
FlowJo software for flow cytometry analysis	Treestar Inc.
Human ProcartaplexTM Panel 1 Multiplex	Thermo Fisher Scientific (Waltham, MA, USA)
ParaFormaldehyde solution (37%)	Sigma-Aldrich (Italy).

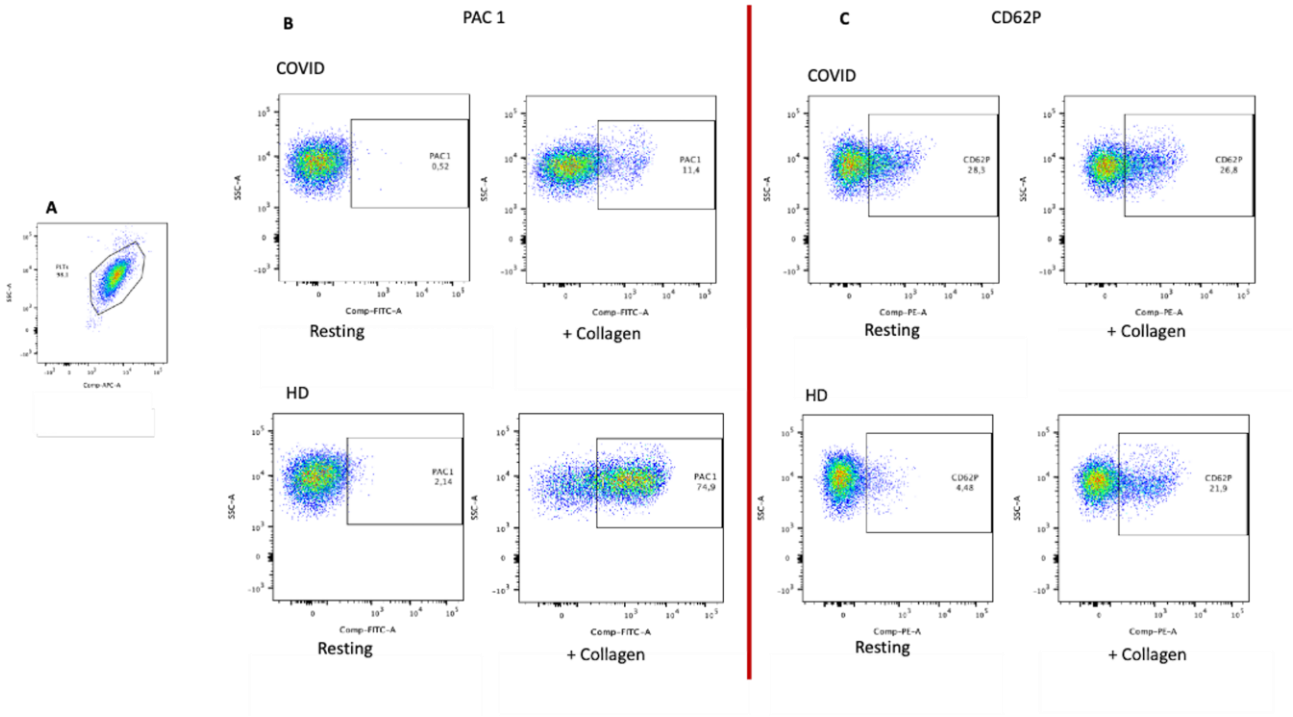
Supplemental figures

Supplemental Figure I



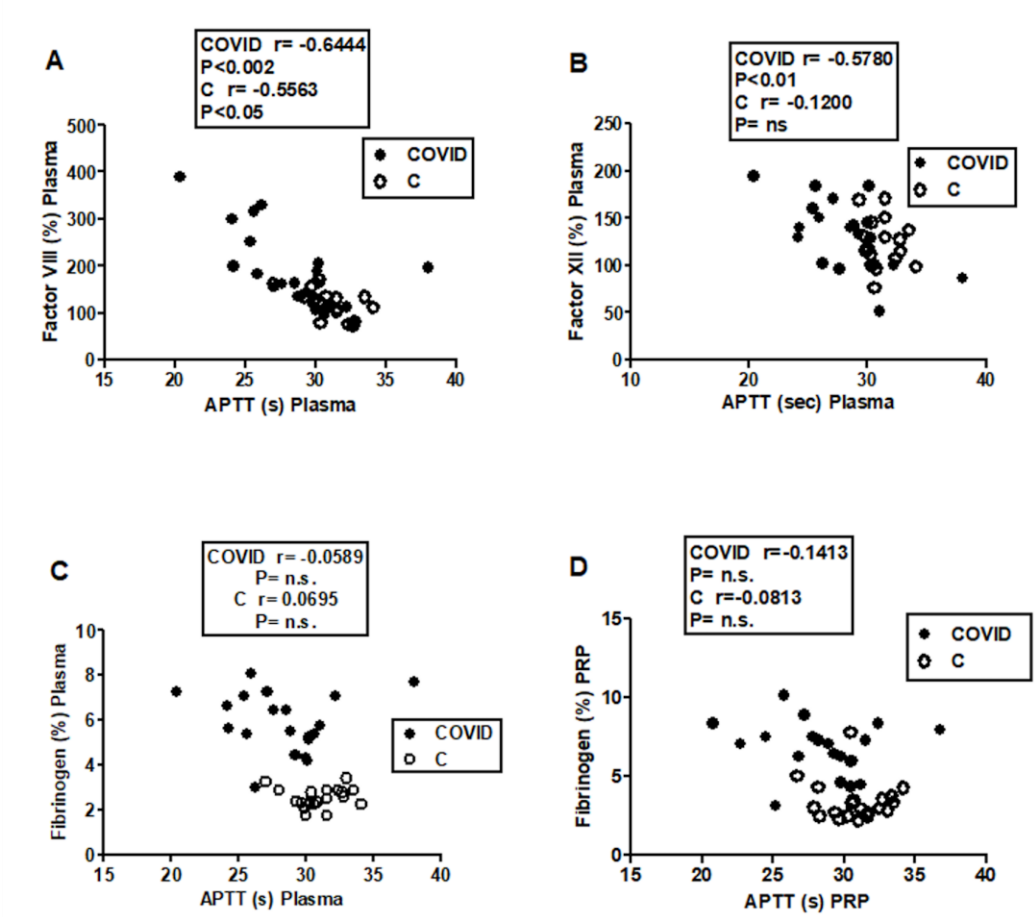
Supplemental Figure I: Gating strategy of whole blood analysis of Platelet-Leukocyte Aggregates (PLA) in COVID patients and in Healthy donors (HD). Leukocytes are labelled with anti-CD45-FITC and plotted versus side light scatter (A). Monocytes and Neutrophils were labelled with anti-CD14 and anti-CD66b, respectively (B). A gated histogram of CD14 monocytes is plotted and a marker is placed just above the FMO control peak (black histogram). The platelet-positive monocytes stain brightly with anti-CD41-PE (grey histogram) (C). A gated histogram of CD66b neutrophils is plotted and analysed in the same manner as the monocytes (D). Histograms representative of the difference between the presence of leukocyte-platelet aggregates in COVID patients (grey histogram) and healthy donor (black histogram) (E-F).

Supplemental Figure II



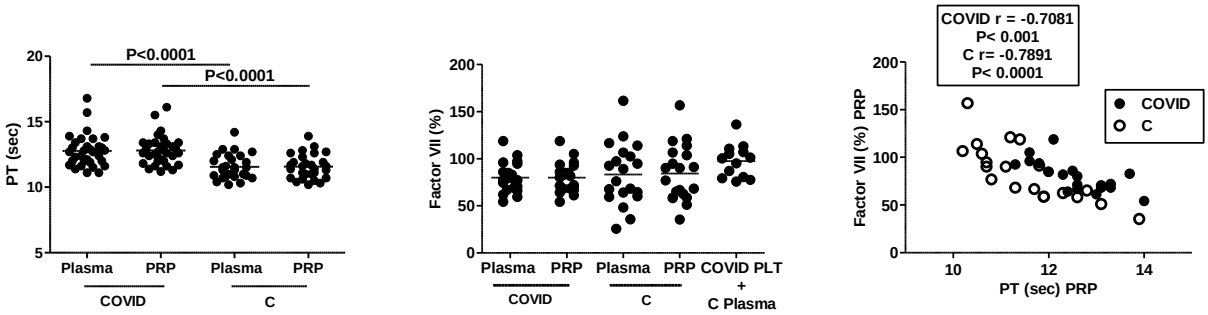
Supplemental Figure II: Gating strategy of flow cytometry detection of activated platelet. Platelets gate was determined by anti CD61 (A). The expression of the active form of the fibrinogen receptor, as detected using the monoclonal antibody PAC1 for the active fibrinogen receptor (B) and CD62P (C) were assessed on the CD61 positive population stimulated or not with collagen.

Supplemental Figure III



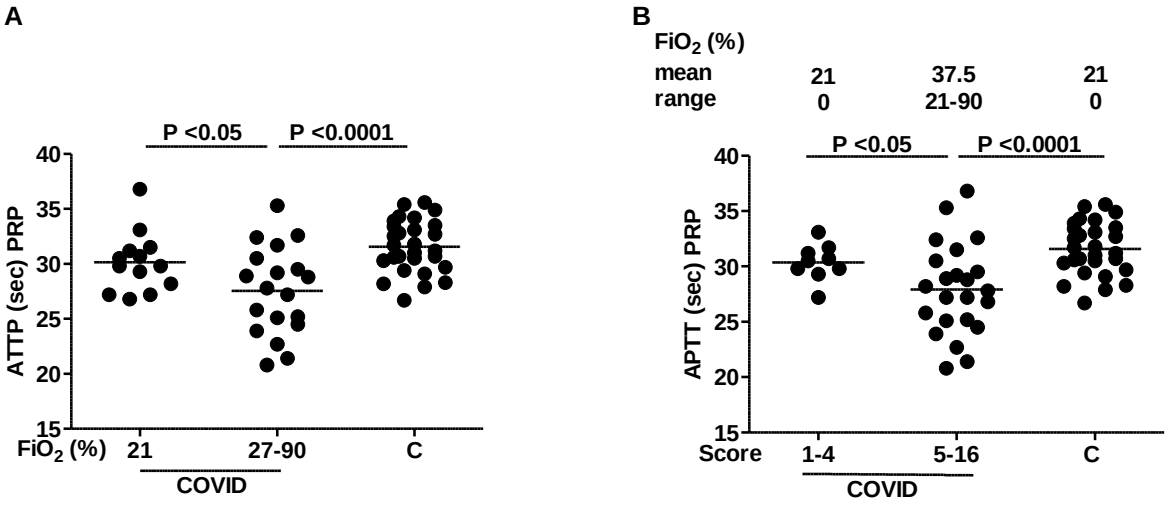
Supplemental Figure III: Factor VIII activity correlates with APTT using plasma collected from COVID-19 patients (n=20) and healthy controls (n=20) (Panel A). Factor XII activity in plasma collected form from COVId-19 patients correlated with APTT (n=20). This is not observed in healthy controls (n=20) (Panel B). Fibrinogen activity does not correlate with APTT neither in plasma nor in PRP from COVID-19 patients (n=20) as well as in control subjects (n=20) (Panel C and D).

Supplemental Figure IV



Supplemental Figure IV: Prothrombin time (PT) tested using plasma and PRP from COVID-19 patients (n=32) was significantly longer compared to healthy controls (n=28) (Panel A). The activity of the coagulation factor VII is similar in plasma and PRP in COVID-19 patients (n=20) and healthy controls (n=20) as well as in washed platelet suspended in control plasma (n=(Panel B) and correlates with APTT in both conditions (Panel C and D)

Supplemenatal Figure V



Supplemental Figure V: The severity of SARS-CoV-2 pneumonia, as assessed either by measuring oxygen requirement (FiO2 >21) or the radiologic score based on the area of lung interstitial alterations is associated with differences in APTT tested with platelet rich plasma (PRP) from the same patients (n=32) (Panels A and B). In comparison with healthy controls (n=28) and patients with limited pneumonia not requiring oxygen supply, those with more severe pneumonia show a significantly shorter APTT.