Induction of soluble platelet activation markers and FXIII deficiency promote COVID-19 severity

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Abstract:

Coagulation dysfunction and thromboembolism emerge as strong comorbidity factors in severe COVID-19 patients. However, the underlying pathomechanisms are largely undefined. Here, we sought to identify the potential molecular mechanisms of SARS-CoV-2 mediated coagulopathy and thromboembolism. A broader investigation was conducted including hospitalized COVID-19 patients with (severe cases that required intensive care) or without pneumonia (moderate cases). Phenotypic and molecular characterizations were performed employing basic coagulation tests, flow cytometry-based multiplex assays, and ELISA.

The investigations revealed induction of plasma P-selectin and CD40 ligand (sCD40L) in moderate COVID-19 cases which were significantly abolished with the progression of COVID-19 severity. Moreover, a profound reduction in plasma tissue factor pathway inhibitor (TFPI) and FXIII were identified particularly in the severe COVID-19. Further analysis revealed a profound induction of fibrinogen in both moderate and severe patients. Interestingly, an elevated plasminogen activator inhibitor-1 more prominently in moderate, and tissue plasminogen activator (tPA) particularly in severe COVID-19 cases were observed. Particularly, the levels of fibrinogen and tPA directly correlated with the severity of COVID-19.

In summary, SARS-CoV-2 infection induces the levels of platelet activation markers soluble P-selectin and sCD40L in hospitalized COVID-19 patients. Furthermore, an attenuated level of TFPI indicates TF pathway activation and, acquired FXIII deficiency likely plays a key role in thrombus instability and promotes thromboembolism in severe cases. The progression of COVID-19 severity could be limited with anti-platelet in combination with recombinantTFPI treatment. Furthermore, thromboembolic events in severe COVID-19 patients could be minimized if treated with recombinantFXIII in combination with LMW heparin.

Keywords; Coagulation, Platelet activation, FXIII, Embolism, TFPI, tPA

Introduction:

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection often leads to respiratory conditions like acute respiratory distress syndrome (ARDS) and pneumonia in severe cases [1, 2]. Preliminary cohort studies show a relatively higher incidence (35–45%) of venous thromboembolic events, particularly in hospitalized COVID-19 patients [3, 4]. Acute respiratory disease progression includes an early infection phase in which the virus attacks epithelial cells using angiotensin-converting enzyme 2 (ACE2) receptors, which leads to pneumonia and induction of severe systemic inflammation [5]. Coagulopathy is one of the most concerning features in severe COVID-19 patients which is characterized by mildly prolonged prothrombin and activated partial thromboplastin times, elevated D-dimer, and fibrinogen levels [6, 7]. The elevated coagulopathy markers are found to be associated with a higher mortality rate [8, 9].

Accumulating evidences suggest SARS-CoV-2 -mediated endothelial damage releases a variety of soluble markers of endothelial dysfunction including von Willebrand factor (VWF), soluble E-selectin, P-selectin, thrombomodulin [10, 11]. Such biologically active molecules eventually promote platelet activation. Platelet receptors regulate not only thrombosis but also initiate delayed cellular activities underlying inflammatory response against diverse pathogens, including viruses [12]. Tissue factor pathway inhibitor (TFPI) is a physiological inhibitor of TF and plays a critical role in balancing the initiation phase of coagulation. TFPI is a serine protease inhibitor that inhibits TF-FVIIa and prothrombinase complex (FXa/FVa) and regulates thrombin generation[13, 14].

In a variety of infections, endothelial cells release proinflammatory cytokines such as TNF-α, IL-1, and IL-6 which in turn not only increases the expression of TF on the endothelial lining but also increases the plasminogen activator inhibitor-1 (PAI-1). Endotoxin-induced expression of TF and PAI-1 by endothelium thus may provide a stimulus that promotes thrombotic complication [15, 16]. Several viral infections including dengue and influenza were found to be associated with platelet activation and inflammation through different molecular pathways[17]. The inflammatory reaction can also lead to a release of reactive oxygen species (ROS) and proteases which contribute to endothelial damage and further pathological remodeling of the venous wall [18].

Studies have revealed that patients infected with SARS-CoV-1 or MERS virus develop fibrin/thrombi within the pulmonary vasculature [19]. Studies with SARS-CoV-1 infected mice model further suggest the abnormal expression of procoagulant genes such as thrombin, VII, XI, XII, and plasminogen activators especially in those who had fatal consequences [20, 21]. The SARS-CoV-2 infection leads to coagulation dysfunction by increasing the plasma procoagulant factor levels including fibrinogen and D-dimer [8, 9]. Sepsis-induced coagulopathy (SIC) and

disseminated intravascular coagulopathy (DIC) have been documented with severe disease, especially in non-survivors COVID-19 cases [6].

Here we have performed a broader investigation and report lower levels of plasma TFPI which was associated with FXIII deficiency in severe COVID-19 patients. Moderate COVID-19 patients exhibited increased levels of plasma P-selectin and CD40 ligand (sCD40L) and the levels declined with the progression of the severity of COVID-19. Fibrinolysis pathway analysis revealed a similar pattern of PAI-1, a higher level in moderate vs. severe patients, and an elevated level of tPA only in severe COVID-19 cases. These findings provide strong evidence of platelet activation and thrombosis induction in moderate patients. COVID-19 severity advances with the activation of TF pathway and, induction of thromboembolism due to FXIII deficiency.

Patients and Methods:

The study was conducted on COVID-19 patients, hospitalized in Rashid Hospital, Dubai between May-June 2020. SARS-CoV-2 infection was confirmed through the reverse transcription-polymerase chain reaction (RT-PCR). A total of 30 (15 moderate + 15 severe) hospitalized COVID-19 cases and 10 healthy controls (8 Males: 2 Females), age ranging from 25- 43 years, were included in the study. The family history and blood samples were collected from patients and healthy controls after getting written informed consent. The ethical approval to conduct the proposed studies was taken from the institutional ethical review boards of the University of Sharjah and Dubai Health Authority.

Hospitalized COVID-19 patients with elevated D-dimer were included in the study. Patients were categorized into moderate (without pneumonia or the requirement of intensive care) and severe (presented pneumonia and/or acute respiratory distress syndrome confirmed by Chest X-ray or CT scan and, required intensive care). Patients with a preexisting history of cardiovascular diseases including those with myocardial infarction, stroke, or deep vein thrombosis (DVT) were excluded from the study. Patients were also excluded if received thromboprophylaxis like low molecular weight (LMW) heparin or other anti-coagulant.

Blood sample collection and plasma preparation:

Blood samples from COVID-19 patients and healthy controls were collected in EDTA-coated vials. Plasma was prepared by centrifuging the whole blood at 700*g* for 10 minutes and then transferred to a fresh tube and stored at -80^{0}C until further use.

Clinical laboratory investigation:

Upon hospitalization, basic laboratory investigations including complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, C reactive protein (CRP) were performed. PT, APTT, and D-dimer assays were performed using semi-automated coagulometer STAGO*1 and STAGO-STAIR1, and CBC was measured under Beckman Coulter. The level of CRP was measured using CoBAS.

Flow cytometric detection of coagulation, thrombosis, and fibrinolysis markers:
To detect the levels coagulation, thrombosis and fibrinolysis markers in plasma, we performed multiplex bead-based assays using LegendPlex Human thrombosis (BioLegend #740892) and LegendPlex Human Fibrinolysis Panels (BioLegend #740761) following the manufacturer's instruction. Briefly, diluted plasma samples from COVID-19 patients and healthy controls were used to measure the levels of soluble markers. Data were acquired under BD FACS Aria III using FACS Diva software and data analysis was done using Legendplex software (Biolegend, USA). All the samples were assessed in duplicate and average was taken as a final reading.

Quantification of human tissue factor pathway inhibitor: To quantify the tissue factor pathway inhibitor (TFPI) levels in the plasma samples of the COVID-19 patients and healthy controls, we performed a sandwich enzyme-linked immunosorbent assay (ELISA) using Human TFPI ELISA kit (Abcam# ab274392) according to the manufacturer's instruction. All the samples were assessed in duplicate and average was taken as a final reading.

Statistics:

Data group differences were evaluated for significance using unpaired *t*-test or one-way ANOVA (Graph Pad Prism Software Inc., San Diego, CA). Data are expressed as mean ± SEM. For all tests, a *P*-value <0.05 was considered for statistical significance.

Results:

Phenotypic and clinical presentation:
The age range of recruited patients was between 32- 69 years (28 Males: 2 Females) and the majority of the cases were of Asian background. Laboratory investigations revealed slightly prolonged PT irrespective of the COVID-19 severity. APTT was within the normal range in the moderate and, slightly prolonged in severe cases (**Fig. 1A-B**). The platelet counts were in the normal range in both patient groups (**Fig. 1C**). D-dimer level both in moderate and severe groups was higher than the normal range and the level was significantly higher in the severe vs. moderate COVID-19 patients (**Fig. 1D**). The laboratory investigations further suggested the induction of inflammatory reaction in COVID-19 cases in which an elevated level of plasma c-reactive protein (CRP) was identified in both moderate and severe cases (**Fig. 2A**). Moreover, increased inflammation was further indicated by a significantly higher number of white blood cells (WBCs) including absolute lymphocyte counts (ALC), particularly in severe COVID-19 cases (**Fig. 2B-C**). The level of plasma creatinine was normal and profound induction of ferritin level was seen in both patient groups (**Fig. 2D-E**).

SARS-CoV-2 induces the release of soluble platelet activation marker:

Vascular inflammation often leads to endothelium damage and platelet activation through releasing numerous intermediate biologically active molecules [22-24]. Therefore, we measured the levels of different soluble plasma markers including P-selectin and sCD40L which might play a role in platelet activation, inflammation, and thrombosis in COVID-19. Indeed, P-selectin was found to be markedly elevated in the moderate group which was significantly abolished in the severe COVID-19 cases (**Fig. 3A-B**). Similarly, the level of sCD40L was found significantly elevated in the moderate COVID-19 cases and the level of sCD40L profoundly diminished with the advancement of severity (**Fig. 3C**). These findings strongly suggest that SARS-CoV-2 triggers the release of P-selectin and sCD40L potentially from activated endothelial cells and induces platelet activation. The activated platelet further contributes to the elevated levels of P-selectin and sCD40L and thrombosis.

SARS-CoV-2 suppresses tissue factor pathway inhibitor, FXIII and induces fibrinogen:

Accumulating data sets suggest that SARS-CoV-2 infection cause disseminated intravascular coagulation (DIC) [2, 6]. Therefore next, we assessed the potential dysregulation of intrinsic, extrinsic, and/or common coagulation pathways that might also contribute to thrombin generation and ultimately platelet activation. Our results show comparable FXI levels between moderate and severe cases vs. healthy controls which indicates the minimal role of the intrinsic pathway in SARS-CoV-2 –induced coagulopathy (**Fig. 4A-B**). Moreover, extrinsic or TF pathway analysis revealed a trend of decline in plasma TF pathway inhibitor (TFPI) level in moderate patients which was at significantly lower levels in the severe COVID-19 cases vs. healthy controls (**Fig. 4C**). The assessment of the common coagulation pathway suggests unchanged plasma prothrombin and antithrombin levels in both moderate and severe cases (**Fig. 4D-E**). Interestingly, fibrinogen levels found markedly upregulated in moderate cases in comparison to healthy controls which further significantly elevated with the severity of COVID-19 (**Fig. 4F**). In stark contrast to fibrinogen level, FXIII level was somewhat lower in moderate patients and level significantly decline in the severe patients vs. healthy controls (**Fig. 4G**). These findings strongly suggest that SARS-CoV-2 -infection has minimal impact on the intrinsic coagulation pathway. However, infection dysregulates the extrinsic as well as common pathways by attenuating the plasma TFPI and FXIII levels, and by upregulating the fibrinogen level.

SARS-CoV-2 infection enhances tissue plasminogen activator and fibrinolysis:

Aberrant fibrinolysis and elevated levels of D-dimer are commonly seen in COVID-19 patients particularly those who required intensive care. However, so far it is not clear whether elevated D-dimer is contributed due to SARS-CoV-2 infection mediated dysregulation of either tissue plasminogen activator (tPA) or plasminogen activator inhibitor-1 (PAI-1) or both. In this series, the key components of fibrinolysis pathways were assessed. Interestingly, PAI-1 level was profoundly elevated in the moderate patients and comparatively lower level was observed in

the severe patients (**Fig. 5A-B**). In contrast, the level of tPA in the moderate cases was comparable to healthy controls however, a significantly higher level was observed in the severe COVID-19 cases (**Fig. 5C**). Moreover, aberrant fibrinolysis was confirmed in the recruited moderate and severe COVDI-19 cases by assessing the D-dimer levels through flow cytometry. Indeed, consistent with the tPA level in plasma, the D-dimer level was slightly higher in the moderate cases and the level was profoundly elevated in the severe COVID-19 (**Fig. 5D**). Importantly, the plasminogen level was comparable in moderate and severe patients vs. healthy controls (**Fig. 5E**). Overall, these findings strongly suggest that the increased level of PAI-1 in moderate cases likely causes increased resistance to fibrinolysis, which in turn, contributes to thrombosis. However, elevated levels of tPA, particularly in severe COVID-19 cases, promote hyperfibrinolysis and increases the level of D-dimer formation.

Discussion:

In this study, we report a mild prolonged PT in both non-ICU (moderate) and ICU (severe) patient groups and APTT only in severe patients. Further investigation revealed attenuated levels of TFPI and FXIII, and the level of fibrinogen was significantly higher in severe COVID-19 patients. Endothelial cell and platelet activation markers plasma P-selectin and sCD40L levels were profoundly elevated in moderate cases which levels were significantly abolished in critical cases. The PAI-1 level was elevated in both the patient groups however increased tPA level was seen only in severe COVID-19 cases.

PT and APTT are the laboratory tools that predict the defective extrinsic and intrinsic coagulation pathways [25]. Recent reports on severe COVID-19 patients presented contradictory results on PT and APTT. In contrast to our results, a study has reported prolonged PT and APTT [26], while others have shown shorten PT and APTT in severe vs. moderate patients [27, 28]. Consistent with our findings, Lin et al., have reported a comparable PT and APTT between moderate and severe patients [29]. Moreover, a multicenter study has shown unchanged initial PT and APTT between COVID-19 patients with or without thrombotic complications [30]. Other coagulation parameters like D-dimer and fibrinogen are consistently reported to be elevated [7, 30, 31], however, there are contradictory reports on thrombocytopenia in critical COVID-19 patients [4]. Some studies show mild thrombocytopenia [4, 32, 33] while, consistent with our findings, others have presented normal platelet count in severe COVID-19 patients [30]. Therefore, these initial diagnostic tools may not provide a clear clinical scenario of COVID-19 patients and a deeper investigation of coagulation pathway and molecular markers related to platelet activation might help in better interpretation.

The low level of TFPI strongly suggests the activation of the tissue factor (TF) pathway in these cases. The low level of TFPI in severe patients is probably insufficient to inhibit the initiation of procoagulant response which is induced by SARS-CoV-2 –mediated endotheliopathy. Deficiency

in TFPI may prompt thrombin generation through TF pathway and FX activation that in turn induces platelet activation. Local TF-dependent coagulation pathway activation and concomitant inhibition of fibrinolysis as indicated by the increased level of PAI-1 particularly in moderate COVID-19 cases are clinical features of ARDS. Moreover, such coagulation activation leads to alveolar fibrin deposition which is a pathological feature of early-phase acute lung injury [34]. Studies employing animal model revealed protective effects of various anticoagulants against acute lung injury and ARDS [35]. It is evidenced that recombinant TFPI (recTFPI) has antithrombotic effects in a variety of *ex vivo* and *in vivo* experimental models [36]. Pre-clinical studies have evidenced that infusing a large dose of the recTFPI limits disseminated intravascular coagulation (DIC) induced by TF or *E.Coli* [37, 38]. Moreover, recTFPI has shown antithrombotic efficacy in an *ex vivo* model of thrombogenesis [39]. Consistently, infusion of a variety of TFPI displayed protective effects against venous and arterial thrombosis in animal models [40, 41].

Soluble P-selectin secreted by activated endothelial cell and platelet and acted as a key inflammatory mediator during viral infections including influenza [42, 43] and SARS-CoV-2 [44]. In our study, a higher level of plasma P-selectin in moderate COVID-19 cases, suggests that SARS-CoV-2 infection potentially triggers the release of P-selectin from endothelial cells which eventually contributes to inflammatory reaction and thrombotic complications in turn increases the severity in COVID-19. Platelet-derived sCD40L also provides vital signals to induce B cells and secrete immunoglobulin [45]**.** Another study highlights the influence of platelet- T-cell interactions during SARS-CoV-2 infections by modulating cytokine IL-17 and IFN-γ production[46, 47]. Moreover, platelet: T cell interaction is clinically relevant as sCD40L was reported to enhance CD8+ T cell activity [48]. sCD40L plays a larger role in shaping innate immune responses through its cognate receptor, CD40, which is expressed on neutrophils [49], endothelial cells [50]**,** and platelets [51]. This suggests the possible interaction between platelet and neutrophil in severe COVID-19 cohort which was previously reported in various other pathologic conditions [49]. Such interactions may lead to platelet-mediated neutrophil activation through sCD40L. Moreover, an increased level of sCD40L was found to be associated with plasma CRP level and platelet-monocytes interaction [52]. These studies signify the contribution of sCD40L in the SARS-CoV-2 -induced pathogenesis.

We observed FXIII deficiency in the severe COVID-19 cohort which indicates thromboembolism. Emerging pieces of evidence suggest that FXIII crosslinks fibrin and promote thrombus stability during platelet accumulation and thrombosis [53]. *In vivo* studies employing a ferric chloride-induced venous thrombosis model have shown an increased thrombus embolization in FXIII deficient mice [54]. Moreover, a similar group has recently reported that FXIII supplementation stabilizes deep vein thrombi in mice and limits pulmonary embolism [55]. These findings support the notion that acquired FXIII deficiency is the most likely cause of thrombi instability and embolization in COVID-19 patients. Observed elevated fibrinogen in the COVID-19 probably

resulted due, partly, to the enhanced inflammatory reaction. It is also evident that fibrinogen and FXIII interact during thrombogenesis to activate FXIII and facilitate the delivery of FXIII to the growing thrombus[53]. Moreover, a recent report suggests that FXIII plays a critical role in extravascular fibrinogen crosslinking in an animal model of liver injury [56]. Therefore, further investigation might elaborate if there is any direct correlation between enhanced fibrinogen and decreased FXIII levels in severe COVID-19 patients.

Aberrant fibrinolysis in COVID-19 patients is consistently reported by multiple studies [9, 32, 57]. In our studies, induction of PAI-1 was observed in the COVID-19 patients indicates resistance to thrombus dissolution, at a higher level in moderate cases, which in turn promotes thrombosis. Moreover, elevated levels of tPA particularly in severe COVID-19 patients indicate the activation of hyperfibrinolysis. It is well-correlated with the significantly elevated level of D-dimer in the severe but not in moderate patients. Similar to our finding, a recent study also reported an elevated level of PAI-1 in COVID-19 patients [58]. Consistently, another study reported elevated levels of both PIA-1 and tPA in the hospitalized COVID-19 patients. This study reported that an extremely elevated level of tPA was significantly associated with higher mortality [59]. The higher level of tPA is consistent with our findings where we have seen increased levels of tPA in the severe COVID-19 patient group.

In conclusion, here we report the induction of platelet activation markers P-selectin and sCD40L were found elevated particularly in the moderate COVID-19 cases, and the levels of these markers markedly declined in the severe COVID-19 patients. Consistent with platelet activation, lower levels of plasma TFPI in a cohort of severe COVID-19 patients indicates the activation of TF pathway. We show that the attenuated level of FXIII was inversely correlated with the fibrinogen level in both moderate and severe COVID-19 patients. Moreover, elevated levels of PAI-1 in moderate, and tPA and D-dimer levels in severe cases suggest a switch of fibrinolysis process with the progression of COVID-19 severity. These findings indicate that a combination prophylactic might be a better therapeutic approach to minimize the coagulation-induced severity and, thromboembolism in the COVID-19 patients. A decreased level of TFPI in COVID-19 suggests that severity in patients could potentially be minimized if treated with LMW heparin in combination with recTFPI which safety was tested in the phase-3 clinical trial [60]. Furthermore, considering a phase-III clinical trial (Identifier: NCT00713648) that showed the safety and therapeutic efficacy of recFXIII[61], it may represent a better therapeutic approach to limit the thromboembolic events in severe COVID-19 patient when treated with recFXIII in combination with LMW heparin.

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Author contribution: AOA, AMY, and MNJ performed experiments and collected data, AWA, ME, FA helped with sample and clinical data collection, MK and RH performed data interpretation and helped in manuscript writing, and FA design study, acquired funding, supervised the project, performed data analysis and interpretation, and wrote the manuscript.

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Figure legends;

Figure 1: Mild prolonged PT and APTT, comparable platelet counts, and a higher level of D-dimer in severe. Dot plots show slightly prolonged (**A**) Prothrombin (PT) in both the moderate and severe groups, and (**B**) activated partial thromboplastin times (APTT) in the severe COVID-19 patients. (**C**) The plot shows a normal platelet count which was comparable between moderate and severe groups. (**D**) The scatter plot shows higher levels of D-dimer in both moderate and severe patients and the level was significantly higher in the severe vs. moderate patient group. The dotted lines show upper and lower normal ranges. *, $p<0.05$.

Figure 2: Induction of inflammatory reaction in COVID-19. Dot plots show a significantly higher level of (**A**) C-reactive protein (CRP) in both moderate and severe groups. The plots show an increased number of (**B**) white blood cells (WBCs) and (**C**) absolute lymphocyte count (ALC) in severe COVID-19 patients. (**D**) The plot shows normal creatinine, and (**E**) higher levels of ferritin in both moderate and severe patients groups. The dotted lines show upper and lower normal ranges. *, $p<0.05$; **, $p<0.005$; ***, $p<0.0005$.

Figure 3: Elevation of plasma prothrombotic markers level in COVID-19. (**A**) Representative flow cytometry Dot plots show the levels of P-selectin and soluble CD40 ligand (sCD40L) in moderate and severe COVID-19 patients vs. healthy controls. Scatter plots show elevated levels of (**B**) plasma P-selectin and (**C**) sCD40L in moderate patients and significantly lower level in the severe vs. moderate COVID-19 patients. *, $p<0.05$; ***, $p<0.0005$; ****, $p<0.0001$.

Figure 4: SARS-CoV-2 suppresses TFPI and factor XIII, and induces fibrinogen levels. (**A**) Representative Dot plots show the levels of plasma factor XIII (FXIII), fibrinogen, and prothrombin in COVID-19 patients. (**B**) Flow cytometry-based multiplex assay shows unchanged FIX levels in COVID-19 cases. (**C**) Scatter plot from ELISA shows a significantly low level of tissue factor pathway inhibitor (TFPI) particularly in severe COVID-19 patients vs. healthy controls. Scatter plots from multiplex assay show comparable (**D**) prothrombin and (**E**) antithrombin levels, (**F**) significantly higher levels of fibrinogen, and (**G**) lower levels of FXIII in COVID-19 patients vs. healthy controls. *, $p<0.05$; **, $p<0.005$; ****, $p<0.0001$.

Figure 5: Defective fibrinolysis in COVID-19. (**A**) Representative Dot plots show higher levels of D-dimer, plasminogen activator inhibitor-1 (PAI-1), and tissue plasminogen activator (tPA) in COVID-19 patients. Scatter plots from multiplex assay show (**B**) comparable plasminogen level, (**C**) higher levels of PAI-1 in both moderate and severe cases, (**D**) a significantly higher level of tPA, and (**E**) D-dimer particularly in severe COVID-19 vs. healthy controls. *, $p<0.05$; **, $p<0.005$; ****, $p<0.0001$.

Figure 6: Diagram illustrating the SARS-CoV-2 -mediated induction of plasma CD40L and P-selectin levels, and suppression of tissue factor pathway and FXIII. SARS-CoV-2 -mediated endothelium damage potentially contributes to the elevated level of plasma CD40L (sCD40L) and P-selectin which induces platelet activation. The activated platelet further releases P-selectin and sCD40L and provides positive feedback to platelet plug formation and platelet-mediated thrombin generation. An attenuated level of TFPI activates the tissue factor pathway which likely boosts thrombin level and enhances platelet activation. The elevated level of fibrinogen in COVID-19 patients likely provides the required fibrin however, a decreased level of FXIII indicates the defective fibrin polymerization which potentially results in unstable thrombi formation and embolization. Moreover, an elevated level of PAI-1 prevents fibrinolysis in moderate COVID-19, however, an increased level of tPA in severe COVID-19 likely induces hyperfibrinolysis in turn increased level of D-dimer formation. s**CD40L**; soluble CD40 ligand, **TFPI**; tissue factor pathway inhibitor, **FXIII**; factor XIII, **PAI-1**; plasminogen activator inhibitor-1, **tPA**; tissue plasminogen activator, **+**; positive feedback. Dotted lines; not known if direct or indirect regulation, Solid line; direct regulation.

Figure 1

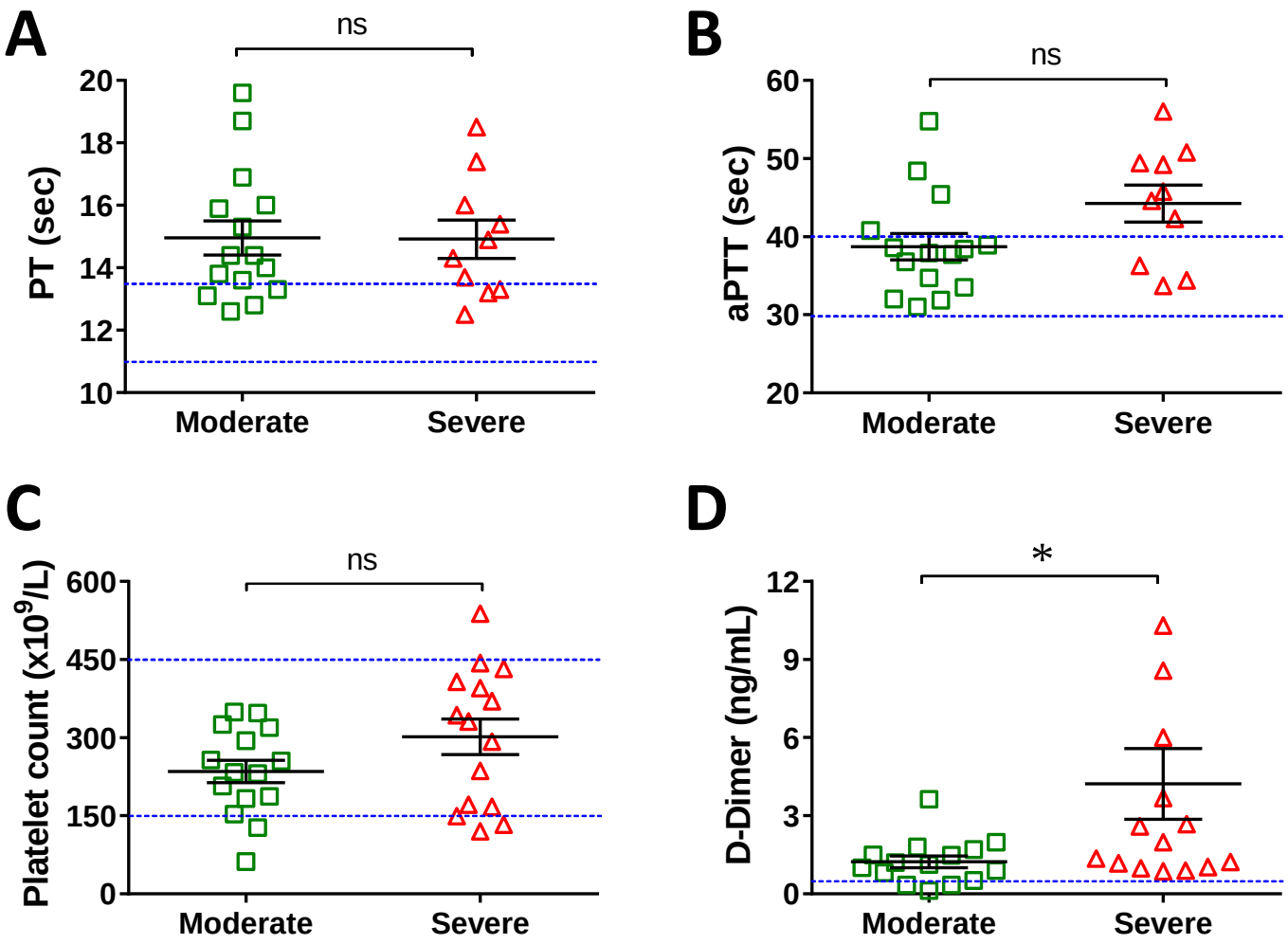
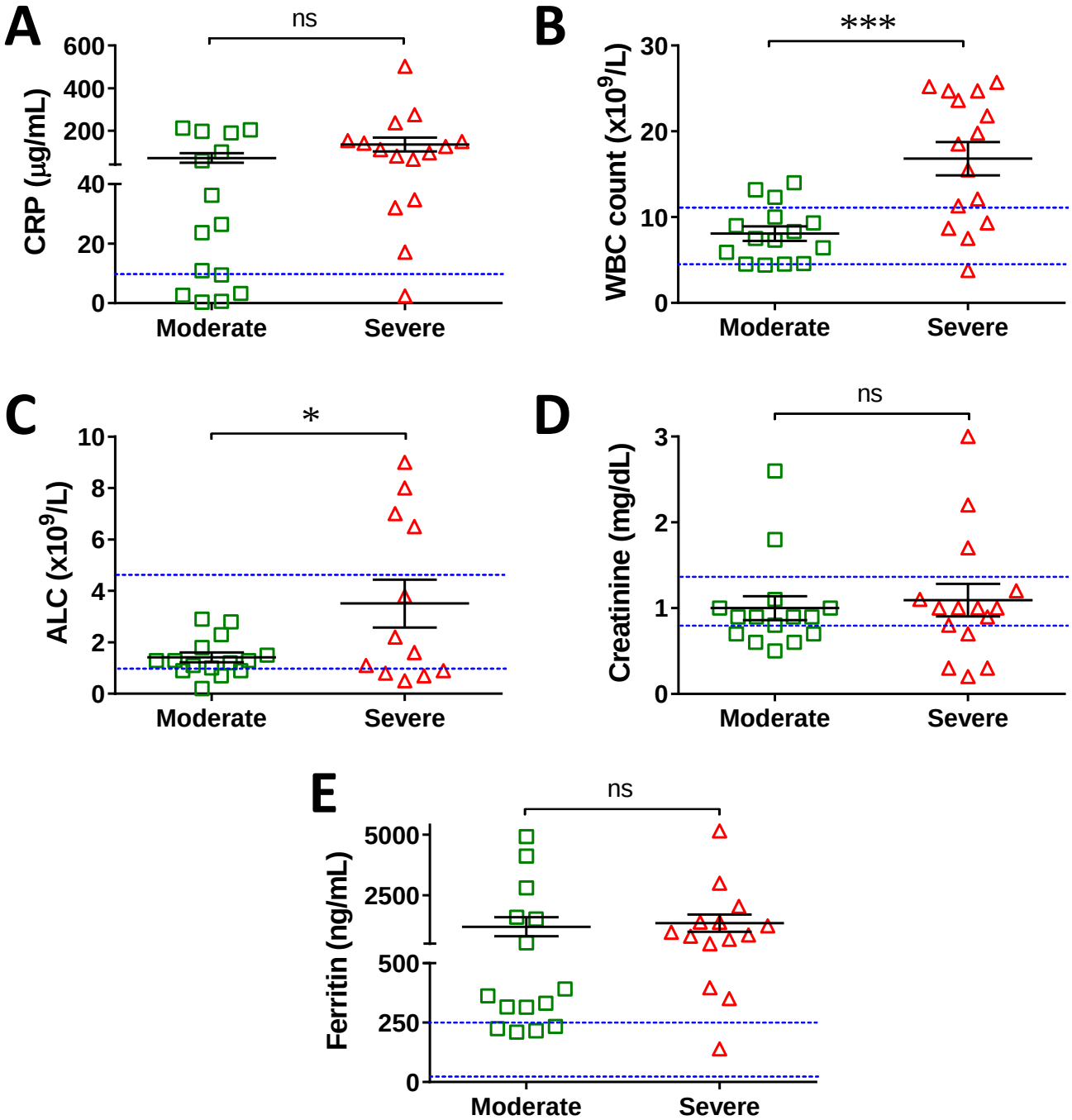

A
ns
PT (sec)
20
18
16
14
12
10
Moderate
Severe
B
ns
aPTT (sec)
60
50
40
30
20
Moderate
Severe
C
ns
Platelet count (x10^9/L)
600
450
300
150
0
Moderate
Severe
D
*
D-Dimer (ng/mL)
12
9
6
3
0
Moderate
Severe

Figure 2


A
ns
CRP (µg/mL)
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400
200
40
20
0
Moderate
Severe
B
***
WBC count (x10^9/L)
30
20
10
0
Moderate
Severe
C
*
ALC (x10^9/L)
10
8
6
4
2
0
Moderate
Severe
D
ns
Creatinine (mg/dL)
3
2
1
0
Moderate
Severe
E
ns
Ferritin (ng/mL)
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2500
500
250
0
Moderate
Severe

Figure 3

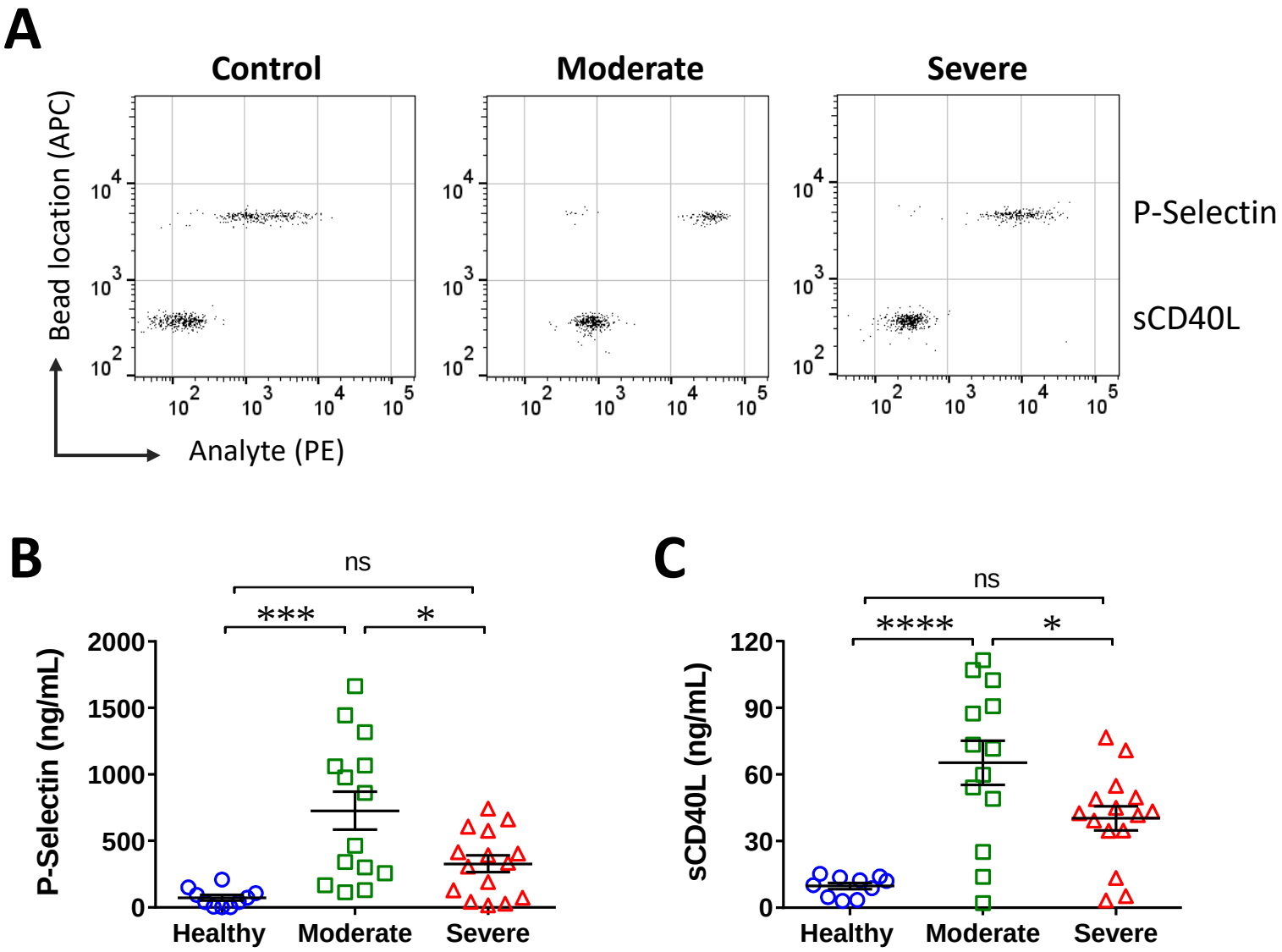

A
Control
Moderate
Severe
Bead location (APC)
Analyte (PE)
P-Selectin
sCD40L
B
ns
***
*
P-Selectin (ng/mL)
2000
1500
1000
500
0
Healthy
Moderate
Severe
C
ns
****
*
sCD40L (ng/mL)
120
90
60
30
0
Healthy
Moderate
Severe

Figure 4

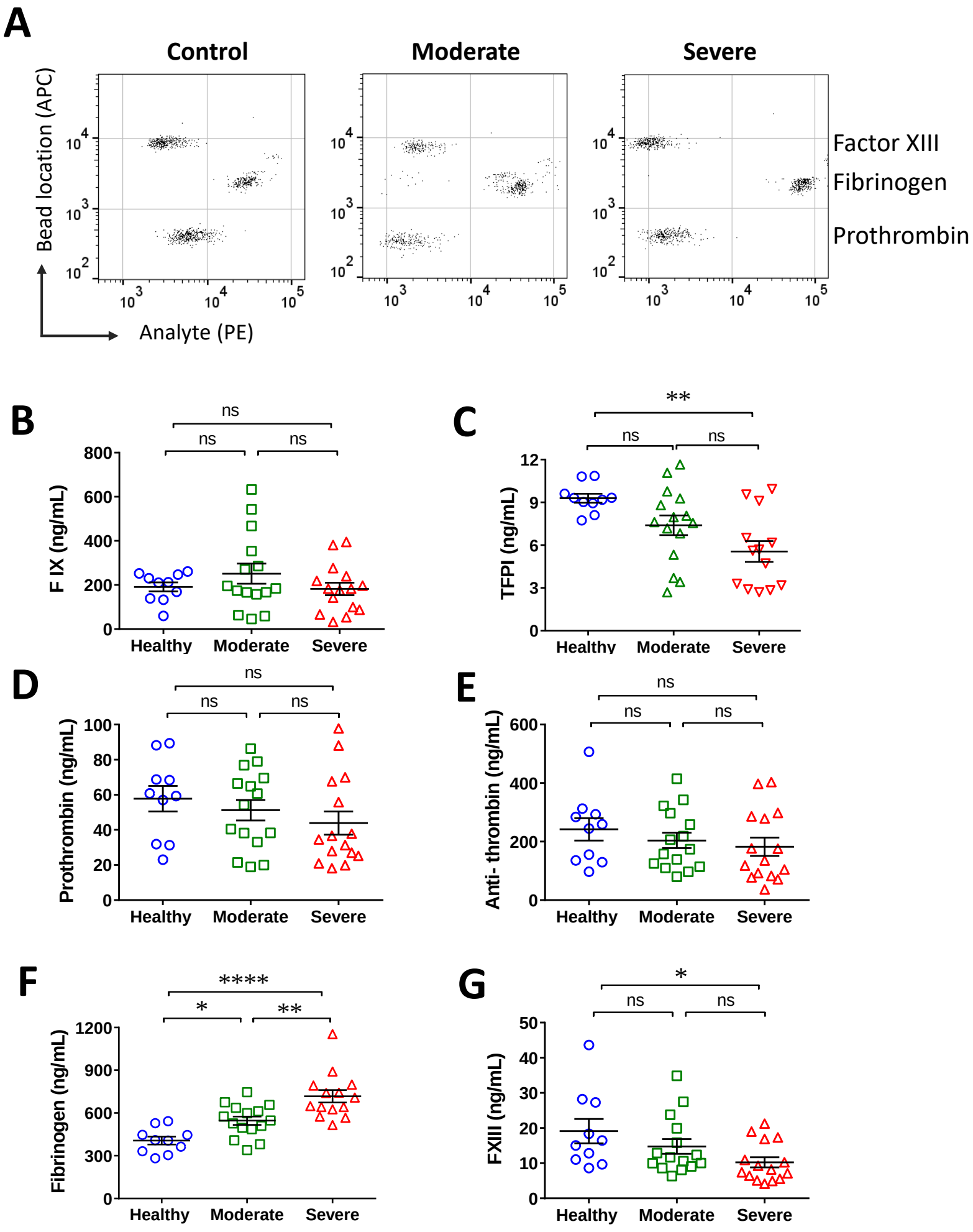

A
Control
Moderate
Severe
Bead location (APC)
Analyte (PE)
Factor XIII
Fibrinogen
Prothrombin
B
F IX (ng/mL)
ns
ns
ns
Healthy
Moderate
Severe
C
TFPI (ng/mL)
**
ns
ns
D
Prothrombin (ng/mL)
E
Anti- thrombin (ng/mL)
F
Fibrinogen (ng/mL)
****
*
**
G
FXIII (ng/mL)
*

Figure 5

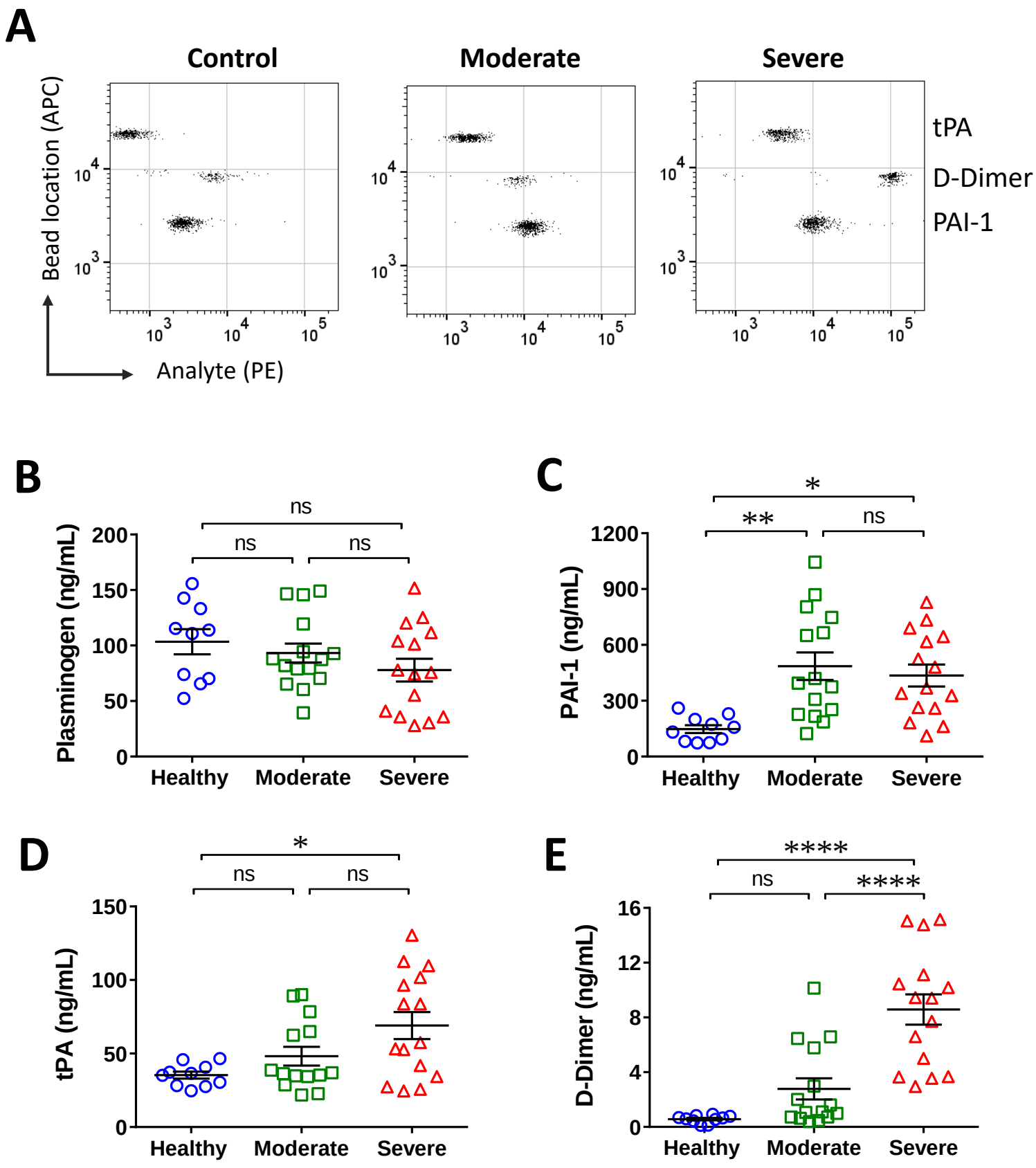
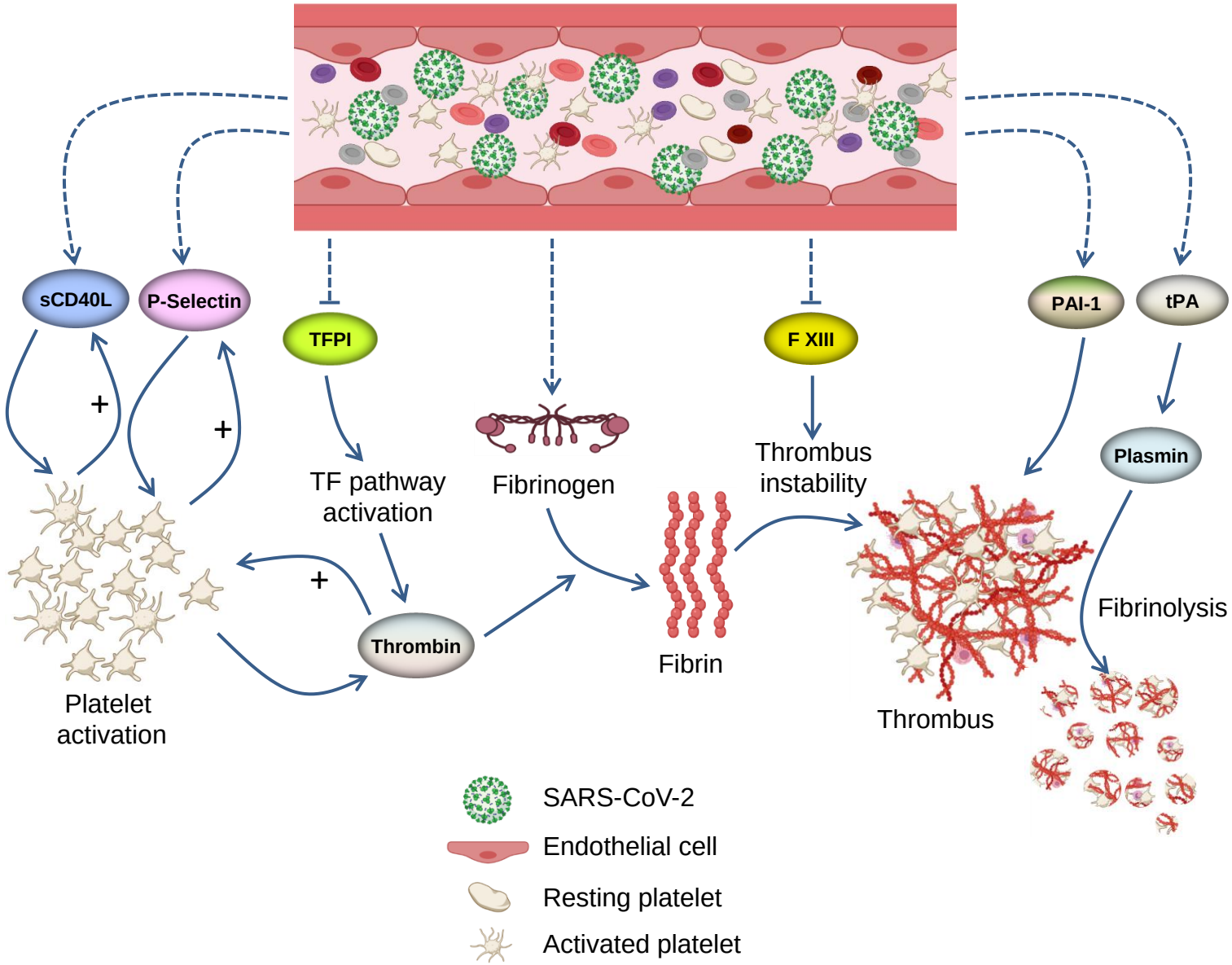

A
Control
Moderate
Severe
Bead location (APC)
Analyte (PE)
tPA
D-Dimer
PAI-1
B
Plasminogen (ng/mL)
ns
ns
ns
Healthy
Moderate
Severe
C
PAI-1 (ng/mL)
*
**
ns
Healthy
Moderate
Severe
D
tPA (ng/mL)
*
ns
ns
Healthy
Moderate
Severe
E
D-Dimer (ng/mL)
****
ns
****
Healthy
Moderate
Severe

Figure 6


sCD40L
P-Selectin
TFPI
F XIII
PAI-1
tPA
+
+
+
TF pathway activation
Fibrinogen
Thrombus instability
Plasmin
Thrombin
Fibrin
Fibrinolysis
Platelet activation
Thrombus
SARS-CoV-2
Endothelial cell
Resting platelet
Activated platelet