
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

blood advances

American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

# Uncoupling of platelet granule release and integrin activation suggests GPIIb/IIIa as therapeutic target in COVID-19

Tracking no: ADV-2022-008666R2

Lukas Weiss (University Hospital Wuerzburg, Germany) Maria Drayss (University Hospital Wuerzburg, Germany) Georgi Manukjan (University Hospital Würzburg, Germany) Maximilian Zeitlhöefler (University Hospital Wuerzburg, Germany) Judith Kleiss (University Hospital of Würzburg, Julius-Maximilians-University Würzburg, Germany) Mathis Weigel (University Hospital Wuerzburg, Germany) Johannes Herrmann (University Hospital of Würzburg, Julius-Maximilians-University Würzburg, Germany) Kristina Mott (University Hospital, University of Würzburg, Germany) Sarah Beck (Rudolf Virchow Center, University of Würzburg, Germany) Philipp Burkard (Institute of Experimental Biomedicine, University Hospital Würzburg, Germany) Thien-Tri Lam (University of Würzburg, Germany) Karina Althaus (Transfusion Medicine , University Hospital, Tuebingen, Germany) Tamam Bakchoul (Institute for Clinical and Experimental Transfusion Medicine, Medical Faculty of Tuebingen, University Hospital of Tuebingen, Germany) Stefan Frantz (University Hospital Würzburg, Germany) Patrick Meybohm (University Hospital of Würzburg, Julius-Maximilians-University Würzburg, Germany) Bernhard Nieswandt (Institute of Experimental Biomedicine I, University Hospital, University of Würzburg, Germany) Dirk Weismann (University Hospital Wuerzburg, Germany) Harald Schulze (University Hospital Wuerzburg, Germany)

**Abstract:**
Thromboembolic events are frequent and life-threating complications of COVID 19, but are also observed in patients with sepsis. Disseminated thrombosis can occur despite anticoagulation, suggesting that platelets play a direct, but yet incompletely understood role. Several studies demonstrated altered platelet function in COVID 19 with in part controversial findings, while underlying disease-specific mechanisms remain ill-defined. We performed a comprehensive cohort study with 111 patients, comprising 37 with COVID-19, 46 with sepsis, and 28 with infection, compared to controls. Platelet phenotype and function were assessed under static and flow conditions, revealing unexpected disease-specific differences. From hospital admission on, platelets in COVID-19 failed to activate integrin GPIIb/IIIa in response to multiple agonists. Dense granule release was markedly impaired due to virtually missing granules, also demonstrated by whole mount electron microscopy. In contrast, alpha-granule marker CD62P exposure was only mildly affected, revealing a subpopulation of PAC-1-/CD62P+ platelets, independently confirmed by automated clustering. This uncoupling of alpha-granule release was not observed in sepsis patients, despite a similar disease severity. We found overall unaltered thrombus formation in COVID 19 and sepsis samples under venous shear rates, which was dependent on the presence of tissue factor. Unexpectedly, under arterial shear rates thrombus formation was virtually abrogated in sepsis, while we detected overall normal-sized and stable thrombi in blood from COVID-19 patients. These thrombi were susceptible to subthreshold levels of GPIIb/IIIa blockers eptifibatide or tirofiban that had only a minor effect in control blood. We provide evidence that low dose GPIIb/IIIa blockade could be a therapeutic approach in COVID-19.

**Conflict of interest:** No COI declared

**COI notes:** The authors do not declare any conflict of interest.

**Preprint server:** No;

**Author contributions and disclosures:** LJW conceptualized the study designed and performed experiments, analyzed data and co-wrote the manuscript. MD, GM, MZ, JK, MW, JH, KM, SB, PB, designed and performed experiments, and analyzed data, TTL, KA, TB, SF, PM and BN provided intellectual input and analyzed data. DW and HS conceptualized the study, designed experiments, analyzed data and co-wrote the manuscript

**Non-author contributions and disclosures:** No;

**Agreement to Share Publication-Related Data and Data Sharing Statement:** For original data, please contact harald.schulze@uni-wuerzburg.de

**Clinical trial registration information (if any):**

# Uncoupling of platelet granule release and integrin activation suggests GPIIb/IIIa as a therapeutic target in COVID-19

Lukas J. Weiss,[1,2] Maria Drayss,[1,2] Georgi Manukjan,[1] Maximilian Zeitlhöfler,[1] Judith Kleiss,[1] Mathis Weigel,[1] Johannes Herrmann,[3] Kristina Mott,[1] Sarah Beck,[4] Philipp Burkard,[1] Thiên-Trí Lâm,[5] Karina Althaus,[6] Tamam Bakchoul,[6] Stefan Frantz,[2] Patrick Meybohm,[3] Bernhard Nieswandt,[1,4] Dirk Weismann*[2] and Harald Schulze*[1]

[1]Institute of Experimental Biomedicine, University Hospital Würzburg, Germany

[2]Department of Internal Medicine I, University Hospital Würzburg, Germany

[3]Department of Anaesthesiology, Intensive Care, Emergency and Pain Medicine, University Hospital Würzburg, Germany

[4]Rudolf Virchow Center, University of Würzburg, Germany

[5]Institute for Hygiene and Microbiology, University of Würzburg, Germany

[6]Centre for Clinical Transfusion Medicine, University Hospital of Tübingen, Germany

* DW and HS contributed equally to this work

Short title: GPIIb/IIIa blockade as a target in COVID-19

Figure/Table count: 6/1 Reference count: 46

Word count: 4410 Abstract count: 249

Correspondence:
Harald Schulze, PhD
University Hospital Würzburg
Institute for Experimental Biomedicine, Chair I,
Josef-Schneider-Str. 2/D15
97080 Würzburg
Germany
Phone: +49-931-201-48329
Fax: +49-931-201-648363
harald.schulze@uni-wuerzburg.de

For original data, please contact harald.schulze@uni-wuerzburg.de.

## Abstract

Thromboembolic events are frequent and life-threating complications of COVID-19, but are also observed in patients with sepsis. Disseminated thrombosis can occur despite anticoagulation, suggesting that platelets play a direct, but yet incompletely understood role. Several studies demonstrated altered platelet function in COVID-19 with in part controversial findings, while underlying disease-specific mechanisms remain ill-defined. We performed a comprehensive cohort study with 111 patients, comprising 37 with COVID-19, 46 with sepsis, and 28 with infection, compared to controls. Platelet phenotype and function were assessed under static and flow conditions, revealing unexpected disease-specific differences. From hospital admission on, platelets in COVID-19 failed to activate integrin GPIIb/IIIa in response to multiple agonists. Dense granule release was markedly impaired due to virtually missing granules, also demonstrated by whole mount electron microscopy. In contrast, alpha-granule marker CD62P exposure was only mildly affected, revealing a subpopulation of PAC-1$^{-}$/CD62P$^{+}$ platelets, independently confirmed by automated clustering. This uncoupling of alpha-granule release was not observed in sepsis patients, despite a similar disease severity. We found overall unaltered thrombus formation in COVID-19 and sepsis samples under venous shear rates, which was dependent on the presence of tissue factor. Unexpectedly, under arterial shear rates thrombus formation was virtually abrogated in sepsis, while we detected overall normal-sized and stable thrombi in blood from COVID-19 patients. These thrombi were susceptible to subthreshold levels of GPIIb/IIIa blockers eptifibatide or tirofiban that had only a minor effect in control blood. We provide evidence that low dose GPIIb/IIIa blockade could be a therapeutic approach in COVID-19.


Key Points:

- Blunted GPIIb/IIIa activation is uncoupled from functional $\alpha$–granule release in COVID-19 patients differing from bacterial sepsis

- Subthreshold doses of GPIIb/IIIa blockers prevent thrombus formation in COVID-19

## Introduction

SARS-CoV-2 is a recently evolved betacoronavirus and trigger of the COVID-19 pandemic with more than 500 million confirmed infections and 6.2 million deaths worldwide by April 2022.[1,2] A subset of infected patients develops life-threatening pneumonia and acute respiratory distress syndrome, but - despite frequently being on strict anticoagulation[3,4] - also disseminated thrombosis, which was confirmed in autopsy studies. Platelets have thus been identified as a major contributor to disease burden, but their exact role for COVID-19-based thrombotic and thromboembolic events remains a matter of debate.[5-7] The interaction between platelets and the immune system was shown to trigger thrombosis by antibody-mediated mechanisms or neutrophil extracellular trap (NET) formation in COVID-19 patients.[8-10] This specific interplay, referred to as immunothrombosis, constitutes also a hallmark of sepsis, a systemic inflammatory disease, which becomes aggravated by disseminated thrombotic events and can exacerbate to disseminated intravascular coagulation (DIC).[11,12] Prevention and therapy of thrombosis are thus indispensable for patients with COVID-19 or sepsis. As most studies focused on anticoagulation, clinical data on the use of antiplatelet medication in both disorders are limited.[13,14] Recent data from the REMAP-CAP trial suggest that especially critically-ill patients with COVID-19 might benefit from antiplatelet therapy.[15] Furthermore, disease-specific differences regarding platelet dysfunction in COVID-19 and sepsis remain ill-defined. Previous studies provided controversial and in part even

contradictory results, describing that platelets are (or become) hyper- or hyporeactive, respectively.[9,16-18]

In this comprehensive platelet function study, we provide results from a total of 111 patients, including 37 patients with confirmed COVID-19, 46 with sepsis and 28 with infection at disease onset ($t_1$). For critically-ill patients, a one-week follow-up ($t_2$) was performed. By flow cytometry, we found that patients with sepsis - compared to COVID-19 patients - showed a markedly reduced $\alpha$-granule release upon stimulation. In contrast, GPIIb/IIIa activation and $\delta$-granule secretion were profoundly deficient in critically-ill patients with both COVID-19 or sepsis. In a microfluidic flow chamber model, thrombus formation was severely impaired only in sepsis patients, while COVID-19 patients showed numerous and overall stable thrombi. Our comprehensive data strongly imply that there is a SARS-CoV-2-specific dysfunctional platelet phenotype, which could be exploited as a therapeutic target. Sub-threshold doses of tirofiban or eptifibatide, which had a minor effect in blood of healthy donors, prevented thrombus formation in blood derived from COVID-19 patients.

## Material and Methods

### Study cohort and sample generation

Patients were recruited at the University Hospital Würzburg (UKW) (Suppl. Fig. 1). The study was approved by the local ethical committee (92/19 and 63/20), additional ethical approval was obtained in the context of a broad consent for the COVID-19 cohort, and conducted according to the declaration of Helsinki and its current amendments. Informed consent was provided by patients or their legal guardian. SARS-CoV-2 diagnosis was confirmed by PCR testing. Sepsis was defined according to modified Sepsis-III criteria by an increase of the diagnostic SOFA-score of 2 points.[19] Exclusion criteria were pregnancy, aplasia or ECMO-therapy. Healthy controls over 18 years without self-reported antiplatelet medication that were free from acute illness were recruited at the UKW at a routinely scheduled blood withdrawal by in-house physicians.

### Data sharing

For original data, please contact harald.schulze@uni-wuerzburg.de.

Additional methods are described in the Supplementary data to this manuscript.

## Results

In this study, we aimed to analyze platelet function in patients with COVID-19 in a direct comparison with sepsis. We recruited (I) 37 patients with COVID-19 and (II) 46 patients with sepsis or septic shock and compared them to (III) 28 patients with infection without sepsis. These 111 patients (Suppl. Fig. 1) were recruited at the day of hospital or ICU admission ($t_1$) and compared to 35 healthy controls based on a normalization scheme (Suppl. Table 1; Suppl. Fig. 2) to allow comparison between cohorts. Blood of ICU patients was additionally analyzed after 4-7 days ($t_2$). Including our follow-up analyses, a total of 159 patient samples were assessed. Most COVID-19 (95%) and sepsis patients (87%) were recruited at the ICU (n=69), indicating critical illness in both cohorts. This was reflected by a similar median SOFA-score (8, IQR 4-9 vs. 6 IQR 4-8) and mortality (20% vs 16%) (Table 1).

### Uncoupled GPIIb/IIIa activation and α-granule release in COVID-19

Thrombocytopenia is a hallmark of sepsis and component of the diagnostic SOFA-score.[20] Accordingly, at $t_1$ thrombocytopenia was more frequent in the sepsis cohort than in the infection cohort (47% vs. 11%). In contrast, only 25% of our COVID-19 patients were thrombocytopenic (Fig. 1A). This discrepancy was preserved at $t_2$, with 59% having a low platelet count in the sepsis compared to 21% in the COVID-19 cohort. Mean platelet volume inversely correlated with platelet counts, implying that larger-sized platelets are a direct consequence of thrombocytopenia-triggered thrombopoiesis ($r^2$ sepsis: 0.43; $p<0.0001$, COVID-

19: 0.07; p<0.048) (Fig. 1B,C). Plasma fibrinogen and D-dimer levels were increased throughout all cohorts (Suppl. Fig. 3A,B). The number of patients who had prolonged international normalized ratio (INR) was higher in the sepsis (n=22) compared to the COVID-19 cohort (n=3) (Suppl. Fig. 3C), which is also reflected by an increased SIC-score in sepsis (Suppl. Fig. 3D).

Platelet activation induces two main cellular responses: the release of internally stored granules (mostly $\alpha$- and $\delta$-granules) and activation of the integrin receptor GPIIb/IIIa. The latter results in a high affinity conformation for fibrinogen,[21] which can be specifically detected by PAC-1 antibody binding. In order to assess whether any disease condition results in pre-activated platelets, we have set resting platelets of healthy controls to a 2% threshold, but could not detect increased PAC-1 binding in any of the patient cohorts (Fig. 1D). The same finding was observed when normalized GeoMFI values were compared (Fig. 1E). CD62P is not expressed on resting platelets and its exposure thus a direct consequence of $\alpha$-granule release. When applying the 2% threshold gate to healthy controls, there was only a minor increase of CD62P in patients with COVID-19 (7.3%), sepsis (3.0%) or infection patients (2.8%) (Fig. 1F). GeoMFI levels were slightly elevated in patients with COVID-19 or sepsis (Fig. 1G), but far from the range observed for activated platelets (grey bar in Suppl. Fig. 4A), suggesting that circulating platelets in our patient cohorts are not fully activated. Integrin activation and granule release typically occur concomitantly, but can also be uncoupled, as recently described for patients with COVID-19.[16] We thus set up a quadrant analysis by gating on $CD62P^+$ and $PAC\text{-}1^+$ single and double-

positive subpopulations (Fig. 1H). We found a shift toward $CD62P^+/PAC1^-$ platelets (displayed in blue), which was more pronounced in COVID-19 compared to sepsis patients (Fig. 1I). Next, we assessed platelet function upon stimulation with ADP [5 µM] or TRAP-6 [5 µM], both binding to G protein-coupled receptors. GPIIb/IIIa activation in response to ADP was markedly downregulated in sepsis and COVID-19 platelets, while infection patients showed an intermediate pattern (Fig. 2A,B). Agonist-triggered CD62P exposure was also largely reduced in sepsis patients (median relative GeoMFI: 0.29), but unexpectedly, only minimally reduced in COVID-19 patients (median relative GeoMFI: 0.83) (Fig. 2C). Similar results were obtained for TRAP-6 (Suppl. Fig. 4B,C). Upon stimulation of the immunoreceptor GPVI with collagen-related peptide (CRP-$X_L$) [0.01 µg/ml], platelets from the infection cohort showed a broad distribution of GPIIb/IIIa activation (median relative GeoMFI: 0.53 compared to 1.0 in healthy controls), while PAC-1 binding was virtually abrogated in both COVID-19 (0.17) and sepsis (0.10) patients (Fig. 2D). This degree of GPIIb/IIIa dysfunction correlated with disease severity, reflected by an increased SOFA-score (Pearson r: -0.63, $p<0.0001$) (Fig. 2E) and robustly separated patients of the infection cohort from the sepsis cohort, already at disease onset (AUC: 0.84) (Fig. 2F). GPIIb/IIIa activation upon stimulation was even more decreased in non-survivors compared to survivors without being statistically significant (CRP-$X_L$ median relative GeoMFI sepsis survival: 0.20; deceased: 0.14; COVID-19 survival: 0.11, deceased: 0.04) (Suppl. Fig. 4D,E). These results underline our previous findings that platelet dysfunction occurs already at sepsis onset,

preceding the drop in platelet count and might help improving an early diagnosis.[22] Similar to ADP, the CD62P exposure upon CRP-$X_L$ stimulation in COVID-19 patients was far less affected (median relative GeoMFI: 0.55), which is in strong contrast to patients with sepsis (median relative GeoMFI: 0.24) (Fig. 2G). Also, in a small subgroup of patients with viral sepsis (EBV, Enterovirus), CD62P exposure after CRP-$X_L$ stimulation was significantly lower than in COVID-19 patients, implying that the underlying pathomechanisms between sepsis and SARS-CoV-2 are distinct (Suppl. Fig. 4F). Quadrant analysis after CRP-$X_L$ stimulation unexpectedly revealed a clear separation of COVID-19 and sepsis (Fig. 2H): we found 21% $CD62P^+/PAC\text{-}1^-$ platelets in patients with COVID-19 after ADP stimulation, compared to only 12% in sepsis patients (blue bars in Fig. 2I). This difference was also observed upon stimulation with CRP-$X_L$ (27% vs. 11%) (Fig. 2J). As our analysis might be biased in respect to the choice of quadrants, we decided to corroborate our findings independently in a subcohort of patients, where we performed an unbiased automated clustering approach. By using FlowSOM software[23,24] we identified an increased subpopulation of $CD62P^+/PAC\text{-}1^-$ platelets upon CRP-$X_L$ or ADP stimulation, predominantly in the COVID-19 cohort (Fig. 2K,L). Taken together, the findings from our comprehensive function analysis provide several lines of evidence that platelets in COVID-19 patients acquire a distinct, disease-specific dysfunctional pattern comprising an uncoupling between GPIIb/IIIa activation and $\alpha$-granule release with a separation from sepsis patients. Next, we asked whether there were differences in respect to a procoagulant platelet phenotype, characterized by

breakdown of the mitochondrial membrane potential or increased phosphatidylserine (PS) exposure on the platelet surface that will trigger and finally boost the coagulation cascade.[25] We assessed the mitochondrial membrane potential by TMRE staining and PS exposure by annexin V (AV) binding in a subset of our sepsis or COVID-19 patients. The TMRE signal intensity was markedly reduced in COVID-19 and sepsis patients, accompanied by an increased fraction of AV-positive platelets (Suppl. Fig. 5A,B), suggesting a procoagulant platelet phenotype in both disorders that contributes to the altered function observed.

**Deficient δ-granule secretion in critical infection**

During the initial phase of platelet activation, δ-granules become exocytosed and release mediators including ADP, serotonin, and polyphosphates. ADP can induce further auto- and paracrine activation, mediating the second wave of platelet aggregation. δ-granule function depends mainly on the amount of granules, their contents, and finally the potential to release the cargo. We assessed δ-granule loading and release by a kinetically resolved flow cytometric assay using mepacrine, which selectively incorporates into δ-granules. We recorded the fluorescence of unstained and mepacrine-loaded platelets and calculated the difference as a surrogate marker for the amount of δ-granules (Fig. 3A). Most patients with sepsis or COVID-19 showed a markedly reduced mepacrine uptake (median relative MFI ctrl: 1.00, infection: 0.81 sepsis $t_1$: 0.53, COVID-19 $t_1$: 0.58) on the day of hospital admission (Fig. 3B,C). Mepacrine

release upon TRAP-6 [5 µM] stimulation was decreased in patients with infection compared to controls, but not to the same extent as in sepsis or COVID-19 (Fig. 3B,D). The proportion of released to loaded mepacrine ("relative mepacrine release") was reduced, suggesting even a combined "uptake-and-release defect" (Fig. 3B,E). In a subset of patients with COVID-19 or sepsis, we additionally performed lumiaggregometry, which monitors the release of δ-granule-derived ATP. Upon stimulation with either TRAP-6 or thrombin, ATP release was markedly reduced in both COVID-19 and sepsis patients compared to controls (Fig. 3F,G; Suppl. Fig. 5C). Next, we determined the number of δ-granules in critically-ill patients by whole-mount transmission electron microscopy. We detected 3 to 5 δ-granules per platelet in controls (n=6), but only 0 to 3 granules in patients with sepsis (n=11) or COVID-19 (n=10) (Fig. 3H,I). Taken together, our data indicate an acquired profound δ-granule deficiency in critically-ill patients with COVID-19 or sepsis, mainly due to a reduced number of δ-granules.

**Low dose GPIIb/IIIa blockade prevents thrombus formation in COVID-19**

Next, we assessed the functional relevance of these defects under flow conditions by using the platelet function analyzer PFA-200. This point-of-care device is considered to provide a surrogate "in vitro bleeding time", reflecting the time until a collagen/epinephrin (Col/Epi) or collagen/ADP (Col/ADP)-coated aperture is closed under a standardized shear rate. Unexpectedly, 18/19 sepsis patients and 23/25 COVID-19 samples revealed full closure of the Col/Epi cartridge, mostly within the reference range (Fig. 4A-B). In addition to the

detected closure time as an endpoint analysis, the aggregation kinetics were further monitored until full closure and displayed as histograms. In healthy donors, the flow rate decreased linearly over time, while we often observed a saw-tooth pattern in blood derived from COVID-19 or sepsis patients (Fig. 4C-D), reflecting possible embolization and thrombus rupture at the aperture. Thrombus formation depends on the interplay of platelet activation with the coagulation cascade.[26,27] We thus evaluated platelet function in conjunction with fibrin formation on collagen- and tissue factor-coated coverslips in a flow chamber using recalcified whole blood. Under venous shear rates, platelet aggregation (Fig. 4E), indicated by platelet surface area coverage (SAC) (Fig. 4F), as well as thrombus volume, morphology and contraction score (Fig. 4G,H; Suppl. Fig. 6A-C) were comparable between COVID-19 or sepsis patients and healthy controls. Thrombin-mediated fibrin formation was slightly reduced in both COVID-19 and sepsis patients (Fig. 4I), most likely due to the anticoagulation therapy. In vitro-heparinized whole blood of healthy controls reduced fibrin formation, but did not impact platelet aggregation and thrombus formation (Fig. 4E-I; Suppl. Fig. 6A-C). Unexpectedly, fibrin(ogen) deposition was higher in sepsis or COVID-19 samples compared to in vitro-heparinized control samples, but not to the same extent as controls, suggesting that hypercoagubility might occur under low shear independent of heparin. Confocal imaging confirmed similar clot composition of these thrombi in patients and controls (Fig. 4J). Unexpectedly, when external tissue factor (TF) was omitted, platelet aggregation, thrombus volume and fibrin formation were markedly reduced in both sepsis and COVID-19 samples when

compared to controls. This implies that the TF-dependent interplay with the coagulation cascade could rescue the reduced platelet function we observed in patients (Fig. 4K-N; Suppl. Fig. 6D-F). We monitored distinct activities of the coagulation cascade by Rotational Thromboelastometry and found a slightly increased coagulability in a subset of sepsis and COVID-19 patients (Suppl. Fig. 7), especially in FIBTEM, when platelet function becomes blocked through cytochalasin D, reflecting a procoagulable state and increased fibrinogen plasma levels.

Under arterial shear rates (Fig. 5A-F), platelet aggregate formation was markedly reduced in sepsis patients (median platelet SAC: 8.2%, MS: 3), while COVID-19 patients revealed an overall stable thrombus formation (median platelet SAC: 18.3%, MS: 3.9). Thrombi were increased in number, but smaller in individual size when compared to controls (Fig. 5A,B). Quantitative imaging of formed thrombi revealed decreased thrombus volume, MLS, and CS scores in both, COVID-19 and sepsis patients (Fig. 5C,D, Suppl. Fig. 8A-C). Fibrin formation was markedly reduced under arterial shear (Fig. 5E). To evaluate whether the anticoagulation administered to ICU patients had any confounding effect in our assays, we added unfractionated heparin [final concentration: 2 U/ml] to whole blood of healthy controls and repeated the flow chamber experiments. In line with our patient results, fibrin(ogen) SAC was abrogated upon heparinization (Fig. 5A,E). We detected quantitatively more, but less contracted thrombi - comparable to COVID-19 samples (Fig. 5A-E; Suppl. Fig. 8A-C), while thrombus volume remained unaltered (median: 1.2 x $10^8$ A.U.). This

implies that anticoagulation therapy led to altered aggregate morphology in COVID-19, but did not account for the reduced thrombus volume. This was confirmed in clots generated under arterial shear by a refined analysis using confocal microscopy: blood from healthy controls formed a dense fibrin(ogen) network and roundish thrombi, while COVID-19 patients exhibited reduced fibrin(ogen) formation and flat thrombi, corresponding to heparinized healthy control samples (Fig. 5F). When thrombus formation was assessed without external TF, platelet aggregation and thrombus volume did not differ between COVID-19 patients and controls, which is in contrast to measurements under venous shear rates (Fig. 5G-J; Suppl. Fig. 8D-F). This observation suggests that robust thrombus formation in COVID-19 samples under arterial shear rates is not primarily mediated by TF. Taken together, our data provide evidence that platelets of COVID-19 patients form stable, but less 3D thrombi, despite the defective granule secretion and virtually absent GPIIb/IIIa activation.

Since COVID-19 patients often suffer from thrombotic complications, we finally questioned whether the abrogated GPIIb/IIIa activation (Fig. 2B,D; Suppl. Fig. 4B) in conjunction with an overall unaltered thrombus formation under flow conditions (Fig. 5A-F) could be a therapeutic target to control thrombus formation. We preincubated whole blood of patients and controls for 20 minutes with increasing doses of the clinically approved GPIIb/IIIa blocker eptifibatide and applied arterial flow conditions in our microfluidic chamber. In healthy and heparinized controls, low doses eptifibatide hardly affected platelet SAC (mean 0.1 µg/ml: 19%; mean 1 µg/ml: 18%) and it required 10 µg/ml to fully

reduce the platelet SAC (mean: 8%). We measured low thrombus volume (mean: 1.8 x $10^7$ A.U.), CS and MLS (both mean: 0), indicating loose aggregates of few platelets adhering to collagen fibers, while fibrin formation was unaffected. Intriguingly, 1 µg/ml eptifibatide was already sufficient to fully prevent thrombus formation in COVID-19 patients, tenfold less compared to the dosage required to affect thrombus formation in heparinized controls (Fig. 6A-E; Suppl. Fig. 9A-C). This implies that the reduced dosage necessary to affect thrombus formation in COVID-19 is less likely due to anticoagulation, but rather intrinsic to the disease condition. Finally, to exclude a specific effect of eptifibatide, we repeated the experiment with tirofiban. Again, a tenfold reduced dosage (50 ng/ml) abrogated thrombus formation in COVID-19 patients, whereas 500 ng/ml was required in blood derived from healthy donors, independent of heparinization (Fig. 6F-J; Suppl. Fig. 9D-F). This confirms our results obtained with eptifibatide. Taken together, our data suggest that low dose GPIIb/IIIa blockade could be a promising target for antithrombotic therapy in COVID-19 patients.

## Discussion

Patients with COVID-19 have an increased risk of thromboembolic events, including VTE, venous or arterial thrombosis.[28] Our study aimed to perform a comprehensive analysis of altered platelet function in patients with COVID-19, especially in direct comparison with sepsis or infection. We provide experimental evidence that platelet responses to various agonists are selectively blunted or dimmed, and that GPIIb/IIIa activation and δ-granule release become uncoupled

from α-granule exocytosis. This separation has already been reported by Manne et al.,[16] where it was primarily attributed to virus-based disorders. Our data suggest that there might be a particular contribution by SARS-CoV-2 for this uncoupling, as our patients with other than SARS-CoV2 viral sepsis did not show this effect, but our numbers are yet too small. In sepsis patients the consumption of coagulation factors and platelets will result in thrombocytopenia and DIC.[29] In the initital phase of COVID-19, however, DIC or thrombocytopenia are less frequently observed. Alterations in coagulation parameters have been broadly studied in both disorders, including differences among them.[30,31] In respect to platelet function, however, a detailed comparison between COVID-19 and sepsis is missing. Moreover,

seminal reports on platelet dysfunction in COVID-19 are partly controversial, concluding that platelets in COVID-19 are either hyporeactive[9,17] or hyperreactive.[16,18,32,33] The results of our study provide comprehensive evidence that these controversial results might be less contradicting: we found platelets of both COVID-19 and sepsis patients to be hyporeactive in respect to integrin GPIIb/IIIa activation when compared to healthy controls or patients with infections. In two studies on platelet function in COVID-19, the authors found increased platelet aggregation in response to suboptimal agonist concentrations.[32,33] In our study, we have mainly used optimal agonist concentrations, which might partly explain the differences. The data derived from our microfluidic flow chamber experiments further corroborate that COVID-19-derived platelets reveal an unexpectedly strong adhesion and thrombus

formation, despite their defective integrin activation and despite the presence of heparin, which is in agreement with the increased platelet adhesion on collagen observed by Zaid *et al*.[32] One possible mechanism of how platelets in COVID-19 patients mediate thrombus formation despite an overall deficient GPIIb/IIIa activation, could be an increased phosphatidylserine exposure on the outer membrane leaflet, which is the base for activation of the tenase and prothrombinase complexes, preceding full activation of the coagulation cascade. We found an increased fraction of procoagulant platelets in untreated whole blood of both sepsis and COVID-19 patients (Suppl. Fig. 5A) and observed AV-positive platelets also in our coagulation flow chamber (Suppl. Fig. 10). Procoagulant platelets in samples of COVID-19 patients have also been described by other groups:[8,34,35] especially purified IgG fractions from COVID-19 patients were reported to harbor this increased capacity in addition to the classical agonists used by us.[8] These IgG-induced procoagulant platelets could thus partly compensate for a deficient platelet function. It is feasible that a consumption of more active platelets *in vivo* (i.e. in platelet-rich thrombi) would ultimately result in an increased fraction of less reactive ("exhausted") platelets subjected to *in vitro* studies, but the overall short platelet halflife makes this option unlikely. This might also be reflected in the only partially altered fraction of "secreted-only" $CD62P^+/PAC\text{-}1^-$ platelet subpopulation that might play a role in the hyper-aggregable platelet phenotype. Further work will be required to decipher the role of distinct platelet subpopulations in sepsis and COVID-19. In a recent work using a murine lipopolysaccharide-induced lung injury model it was

shown that especially GPVI and GPIIb/IIIa function are crucial for inducing a PS exposing procoagulant phenotype.[36] Nevertheless, the criteria defining a procoagulant platelet are so far not well standardized.

We speculate that thrombi in COVID-19 might be susceptible for anti-thrombotic therapy. In our study, we provide experimental evidence that especially GPIIb/IIIa blockade might be effective for prevention and therapy of (micro-)thrombotic events in COVID-19 patients. These drugs are known to increase the bleeding risk in some patients, but very recent findings strongly imply to reconsider this antithrombotic approach in thromboinflammatory conditions. In a recent publication using a murine model of immune-triggered cerebral venous thrombosis (CVT), the authors provided compelling data that especially GPIIb/IIIa blockade could prevent thrombus formation, ischemia, and neurological damage, while anticoagulation using heparin did not have any therapeutic effect.[37] This observation was partly unexpected in its clear result, since GPIIb/IIIa as a target would possibly not be the first thought due to the expected increased risk of bleeding. Similar findings were made in a murine model of thrombotic thrombocytopenic purpura.[38] While data on the use of GPIIb/IIIa blockers in COVID-19 and sepsis are rare, recent case reports described the successful use of eptifibatide or tirofiban in critically-ill patients suffering from acute respiratory distress or myocardial infarction during COVID-19.[39,40] Here, we provide data that platelets of critically-ill patients with COVID-19 are tenfold more susceptible toward GPIIb/IIIa blockade in respect to adhesion in our flow chamber. This suggests that the minimal dosage of GPIIb/IIIa blockers in COVID-19 might need

to be adapted for future clinical trials. CD62P might be a further candidate to target, although a study with COVID-19 patients using Crizanlizumab did not find statistically significant differences in the overall clinical outcome compared to the placebo control cohort. [41]

We monitored platelet function in conjunction with clinical parameters during the course of disease and could demonstrate consistent platelet-based defects. As COVID-19 patients have been continuously recruited from the beginning of the pandemic on until June 2022, our data reflect all SARS-CoV-2 variants predominant in Germany and Europe (Wuhan, α, δ, ο), and did not reveal significant differences between these subtypes, suggesting that platelet dysfunction in COVID-19 is intrinsic to an infection with SARS-CoV-2. One mechanism might be the inflamed lung tissue,[42] which could serve as an activation trigger each time the platelet passes the lung, possibly by direct interaction between platelets and the SARS-CoV-2 spike protein.[43]

Our study has several limitations: (1) The data were generated in a single study center at the University Hospital Würzburg. (2) A large fraction of our patients was recruited from ICUs and receiving pharmacological and interventional therapy. Treatment regimens between sepsis and COVID-19 differed regarding mechanical ventilation, anticoagulation, antiplatelet therapy or hemodialysis, which could potentially skew our findings. In a previous study on platelet function in sepsis, we could not find any effect of obvious confounders like antibiotic treatment, or platelet-bacterial interactions.[22] We evaluated the impact of ventilation or hemodialysis by univariate analysis, without detecting any

statistically significant effect. Of note, we excluded patients who required extracorporeal membrane oxygenation (ECMO) therapy at any time, as ECMO is known to trigger platelet dysfunction and can cause an acquired vWF-deficiency.[44,45] Nevertheless, we are fully aware that other confounders may have an impact on platelet function. (3) Increased plasma fibrinogen levels could possibly outcompete the PAC-1 antibody and reduce signal intensity. We thus performed a correlation of plasma fibrinogen contents with PAC-1 binding and could not find any association of both parameters throughout all our patient cohorts (Suppl. Fig. 11). (4) Most of our COVID-19 patients were given LMWH from day 1 on ICU. While this might be less critical for flow cytometry-based platelet function assays, it has relevant impact on coagulation-based assays, although we could find differences in few patients on fondaparinux or unfractionated heparin. We have thus heparinized blood from healthy donors in vitro in our flow chamber experiments, which could well phenocopy the reduced fibrin formation, but cannot explain the overall stable thrombi found in COVID-19 or sepsis. A subfraction of patients received low dose acetylsalicylic acid (infection: n=8, sepsis: n=4, COVID-19: n=19), which might impact platelet function in some of our assay systems. (5) We cannot exclude that the partly reduced platelet count or lower hematocrit found in some patients might have an effect in our coagulation flow chamber. In pilot experiments with artificially reduced hematocrit or thrombocytopenia, we found only a minor effect on our readouts (data not shown). (6) In our in vitro studies we focused on GPIIb/IIIa inhibitors, as we found that GPIIb/IIIa integrin activation was markedly reduced

upon stimulation with various agonists (ADP, TRAP-6, CRP-$X_L$), suggesting a generalized platelet activation defect. The amount of blood available for experiments for each patient was limited due to ethical reasons, so we could not perform each experiment with every patient. We can also not exclude that procoagulant platelet- or MK-derived microparticles contribute to our findings. Of note, circulating platelet-derived extracellular vesicles were also described to be a hallmark finding of COVID-19[46], but have not been assessed in this study.

In summary, our study provides evidence that in COVID-19 platelet $\alpha$-granule release is uncoupled from an acquired defective GPIIb/IIIa activation, in contrast to the extensive platelet function defect found in patients with sepsis. This correlated with a robust thrombus formation in flow-based assays, which was prevented by eptifibatide or tirofiban, but not with heparin. Our findings suggest that low dose GPIIb/IIIa blockade could be a promising therapeutic target for patients with COVID-19, which should be further investigated in clinical trials.

**Authors contribution:**

LJW conceptualized the study, designed and performed experiments, analyzed data and wrote the manuscript. MD, GM, MZ, JK, MW, JH, KM, SB, PB, designed and performed experiments, andA analyzed data, TTL, KA, TB, SF, PM and BN provided intellectual input and analyzed data. DW and HS

conceptualized the study, designed experiments, analyzed data and wrote the manuscript.

**Funding**

This study was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation), grant number 374031971-TR 240 to BN & HS, project number 453989101-SFB 1525 to LJW, BN and SF, DFG equipment grant 426173797 and by IZKF grant A-442 to HS & DW. MD was funded by IZKF grant Z-02/84.

**Acknowledgments:**

We thank Nadine Winter for excellent technical and logistic support and Daniela Naumann for her help with the WM-TEM. The investigators thank Sabrina Krauss for her service as consulting physician.

**Conflicts of interest**

The authors do not declare any conflict of interest.

**References:**

1. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579(7798):265-269.

2. WHO. World Health Organisation COVID-19 Dashboard.; 2022. (last accessed 10.08.2022)

3. Cuker A, Tseng EK, Nieuwlaat R, et al. American Society of Hematology living guidelines on the use of anticoagulation for thromboprophylaxis in patients

with COVID-19: May 2021 update on the use of intermediate-intensity anticoagulation in critically ill patients. *Blood Adv*. 2021;5(20):3951-3959.

4. Spyropoulos AC, Levy JH, Ageno W, et al. Scientific and Standardization Committee communication: Clinical guidance on the diagnosis, prevention, and treatment of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost*. 2020;18(8):1859-1865.

5. Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019. *Crit Care Med*. 2020;48(9):1358-1364.

6. Pellegrini D, Kawakami R, Guagliumi G, et al. Microthrombi as a Major Cause of Cardiac Injury in COVID-19: A Pathologic Study. *Circulation*. 2021;143(10):1031-1042.

7. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med*. 2020;383(2):120-128.

8. Althaus K, Marini I, Zlamal J, et al. Antibody-induced procoagulant platelets in severe COVID-19 infection. *Blood*. 2021;137(8):1061-1071.

9. Nicolai L, Leunig A, Brambs S, et al. Immunothrombotic Dysregulation in COVID-19 Pneumonia Is Associated With Respiratory Failure and Coagulopathy. *Circulation*. 2020;142(12):1176-1189.

10. Zuo Y, Estes SK, Ali RA, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. *Sci Transl Med*. 2020;12(570).

11. Stark K, Massberg S. Interplay between inflammation and thrombosis in cardiovascular pathology. *Nat Rev Cardiol*. 2021;18(9):666-682.

12. Gando S, Levi M, Toh CH. Disseminated intravascular coagulation. *Nat Rev Dis Primers*. 2016;2:16037.

13. Group RC. Aspirin in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2022;399(10320):143-151.

14. Parisi R, Costanzo S, Di Castelnuovo A, de Gaetano G, Donati MB, Iacoviello L. Different Anticoagulant Regimens, Mortality, and Bleeding in Hospitalized Patients with COVID-19: A Systematic Review and an Updated Meta-Analysis. *Semin Thromb Hemost*. 2021;47(4):372-391.

15. Bradbury CA, Lawler PR, Stanworth SJ, et al. Effect of Antiplatelet Therapy on Survival and Organ Support-Free Days in Critically Ill Patients With COVID-19: A Randomized Clinical Trial. *Jama*. 2022;327(13):1247-1259.

16. Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood*. 2020;136(11):1317-1329.

17. Schrottmaier WC, Pirabe A, Pereyra D, et al. Platelets and Antiplatelet Medication in COVID-19-Related Thrombotic Complications. *Front Cardiovasc Med*. 2021;8:802566.

18. Hottz ED, Azevedo-Quintanilha IG, Palhinha L, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. *Blood*. 2020;136(11):1330-1341.

19. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*. 2016;315(8):801-810.

20. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med*. 1996;22(7):707-710.

21. Rivera J, Lozano ML, Navarro-Núñez L, Vicente V. Platelet receptors and signaling in the dynamics of thrombus formation. *Haematologica*. 2009;94(5):700-711.

22. Weiss LJ, Manukjan G, Pflug A, et al. Acquired platelet GPVI receptor dysfunction in critically ill patients with sepsis. *Blood*. 2021;137(22):3105-3115.

23. Quintelier K, Couckuyt A, Emmaneel A, Aerts J, Saeys Y, Van Gassen S. Analyzing high-dimensional cytometry data using FlowSOM. *Nat Protoc*. 2021;16(8):3775-3801.

24. Veninga A, Baaten C, De Simone I, et al. Effects of Platelet Agonists and Priming on the Formation of Platelet Populations. *Thromb Haemost*. 2022;122(5):726-738.

25. Jackson SP, Schoenwaelder SM. Procoagulant platelets: are they necrotic? *Blood*. 2010;116(12):2011-2018.

26. Sang Y, Roest M, de Laat B, de Groot PG, Huskens D. Interplay between platelets and coagulation. *Blood Rev*. 2021;46:100733.

27. Swieringa F, Spronk HMH, Heemskerk JWM, van der Meijden PEJ. Integrating platelet and coagulation activation in fibrin clot formation. *Res Pract Thromb Haemost*. 2018;2(3):450-460.

28. Katsoularis I, Fonseca-Rodríguez O, Farrington P, Lindmark K, Fors Connolly AM. Risk of acute myocardial infarction and ischaemic stroke following COVID-19 in Sweden: a self-controlled case series and matched cohort study. *Lancet*. 2021;398(10300):599-607.

29. Iba T, Levy JH, Warkentin TE, Thachil J, van der Poll T, Levi M. Diagnosis and management of sepsis-induced coagulopathy and disseminated intravascular coagulation. *J Thromb Haemost*. 2019;17(11):1989-1994.

30. Bouck EG, Denorme F, Holle LA, et al. COVID-19 and Sepsis Are Associated With Different Abnormalities in Plasma Procoagulant and Fibrinolytic Activity. *Arterioscler Thromb Vasc Biol*. 2021;41(1):401-414.

31. Campbell RA, Hisada Y, Denorme F, et al. Comparison of the coagulopathies associated with COVID-19 and sepsis. *Res Pract Thromb Haemost*. 2021;5(4):e12525.

32. Zaid Y, Puhm F, Allaeys I, et al. Platelets Can Associate with SARS-Cov-2 RNA and Are Hyperactivated in COVID-19. *Circ Res*. 2020;127(11):1404-1418.

33. Barrett TJ, Bilaloglu S, Cornwell M, et al. Platelets contribute to disease severity in COVID-19. *J Thromb Haemost*. 2021;19(12):3139-3153.

34. Denorme F, Manne BK, Portier I, et al. COVID-19 patients exhibit reduced procoagulant platelet responses. *J Thromb Haemost*. 2020;18(11):3067-3073.

35. Khattab MH, Prodan CI, Vincent AS, et al. Increased procoagulant platelet levels are predictive of death in COVID-19. *Geroscience*. 2021;43(4):2055-2065.

36. Kaiser R, Escaig R, Kranich J, et al. Procoagulant platelet sentinels prevent inflammatory bleeding through GPIIBIIIA and GPVI. *Blood*. 2022;140(2):121-139.

37. Stegner D, Göb V, Krenzlin V, et al. Foudroyant cerebral venous (sinus) thrombosis triggered through CLEC-2 and GPIIb/IIIa dependent platelet activation. *Nature Cardiovascular Research*. 2022;1(2):132-141.

38. Shao B, Hoover C, Shi H, et al. Deletion of platelet CLEC-2 decreases GPIbα-mediated integrin αIIbβ3 activation and decreases thrombosis in TTP. *Blood*. 2022;139(16):2523-2533.

39. Viecca M, Radovanovic D, Forleo GB, Santus P. Enhanced platelet inhibition treatment improves hypoxemia in patients with severe Covid-19 and hypercoagulability. A case control, proof of concept study. *Pharmacol Res*. 2020;158:104950.

40. Merrill PJ, Bradburne RM. Successful Use of Glycoprotein IIb/IIIa Inhibitor Involving Severely Ill COVID-19 Patient. *Perm J*. 2021;25.

41. Leucker TM, Osburn WO, Reventun P, et al. Effect of Crizanlizumab, a P-Selectin Inhibitor, in COVID-19: A Placebo-Controlled, Randomized Trial. *JACC Basic Transl Sci*. 2021;6(12):935-945.

42. Rendeiro AF, Ravichandran H, Bram Y, et al. The spatial landscape of lung pathology during COVID-19 progression. *Nature*. 2021;593(7860):564-569.

43. Zhang S, Liu Y, Wang X, et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. *J Hematol Oncol*. 2020;13(1):120.

44. Balle CM, Jeppesen AN, Christensen S, Hvas AM. Platelet Function During Extracorporeal Membrane Oxygenation in Adult Patients. *Front Cardiovasc Med*. 2019;6:114.

45. Kalbhenn J, Schlagenhauf A, Rosenfelder S, Schmutz A, Zieger B. Acquired von Willebrand syndrome and impaired platelet function during venovenous extracorporeal membrane oxygenation: Rapid onset and fast recovery. *J Heart Lung Transplant*. 2018;37(8):985-991.

46. Cappellano G, Raineri D, Rolla R, et al. Circulating Platelet-Derived Extracellular Vesicles Are a Hallmark of Sars-Cov-2 Infection. *Cells*. 2021;10(1).

## Table 1: Cohort characteristics

| | infection (n=28) | sepsis (n=46) | | COVID-19 (n=37) | |
|---|---|---|---|---|---|
| | $t_1$ (n=28) | $t_1$ (n=45) | $t_2$ (n=29) | $t_1$ (n=28) | $t_2$ (n=29) |
| Age, median (IQR) | 71.5 (66-81)) | 61.5 (51-77) | | 64 (54-71) | |
| BMI, median (IQR) | 25.1 (23.9-29.2) | 25 (22.0-28.8) | | 27.8 (25.4-29.4) | |
| 28 day-mortality [n] | 0 | 9 (20%) | | 6 (16%) | |
| Septic Shock [n] | 0 | 15 (33%) | | 0 | |
| DIC [n] | 0 | 5 (11%) | | 2 (5%) | |
| **Comorbidity [n]** | | | | | |
| COPD | 4 (14%) | 5 (11%) | | 1 (3%) | |
| Diabetes | 6 (21%) | 8 (17%) | | 7 (19%) | |
| CKD | 5 (18%) | 11 (24%) | | 3 (8%) | |
| CHF | 7 (25%) | 9 (20%) | | 3 (8%) | |
| **Infection site [n]** | | | | | |
| Respiratory | 9 (32%) | 26 (57%) | | 37 (100%) | |
| Urinary | 10 (36%) | 5 (11%) | | 0 | |
| Other | 9 (32%) | 15 (33%) | | 0 | |
| **Microbiology [n]** | | | | | |
| Gram-negative | 6 | 15 | | / | |
| Gram-positive | 3 | 7 | | / | |
| Gram-positive and Gram-negative | / | 2 | | / | |
| Viral | 1 | 2 | | 37 | |
| Unknown | 18 | 20 | | / | |
| **Laboratory values, median (IQR)** | | | | | |
| PLT [150-350 /nl] | 195 (166-259) | 181 (79-285) | 139 (77-243) | 213 (144-287) | 266 (177-349) |
| RBC [4.18-5.48 /nl] | 3.44 (3.12-3.78) | 3.24 (2.7-3.53) | 2.94 (2.59-3.00) | 3.62 (3.05-4.12) | 3.4 (3.09-4.11) |
| WBC [5-12 /nl] | 15.9 (9.4-20.3) | 14.2 (9.1-18.9) | 8.5 (5.8-15.5) | 9.2 (7.0-11.9) | 9.5 (7.6-12.6) |
| Hb [14-18g /dl] | 12.4 (10.0-12.9) | 9.7 (8.3-11.3) | 9.5 (8.9-11) | 10.3 (8.8-12.1) | 10.1 (9.2-12.1) |
| Hct [42-50 %] | 35.2 (29.5-38.4) | 29.8 (25.7-33.3) | 28.8 (25.9-31.0) | 31.9 (28.4-34.9) | 31.5 (29.5-36.3) |
| Creatinine [0-1.17 g/dl] | 1.13 (0.90-1.4) | 1.61 (0.96-2.84) | 1.32 (0.75-2) | 0.94 (0.70-1.5) | 1.07 (0.78-1.42) |
| Bilirubin [0.1-1.2 mg/dl] | 0.7 (0.4-0.8) | 0.8 (0.5-1.2) | 0.65 (0.3-2) | 0.5 (0.3-0.6) | 0.5 (0.3-0.7) |
| $paO_2/FiO_2$ [mmHg] | >400 (363->400) | 246 (162-376) | >400 (188->400) | 165 (116-195) | 159 (121-196) |
| CRP [0-0.5 mg/dl] | 10.8 (6.5-16.2) | 19.7 (14.3-26.9) | 8.5 (3.3-15) | 14.7 (6.6-22.2) | 8.3 (2.6-16.7) |
| PCT [0-0.5 ng/dl] | 1.2 (0.2-8.3) | 12.0 (1.6-46.8) | 4.3 (0.9-7.0) | 1.7 (0.1-3.3) | 0.3 (0.1-1.5) |
| Fibrinogen [1.9-3.9 g/l] | 5.5 (4.7-7.7) | 5.6 (4.4-6.7) | 6 (4.3-7) | 5.8 (5.0-7.7) | 5.8 (4.6-6.6) |
| INR [0.85-1.18] | 1.07 (0.98-1.38) | 1.17 (1.07-1.39) | 1.05 (0.95-1.18) | 1.00 (0.98-1.08 | 1 (0.94-1.05) |
| **Scores, median (IQR)** | | | | | |
| SOFA | 1 (0-1) | 8 (4-9) | 3 (2-6) | 6 (4-8) | 5 (3-7) |
| SIC | 1 (1-2) | 4 (2-4) | 3 (1-5) | 2 (2-3) | 2 (2-2) |
| **Devices [n]** | | | | | |
| Ventilation | 0 | 21 (47%) | 9 (31%) | 17 (61%) | 21 (72%) |
| CVVHDF | 0 | 8 (18%) | 1 (3%) | 0 | 3 (10%) |
| **Anticoagulation [n]** | | | | | |
| LMWH | 11 (40%) | 22 (48%) | | 27 (73%) | |
| Heparin | 2 (7%) | 9 (20%) | | 6 (16%) | |
| Fondaparinux | 0 | 1 (2%) | | 1 (3%) | |
| **Antiplatelet therapy [n]** | | | | | |
| ASS | 8 (29%) | 4 (9%) | | 9 (24%) | |
| **Medication [n]** | | | | | |
| Noradrenaline | 0 | 29 (63%) | | 17 (46%) | |
| Dobutamine | 0 | 3 (7%) | | 1 (3%) | |

**Abbreviations:**
IQR: Interquartile range
BMI: Body mass index
DIC: Disseminated intravascular coagulation
COPD: Chronic obstructive pulmonary disease
CKD: Chronic kidney disease
CHF: Chronic heart failure
PLT: Platelet
RBC: Red blood cell count
WBC: White blood cell count
Hb: Hemoglobin
Hct: Hematocrit
CRP: C-reactive peptide
PCT: Procalcitonin
INR: International normalized ratio
SOFA: Sequential organ failure assessment
SIC: Sepsis-induced coagulopathy
CVVHDF: continuous venovenous hemodialfiltration
LMWH: Low molecular weight heparin
ASS: Acetylsalicylic acid

## Figure legends:

**Figure 1: Increased GPIIb/IIIa activation and CD62P exposition in resting platelets of sepsis and COVID-19 patients**
Characteristics of healthy controls (ctrl), patients with infection (inf), sepsis or COVID-19 are displayed at: $t_1$: hospital/ICU admission day; $t_2$: day 4 to 7. **(A)** Platelet counts, **(B)** mean platelet volume and **(C)** platelet count vs. MPV are displayed. **(D-G)** Platelet preactivation due to **(D-E)** GPIIb/IIIa activation or **(F-G)** CD62P exposure was assessed under resting conditions by flow cytometry. Events above 2% threshold applied for healthy donors are displayed in **D** and **F**. Relative GeoMFI are shown in **E** and **G**. **(H-I)** Quadrant analysis of CD62P and PAC-1 positive events under resting conditions. **(A-C)** Reference ranges are shown as dashed lines. **A,B** display median**, D-G** median ± 95%CI and **I** mean ± 95% CI. Differences were analyzed using Kruskal-Wallis test. ns: nonsignificant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. PLTs: platelets, MPV: mean platelet volume, APC: allophycocyanin, FITC: fluorescein isothiocyanate, GeoMFI: geometric mean fluorescence intensity.

**Figure 2: Uncoupled GPIIb/IIIa activation and α-granule release in COVID-19**
Characteristics of healthy controls (ctrl), patients with infection (inf), sepsis or COVID-19 are displayed at: $t_1$: hospital/ICU admission day; $t_2$: day 4 to 7. **(A-C)** Whole blood was preincubated with ADP [5 µM] or **(D-G)** CRP-$X_L$ [0.01 µg/ml] for 5 minutes and **(A,B, D-F)** GPIIb/IIIa activation and **(C, G)** CD62P exposition were measured flow cytometrically. **(E)** CRP-$X_L$ induced GPIIb/IIIa activation vs. SOFA-score, red area indicates patients fulfilling sepsis III-criteria. **(F)** ROC-curve analysis of GPIIb/IIIa activation between infection and sepsis patients at $t_1$ upon CRP-$X_L$ stimulation. **(H-J)** Quadrant analysis of CD62P- and PAC-1-positive events upon **(I)** ADP or **(J)** CRP-$X_L$ stimulation. **(K-L)** Automated clustering analysis of sepsis (n=9) and COVID-19 (n=9) patients upon CRP-$X_L$ stimulation. Representative curves are shown in panels **A, H, K**. All graphs show median ± IQR except **I, J** displaying mean ± 95% CI. Differences were analyzed using **(B-J)** Kruskal-Wallis test or **(L)** Kolmogorov-Smirnov test. ns: nonsignificant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. APC: allophycocyanin, FITC: fluorescein isothiocyanate, GeoMFI: geometric mean fluorescence intensity, AUC: area under the curve

**Figure 3: δ-granule deficiency in patients with sepsis or COVID-19**
Characteristics of healthy controls (ctrl), patients with infection (inf), sepsis or COVID-19 are displayed at: $t_1$: hospital/ICU admission day; $t_2$: day 4 to 7. **(A-E)** Schematic visualization of mepacrine assay depicted in **A**. Mepacrine **(C)** uptake and **(D)** release upon TRAP-6 stimulation [5 µM] were quantified in whole blood using flow cytometry. **(E)** Relative mepacrine release was determined by calculating the relation of mepacrine uptake/release. **(F-G)** ATP release upon TRAP-6 stimulation [10 µM] was determined in PRP by lumiaggregometry (minimal platelet count >150 /nl). **(H-I)** Number of δ-granules was determined in PRP using WM-TEM. **(H)** Representative images are shown, red arrows indicate δ-granules. Scale bars indicate 1 µm. **(I)** Dots show median δ-granule number per platelet calculated in a two investigator blinded approach. Representative figures are displayed in **B, F, H**. All graphs show median ± 95%CI. Differences were analyzed using Kruskal-Wallis test. ns: nonsignificant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

**Figure 4: Unaltered in vitro bleeding time in sepsis or COVID-19**
**(A-D)** In vitro bleeding time was mimicked using PFA-200 in whole blood (minimal platelet count >150 /nl). Representative figures are displayed in **C, D**. Arrows indicate saw-tooth pattern like events. Reference ranges are highlighted in **A, B**. **(E-N)** Thrombus and fibrin formation were assessed under venous shear (200 $s^{-1}$) using recalcified whole blood on **(E-J)** collagen/TF or **(K-N)** collagen coated spots. Samples were stained for platelets (α-GPIbβ-Alexa647: magenta) and fibrin(ogen) (cyan). **(F,K)** Surface area coverage (%SAC) of platelets, **(G,L)** platelet fluorescence intensity in arbitrary units (A.U.), **(H,M)** morphological score evaluated by two blinded investigators and **(I,N)** fibrin(ogen) SAC are displayed. Representative images from one focal plane are depicted in panel **E**. Scale bars indicate 50 µm. **(J)** Thrombi were imaged in one focal plane using confocal microscopy. Scale bar in upper images indicates 50 µm and 25 µm in lower images. All graphs show median ± 95%CI. Differences were analyzed using Kruskal-Wallis test. ns: nonsignificant.

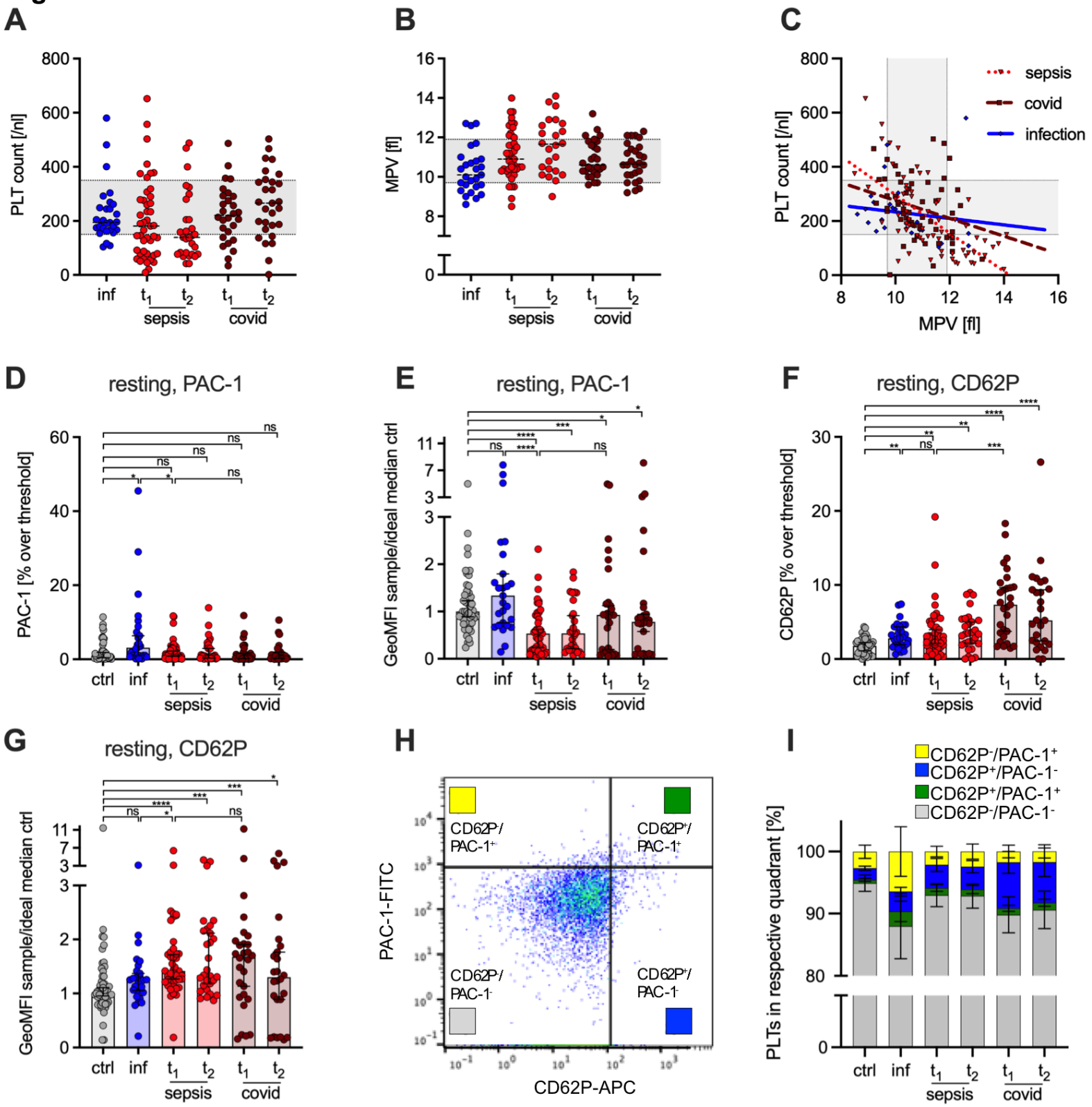
**Figure 5: Robust thrombus formation despite anti-coagulation in COVID-19**
**(A-J)** Thrombus and fibrin formation were assessed under arterial shear (1000 $s^{-1}$) using recalcified whole blood on **(A-F)** collagen/TF **(G-J)** or collagen coated spots. Samples were stained for platelets (α-GPIbβ-Alexa647: magenta) and fibrin(ogen) (cyan). **(B,G)** Surface area coverage (%SAC) of platelets**, (C,H)** platelet fluorescence intensity in arbitrary units (A.U.) **(D,I)** morphological score evaluated by two blinded investigators and **(E,J)** fibrin(ogen) SAC are displayed. Representative images from one focal plane are depicted in panel **A** and scale bars indicate 50 µm. **(F)** Thrombi were imaged in one focal plane using confocal microscopy, scale bar in upper images indicates 50 µm and 25 µm in lower images. All graphs show median ± 95%CI. Differences were analyzed using

Kruskal-Wallis test. ns: nonsignificant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

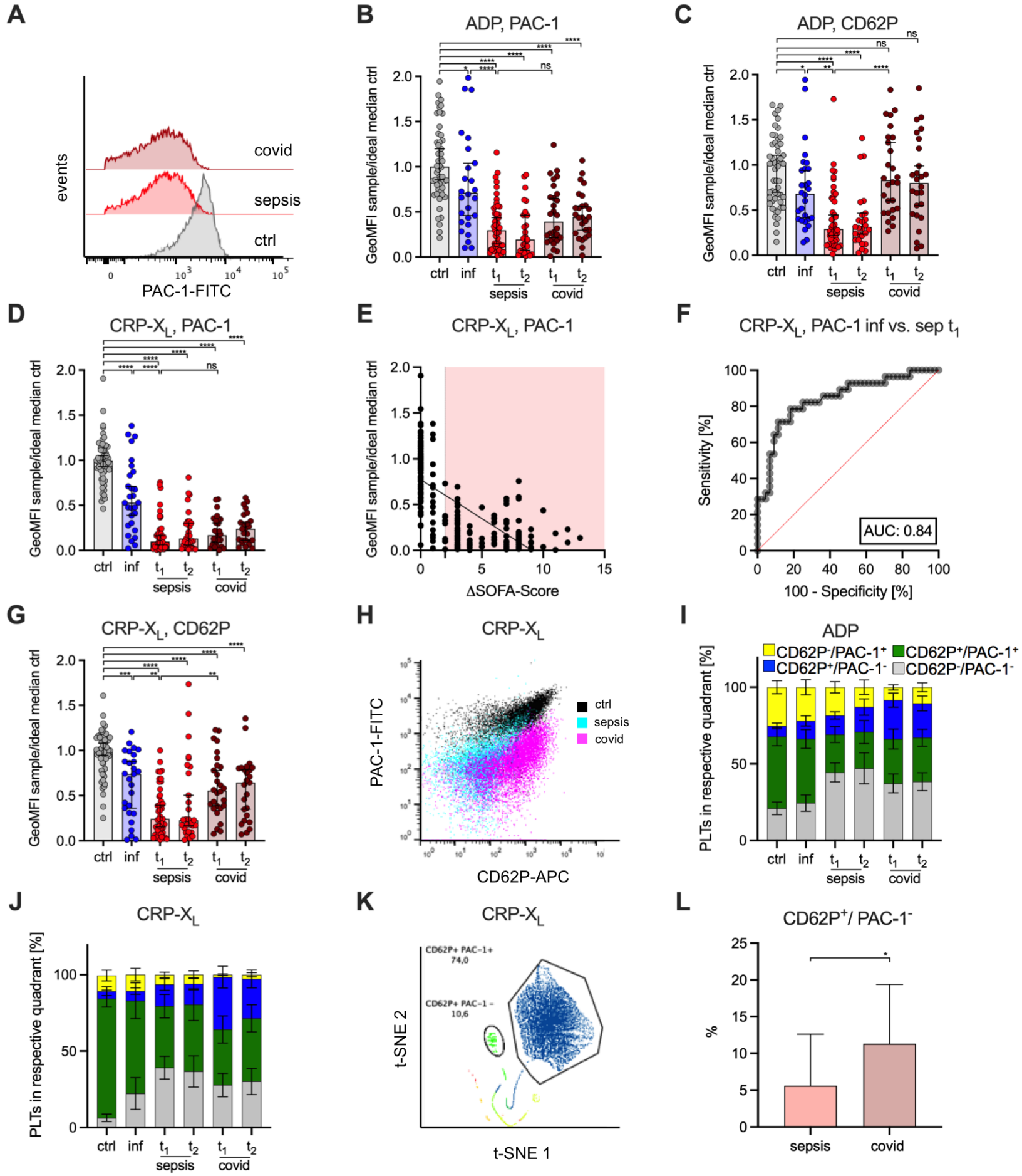
**Figure 6: Low dose GPIIb/IIIa blockade reduces thrombus formation in COVID-19 patients**

Characteristics of controls (ctrl) (n=9) in vitro heparinized controls (n=8) and patients with COVID-19 (n=10) are displayed. Thrombus and fibrin formation on collagen/TF spots were assessed under arterial shear (1000 $s^{-1}$) in recalcified whole blood after preincubation with **(A-E)** eptifibatide or **(F-J)** tirofiban. Platelets are displayed in magenta and fibrin(ogen) in cyan. **(B,G)** Surface area coverage (%SAC) of platelets**, (C,H)** platelet fluorescence intensity in arbitrary units (A.U.), **(D,I)** morphological score depicted by two blinded investigators and **(E,J)** fibrin(ogen) SAC are displayed. Representative images from one focal plane are depicted in panels **A** and **F**, scale bars indicate 50 µm. All graphs show mean ± SEM. Differences were analyzed using Kruskal-Wallis test. ns: non-significant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

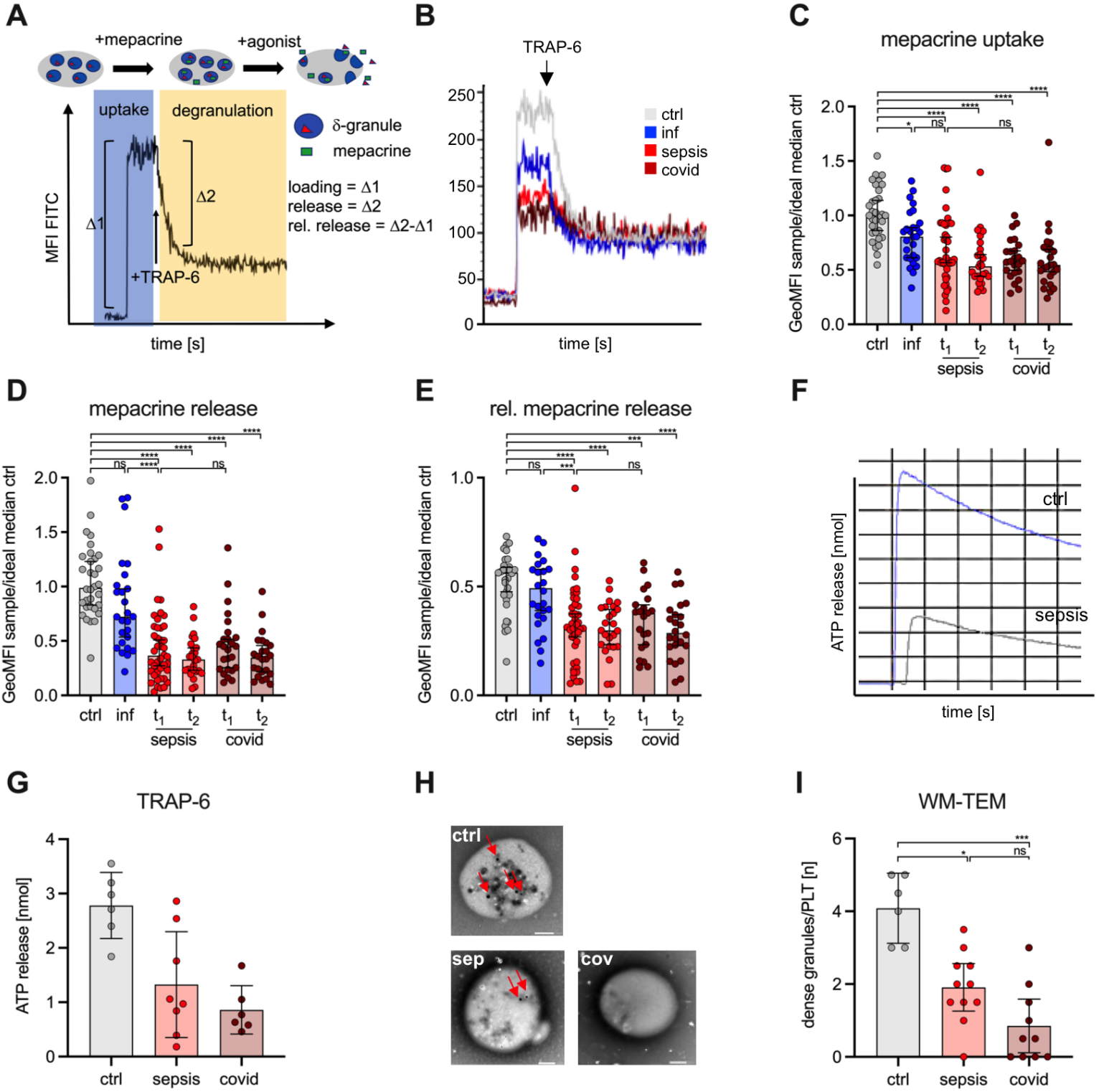
**Figure 1**


A
PLT count [/nl]
inf
sepsis
covid
B
MPV [fl]
C
PLT count [/nl]
MPV [fl]
sepsis
covid
infection
D
resting, PAC-1
PAC-1 [% over threshold]
ctrl
inf
E
resting, PAC-1
GeoMFI sample/ideal median ctrl
F
resting, CD62P
CD62P [% over threshold]
G
resting, CD62P
GeoMFI sample/ideal median ctrl
H
PAC-1-FITC
CD62P-APC
CD62P-/PAC-1+
CD62P+/PAC-1+
CD62P-/PAC-1-
CD62P+/PAC-1-
I
CD62P-/PAC-1+
CD62P+/PAC-1-
CD62P+/PAC-1+
CD62P-/PAC-1-
PLTs in respective quadrant [%]

# Figure 2


A
events
covid
sepsis
ctrl
PAC-1-FITC
B
ADP, PAC-1
GeoMFI sample/ideal median ctrl
ctrl inf t1 t2 t1 t2
sepsis covid
C
ADP, CD62P
GeoMFI sample/ideal median ctrl
ctrl inf t1 t2 t1 t2
sepsis covid
D
CRP-XL, PAC-1
GeoMFI sample/ideal median ctrl
ctrl inf t1 t2 t1 t2
sepsis covid
E
CRP-XL, PAC-1
GeoMFI sample/ideal median ctrl
ΔSOFA-Score
F
CRP-XL, PAC-1 inf vs. sep t1
Sensitivity [%]
100 - Specificity [%]
AUC: 0.84
G
CRP-XL, CD62P
GeoMFI sample/ideal median ctrl
ctrl inf t1 t2 t1 t2
sepsis covid
H
CRP-XL
PAC-1-FITC
CD62P-APC
ctrl
sepsis
covid
I
ADP
CD62P-/PAC-1+
CD62P+/PAC-1+
CD62P+/PAC-1-
CD62P-/PAC-1-
PLTs in respective quadrant [%]
ctrl inf t1 t2 t1 t2
sepsis covid
J
CRP-XL
PLTs in respective quadrant [%]
ctrl inf t1 t2 t1 t2
sepsis covid
K
CRP-XL
CD62P+ PAC-1+
74,0
CD62P+ PAC-1 -
10,6
t-SNE 2
t-SNE 1
L
CD62P+/ PAC-1-
%
sepsis
covid

# Figure 3


A
+mepacrine
+agonist
uptake
degranulation
δ-granule
mepacrine
loading = Δ1
release = Δ2
rel. release = Δ2-Δ1
MFI FITC
Δ1
Δ2
+TRAP-6
time [s]
B
TRAP-6
ctrl
inf
sepsis
covid
time [s]
C
mepacrine uptake
GeoMFI sample/ideal median ctrl
ctrl
inf
sepsis
covid
D
mepacrine release
GeoMFI sample/ideal median ctrl
E
rel. mepacrine release
GeoMFI sample/ideal median ctrl
F
ATP release [nmol]
ctrl
sepsis
time [s]
G
TRAP-6
ATP release [nmol]
H
ctrl
sep
cov
I
WM-TEM
dense granules/PLT [n]

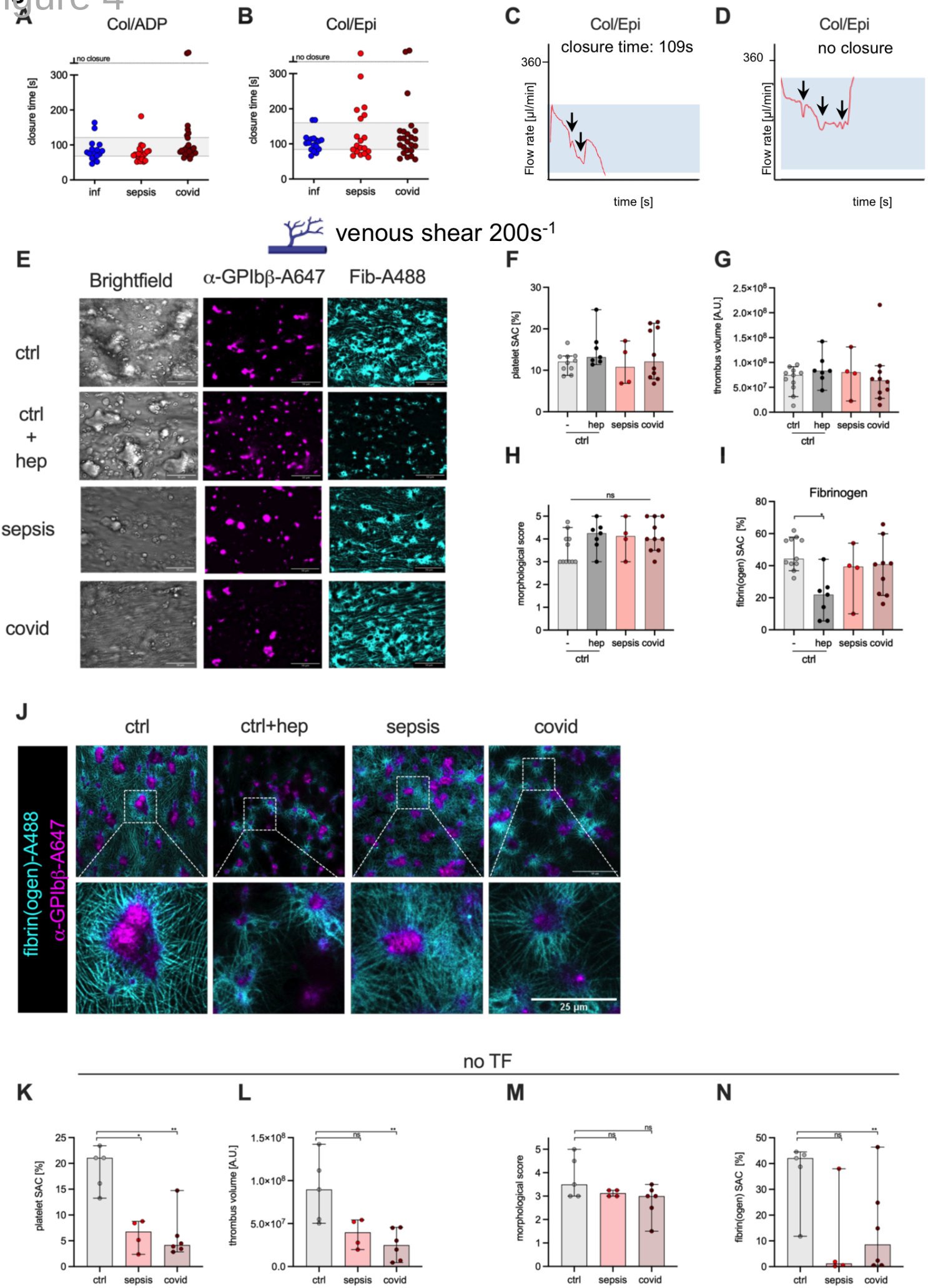
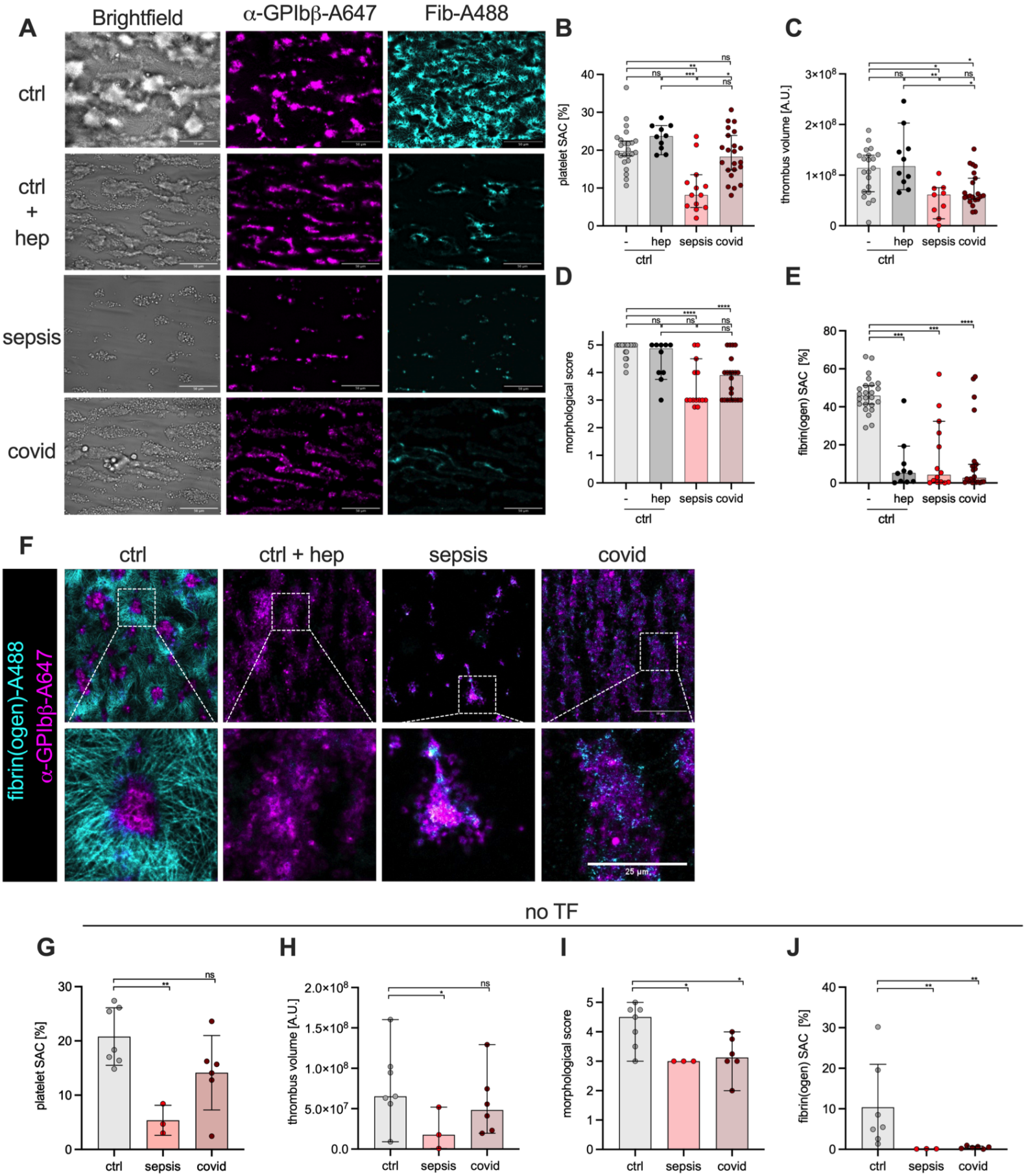
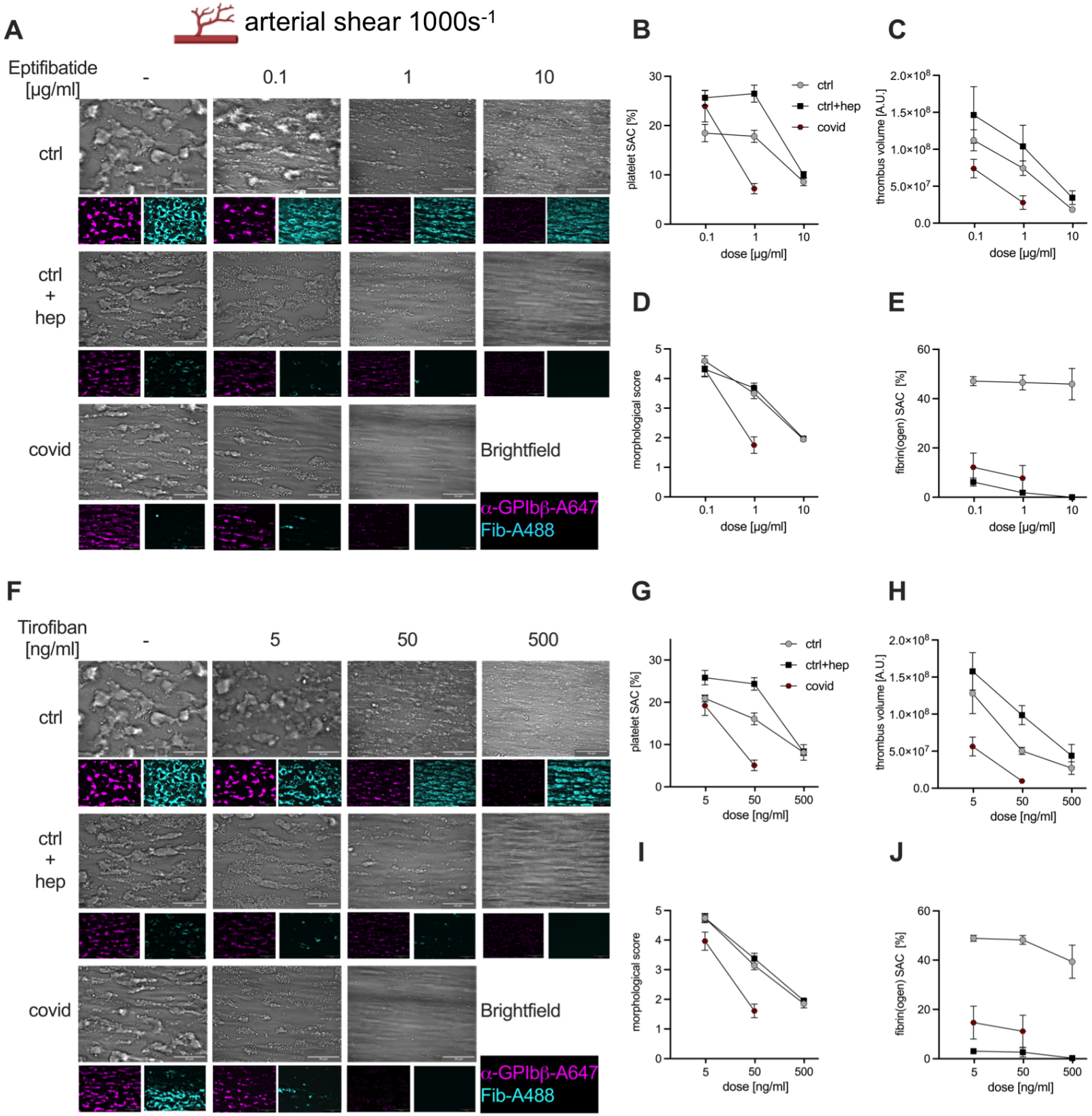

A
Col/ADP
no closure
closure time [s]
300
200
100
0
inf
sepsis
covid
B
Col/Epi
no closure
closure time [s]
300
200
100
0
inf
sepsis
covid
C
Col/Epi
closure time: 109s
360
Flow rate [µl/min]
time [s]
D
Col/Epi
no closure
360
Flow rate [µl/min]
time [s]
venous shear 200s-1
E
Brightfield
α-GPIbβ-A647
Fib-A488
ctrl
ctrl + hep
sepsis
covid
F
platelet SAC [%]
30
20
10
0
-
hep
sepsis
covid
ctrl
G
thrombus volume [A.U.]
2.5×10^8
2.0×10^8
1.5×10^8
1.0×10^8
5.0×10^7
0.0
ctrl
hep
sepsis
covid
ctrl
H
morphological score
ns
5
4
3
2
1
0
-
hep
sepsis
covid
ctrl
I
Fibrinogen
fibrin(ogen) SAC [%]
80
60
40
20
0
-
hep
sepsis
covid
ctrl
J
ctrl
ctrl+hep
sepsis
covid
fibrin(ogen)-A488
α-GPIbβ-A647
25 µm
no TF
K
platelet SAC [%]
25
20
15
10
5
0
ctrl
sepsis
covid
L
thrombus volume [A.U.]
1.5×10^8
1.0×10^8
5.0×10^7
0.0
ctrl
sepsis
covid
M
morphological score
5
4
3
2
1
0
ctrl
sepsis
covid
N
fibrin(ogen) SAC [%]
50
40
30
20
10
0
ctrl
sepsis
covid

**Figure 5** arterial shear 1000s$^{-1}$



# Figure 6


A
arterial shear 1000s-1
Eptifibatide [µg/ml]
-
0.1
1
10
ctrl
ctrl + hep
covid
Brightfield
α-GPIbβ-A647
Fib-A488
B
platelet SAC [%]
dose [µg/ml]
ctrl
ctrl+hep
covid
C
thrombus volume [A.U.]
dose [µg/ml]
D
morphological score
dose [µg/ml]
E
fibrin(ogen) SAC [%]
dose [µg/ml]
F
Tirofiban [ng/ml]
-
5
50
500
ctrl
ctrl + hep
covid
Brightfield
α-GPIbβ-A647
Fib-A488
G
platelet SAC [%]
dose [ng/ml]
ctrl
ctrl+hep
covid
H
thrombus volume [A.U.]
dose [ng/ml]
I
morphological score
dose [ng/ml]
J
fibrin(ogen) SAC [%]
dose [ng/ml]