2020-12-23

SARS-CoV-2 Receptors are Expressed on Human Platelets and the Effect of Aspirin on Clinical Outcomes in COVID-19 Patients [preprint]

Aditya Sahai
Case Western Reserve University

Et al.

1   **SARS-CoV-2 Receptors are Expressed on Human Platelets and the Effect of Aspirin on Clinical**

2   **Outcomes in COVID-19 Patients**

3

4   Aditya Sahai MD [1‡] , Rohan Bhandari MD[1,2‡], Milka Koupenova PhD[3], Jane E. Freedman MD[3],

5   Matthew Godwin BS[1], Thomas McIntyre PhD[2,4], Mina K. Chung MD[1,2,4],

6   Jean-Pierre Iskandar MD[5], Hayaan Kamran MD[1], Essa Hariri MD[5], Anu Aggarwal PhD[2],

7   Ankur Kalra MD[1], John R. Bartholomew MD[1,4], Keith R. McCrae MD[4,6], Ayman Elbadawi MD[7],

8   Lars G. Svensson MD PhD[1,4], Samir Kapadia MD[1,4] and Scott J. Cameron MD PhD[1,2,4] *

9   **Running Title:** Platelets Express SARS-CoV-2 Receptors & Aspirin Does Not Protect in COVID-19

10

11   **Author Affiliations:**
12   [1]   Heart Vascular and Thoracic Institute, Cleveland Clinic, Cleveland, OH
13   [2]   Department of Cardiovascular and Metabolic Sciences, Cleveland Clinic, Cleveland, OH
14   [3]   Department of Medicine, University of Massachusetts Medical School, Worcester, MA.
15   [4]   Case Western Reserve University Cleveland Clinic Lerner College of Medicine, Cleveland,
16   OH
17   [5]   Department of Medicine, Cleveland Clinic, Cleveland, OH
18   [6]   Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH
19   [7]   Division of Cardiovascular Medicine, University of Texas Medical Branch, Galveston, TX
20
21   ‡Equally contributing authors

22   **Word Count:** 2995 (main text), 199 (abstract)

23   **References**: 55

24   **Figures:** 4

25   **Tables:** 2

26
27   **Address for Correspondence***: Cleveland Clinic Foundation, Department of Cardiovascular
28   Medicine, Section of Vascular Medicine, J3-5, 9500 Euclid Avenue, Cleveland, OH, 44195, USA.
29   Telephone: (216) 316-6794. Email: cameros3@ccf.org
30
31   **Key Words:** Platelets, SARS-CoV-2, COVID-19, Thrombosis, ACE2, TMPRSS2
32

33 **Abstract**

34 Coronavirus disease-2019 (COVID-19) caused by SARS-CoV-2 is an ongoing viral pandemic marked by

35 increased risk of thrombotic events. However, the role of platelets in the elevated observed thrombotic

36 risk in COVID-19 and utility of anti-platelet agents in attenuating thrombosis is unknown. We aimed to

37 determine if human platelets express the known SARS-CoV-2 receptor-protease axis on their cell surface

38 and assess whether the anti-platelet effect of aspirin may mitigate risk of myocardial infarction (MI),

39 cerebrovascular accident (CVA), and venous thromboembolism (VTE) in COVID-19. Expression of ACE2

40 and TMPRSS2 on human platelets were detected by immunoblotting and confirmed by confocal

41 microscopy. We evaluated 22,072 symptomatic patients tested for COVID-19. Propensity-matched

42 analyses were performed to determine if treatment with aspirin or non-steroidal anti-inflammatory

43 drugs (NSAIDs) affected thrombotic outcomes in COVID-19. Neither aspirin nor NSAIDs affected

44 mortality in COVID-19. However, both aspirin and NSAID therapies were associated with increased risk

45 of the combined thrombotic endpoint of (MI), (CVA), and (VTE). Thus, while platelets clearly express

46 ACE2-TMPRSS2 receptor-protease axis for SARS-CoV-2 infection, aspirin does not prevent thrombosis

47 and death in COVID-19. The mechanisms of thrombosis in COVID-19, therefore, appears distinct and the

48 role of platelets as direct mediators of SARS-CoV-2-mediated thrombosis warrants further investigation.

49

50

51

52

53

54

55

56

Introduction

COVID-19 is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and curiously displays a propensity for thrombosis in multiple vascular beds. COVID-19-related thrombosis may contribute to severe organ injury and death. The incidence of thrombotic events was as high as 31% in one cohort [1]. Clinical and autopsy studies of COVID-19 patients suggest an increased risk of microthrombi, venous thromboembolism (VTE), and ischemic stroke[2,3]. Activated platelets are circulating mediators of thrombosis and, therefore, may serve as a logical therapeutic target in COVID-19. Two registered clinical trials (NCT04363840 and NCT04365309) will prospectively evaluate patient outcomes following low dose aspirin in the context of SARS-CoV-2 infection.

SARS-CoV-2 utilizes an spike glycoprotein to bind to the host transmembrane angiotensin-converting enzyme 2 (ACE2) and is then cleaved by the serine protease TMPRSS2 to coordinate entry into the host cell[4,5]. Therefore, co-expression of ACE2 and TMPRSS2 may be important for host cell entry and infectivity of SARS-CoV-2. Importantly, human tissue distribution of ACE2 and TMPRSS2 mirrors organ system involvement in COVID-19 and includes the lungs[6-11], vascular endothelium[9-12], heart[11,13,14], kidneys[8,10,13], liver[8,10], digestive tract[8,10,11,15], nasal epithelium[7,10,11] and central nervous system[10,14]. Single-stranded RNA (ssRNA) viruses, including influenza, are engulfed by platelets and may contribute to immuno-thrombosis indirectly through developing neutrophil extracellular traps (NETs) by engaging the platelet toll-like receptor 7 (TLR7)[16]. SARS-CoV-2, another SSRNA virus, utilizes platelets to modulate immunologic responses including the development of neutrophil extracellular traps (NETs) that are emerging as pro-thrombotic responses in patients with COVID-19[17]. Further, elevation of soluble P-selectin and sCD40L in blood from patients with COVID-19 compared to controls provides indirect evidence of platelet activation in COVID-19 coagulopathy[18]. SARS-CoV-2 is a ssRNA virus, and therefore may directly augment platelet activation causing myocardial infarction (MI), stroke, and VTE.

81

82   A recent report demonstrated that COVID-19 patients have a divergent platelet transcriptome from

83   healthy individuals, and aspirin suppresses COVID-19 platelet activation *in vitro*[19]. The platelet surface

84   receptor for SARS-CoV-2 was not clarified in this study, while a similar investigation by another group

85   identified mRNA for SARS-CoV-2 in human platelets[20]. Thus, our goal was to determine if platelets

86   express known SARS-CoV-2 receptor proteins and, as with influenza previously, contribute to thrombotic

87   events in patients. In the absence of clinical trial data, we sought to evaluate the potential benefit in

88   mitigating thrombotic responses *in vivo* with use of aspirin or other NSAID antiplatelet therapies by

89   propensity matching patients using real-world data.

90

91   **Methods**

92   **Platelet Isolation**

93   Healthy volunteers without any known medical history or on antiplatelet therapy donated blood

94   specimens in accordance with and approved by the Cleveland Clinic Foundation Institutional Review

95   Board (IRB) approval. For each subject, venous blood was drawn by a medical professional into citrate

96   plasma tubes, then centrifuged in a tabletop centrifuge at 1100 RPM for 15 minutes. The platelet rich

97   plasma (PRP), collected well above the buffy coat, was decanted and the platelets were centrifuged at

98   2600 RPM for an additional 5 minutes. These washed platelets were then used in immunoblotting and

99   fluorescence-activated cell sorting (FACS) analyses.

100

101   **Immunoblotting**

102   Washed platelets from healthy subjects or patients with coronary artery disease (CAD) enrolled at the

103   Cleveland Clinic main campus in Ohio were isolated and proteins separated by SDS-PAGE as we have

104   previously documented [21,22] and in accordance with IRB protocols (#19-1451 for patients and #20-413

105   for healthy volunteers). We utilized human brain lysate, human placenta, and engineered human heart

106   tissue as positive controls for TMPRSS2 and ACE2. Human brain lysate is commercially available (Novus

107   #NB820-59177). Human placenta lysate was prepared as follows: placental villous tissue was collected

108   immediately upon uncomplicated, full-term (37–42 weeks' gestation), elective C-section deliveries at

109   MetroHealth Hospital in Cleveland, Ohio and approved by the Cleveland Clinic and MetroHealth IRB

110   (#16–1311 and #16–00335, respectively). This tissue was normally discarded placentas with intact fetal

111   membranes, and following inclusion in the study no protected health information, identifiers, or clinical

112   data were collected. A waiver of consent was approved by the Cleveland Clinic Foundation IRB as

113   the placentas were collected anonymously. Engineered human heart tissue was obtained as follows:

114   human-induced pluripotent stem cells (generated by the California Institute of Regenerative Medicine)

115   were differentiated into beating ventricular-like cardiomyocytes (iCMs) and grown in a monolayer. To

116   enhance maturation, iCMs were subsequently grown as engineered heart tissues as we have previously

117   described[23]. Immunoblotting was conducted using anti-TMPRSS2 (abcam #92323), anti-ACE2 (Abcam

118   #15348), anti-tubulin (CST #3873S), and anti-GAPDH (CST #5174) antibodies. The mean ratio of

119   TMPRSS2 or ACE2 to loading control ± SEM is documented, unless stated otherwise. Primary antibody

120   was used as in a 1:10000 titer overnight at 4°C in 3% bovine serum albumin/Tris-buffered saline-Tween

121   20. Secondary antibody (GE Healthcare, Buckinghamshire, UK) was used in a 1:2000 titer in 5%

122   milk/Tris-buffered saline-Tween for 1 hour at room temperature. Final autoradiographic films (Bioblot

123   BXR, Laboratory Product Sales, Rochester, NY) were quantified by densitometry using ImageJ software

124   (National Institutes of Health). All experiments were performed in accordance with relevant guidelines

125   and regulations.

126

127

128

Confocal Microscopy

Venous blood drawn into and separated as citrated plasma was lysed and fixed with BD FACS lysing

solution (BD Biosciences, NJ, USA, cat# 349202) for 10 mins. The platelet pellet was washed with 1X

PBS, centrifuged at 1500g for 7 mins, resuspended in HEPES-buffered Tyrode solution supplemented

with 2% FBS and then stained for 1 hour with the following: CD41 to confirm platelets (ThermoFisher

eBio cat #11-0419-42), ACE2 antibody (Novus cat#NBP2-72117AF647), TMPRSS2 antibody (SantaCruz

cat#sc-515727 AF488) and DAPI to eliminate any DNA components. Mounted slides were resolved by

fluorescent microscopy using a Scanning Disk Nikon A1 confocal microscope with 100x objective lens.

All experiments were performed in accordance with relevant guidelines and regulations.

Study Design

Quality-assured clinical data from ambulatory and hospitalized Cleveland Clinic patients treated in

Northeast Ohio and South Florida was used to appraise data on 22,072 symptomatic patients evaluated

for COVID-19 with the goal of determining whether current aspirin use protects patients from death

and/or the secondary composite outcome of MI, thrombotic stroke, and/or VTE. Positive testing for a

SARS-CoV-2 amplicon by nasopharyngeal RT-PCR was used to determine infection status. The electronic

medical record and hospital Medication Administration Record (MAR) was used to confirm new or

ongoing administration of 81 mg aspirin or other NSAIDs for both outpatients and inpatients.

Statistical Analysis

Categorical factors are summarized using frequencies and percentages, while continuous factors are

described using median and ranges. Initial descriptive analyses were performed. Comparisons were

made between those with known death status and those with missing death information to identify if

any differences exist in these cohorts. Then among those with known death status, differences in COVID

153   positive and COVID negative patients were assessed. Finally, after stratifying by COVID status,

154   comparisons of those with and without aspirin use were performed. For all tables, continuous measures

155   were compared using nonparametric Wilcoxon rank sum tests, while categorical factors were compared

156   using Pearson chi-square tests or Fisher exact tests, for rare events.

157

158   Given the differences across many covariates, propensity score matching was performed to account for

159   differences between those with and without aspirin use. This approach used two steps. First, multiple

160   imputation was performed on all demographic and covariate measures within COVID status stratified

161   datasets, using fully conditional specification methods. Ten imputed datasets were created. Then

162   propensity score models were fit for each dataset, with aspirin use as the response and all other

163   measures as predictors. Predicted probability of aspirin use from each model was calculated, and these

164   probabilities were averaged across models for each patient. Greedy matching was then performed

165   using a caliper of 0.2 standard deviations of the logit to create matched datasets for both COVID positive

166   and negative patients. A small number of aspirin users could not be matched well and were excluded

167   from the matched analysis. Comparisons of outcomes were performed using mixed effect logistic

168   regression models to account for the matching process. Overlap weighting propensity score analyses

169   were also performed[24] which data with the same conclusions. This analysis was repeated using NSAID

170   groups. For significant effects, E-values[25] that represent the magnitude of the association between an

171   unobserved covariate and both the medication group and outcome necessary to make the result non-

172   significant was also calculated. Analyses were performed using SAS software (version 9.4; Cary, NC). A

173   significance level of 0.05 was assumed for all tests.
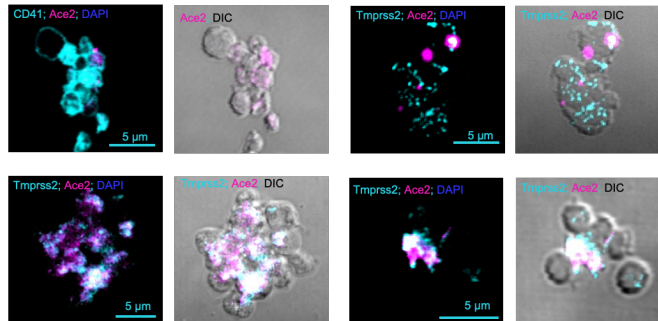
174

175

176

177 **Results**

178 Expression of ACE2 (n=6) and TMPRSS2 (n=3) on the platelet surface was observed by confocal

179 microscopy (Figure 1). Expression of TMPRSS2 in healthy subjects (mean age 40.1 ± 2.8 years, n=20) was

180 also confirmed by immunoblotting at the expected molecular weight of ~50 KDa.

181

Figure 1. Expression of ACE2 and TMPRSS2 in Platelets by Confocal Microscopy.   Platelets isolated from venous blood of healthy individuals was stained for 1h with the following antibodies: CD41 (platelet-specific marker), ACE2, TMPRSS2, and DAPI to eliminate any DNA components. Mounted slides were resolved by confocal fluorescent microscopy using a 100x objective lens. Images are representative of n=6 donors for ACE2 and n=3 for TMPRSS2. Each image represents a different donor. The scale bar is noted.

182

183

184 Utilizing human brain as a positive control, TMPRSS2 expression was standardized to a loading control

185 with no correlation between age and platelet TMPRSS2 expression (Figure 2A; r^2=0.058, p=0.30). Since

186 ACE2 exists as multiple glycosylated proteins of variable molecular weight[26-28], human brain[29], human

187 placenta[30], and engineered heart tissue[31] were utilized as positive controls to confirm predominant

188 migration at ~100 kDa as expected. Given that patients with confirmed CAD receive antiplatelet

189 medications according to established guidelines, TMPRSS2 expression for healthy controls (n=20) was

190 compared to patients with coronary artery disease (CAD, n=10) and, while numerically greater in CAD,

191 was without a statistically significant difference (Figure 2A, p=0.15).

192

8

193   Similarly, expression of ACE2 in healthy subjects (n=20) was confirmed by immunoblotting.   ACE2

194   expression standardized to tubulin did not correlate with age (Figure 2B; r^2=0.0039, p=0.79).
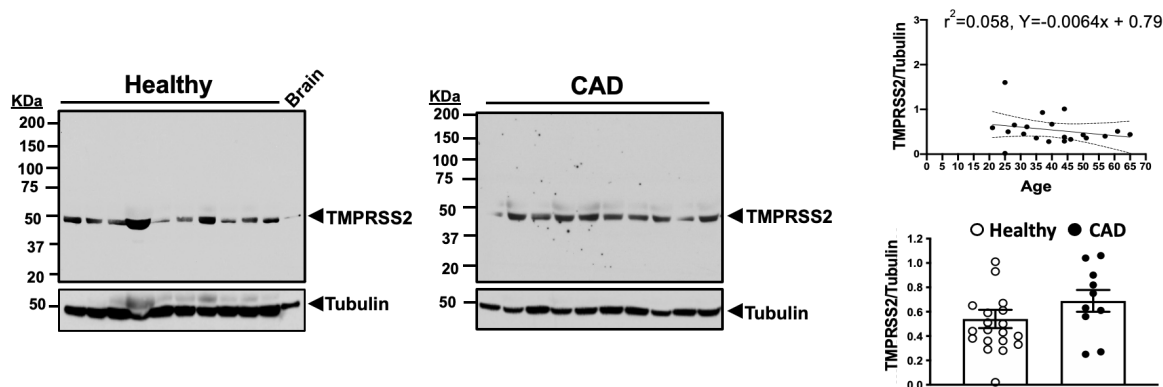

Figure 2A. Expression of TMPRSS2 in Platelets: Washed platelets from healthy individuals (mean age 40.1 ± 2.8 years, n=20) were isolated and proteins separate by SDS-PAGE with molecular weight shown in KiloDaltons (KDa). Immunoblotting was conducted an using an anti-TMPRSS2 antibody or anti-tubulin immunoblotting as a loading control. The ratio of protein to loading control is expressed as a function of age and the correlation coefficient is noted (r ± 95% CI, P=0.30). Human brain lysate served as a positive control for TMPRSS2 migrating at the expected molecular weight (~50 KDa). Data shown are representative of 20 healthy individuals (10 male and 10 female) and 10 patients with coronary artery disease (CAD). The mean ratio of TMPRSS2/Tubulin ± SEM is noted, P=0.145 between healthy and CAD by Mann Whitney *U*). For clarity of presentation, the tubulin blot was cropped just above and below the 50 KDa marker line.

195

196   Platelet ACE2 in healthy subjects (n=20) was compared to patients with CAD (n=10) and, again, while

197   numerically higher in CAD, was without a statistical difference (Figure 2B, p=0.11). Further, we did not

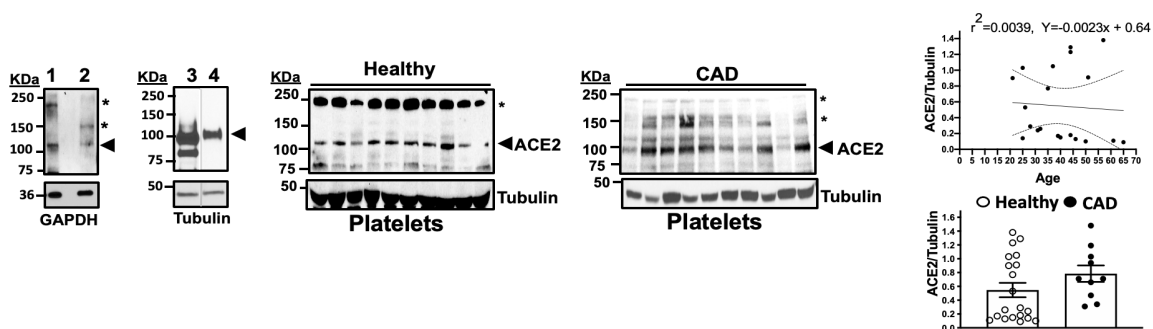198   observe sex-specific differences in platelet expression of ACE2 or TMPRSS2 (20 men and 20 women in

Figure 2B. Expression of ACE2 in Platelets: Washed platelets from healthy individuals (mean age 40.1 ± 2.8 years, n=20) were isolated and proteins separate by SDS-PAGE with molecular weight shown in KiloDaltons (KDa). Lane 1 is human platelet lysate, lane 2 is human brain lysate, lane 3 is human placenta lysate, lane 4 is lysate from engineered human heart tissue. Immunoblotting was conducted using an using anti-ACE2 antibody. Anti-tubulin and anti-GAPDH are loading controls. ACE2 migrates at the expected molecular weight (~100 KDa) shown by an arrowhead with glycosylated forms indicated by *. The ratio of ACE2 protein to loading control is expressed as a function of age and the correlation coefficient is noted (r ± 95% CI, P=0.79). Data shown are representative of 20 healthy individuals (10 male and 10 female) and 10 patients with coronary artery disease (CAD). The mean ratio of ACE2/Tubulin ± SEM is noted, P=0.112 between healthy and CAD by Mann Whitney *U*). For clarity of presentation, the tubulin and GAPDH blots are cropped just above and below the 50 Kda and 36 KDa marker lines, respectively and the ACE2 blot is cropped just below the 75 KDa marker. The grey partition line for ACE2 and tubulin are from the same blot separated by three lanes.

9

199   each group). Full size, uncropped immunoblots for ACE2, TMPRSS2, and loading controls are found in

200   Supplemental Figure 1A-C.

201

202   22,072 patients tested for COVID-19 at two Cleveland Clinic hospitals between March 13, 2020 to May

203   13, 2020 were evaluated. Within this cohort, 11,507 patients had complete clinical data and 1,994

204   tested positive for the SARS-CoV-2 amplicon by RT-PCR testing. Amongst these 1,994 patients, 1,709

205   were not exposed and 285 patients were exposed to aspirin. In an attempt to differentiate an anti-

206   platelet drug effect with aspirin from a more general NSAID class effect, we propensity-matched

207   patients 1,445 patients not exposed and 465 patients exposed to NSAID therapy (Figure 3).

208

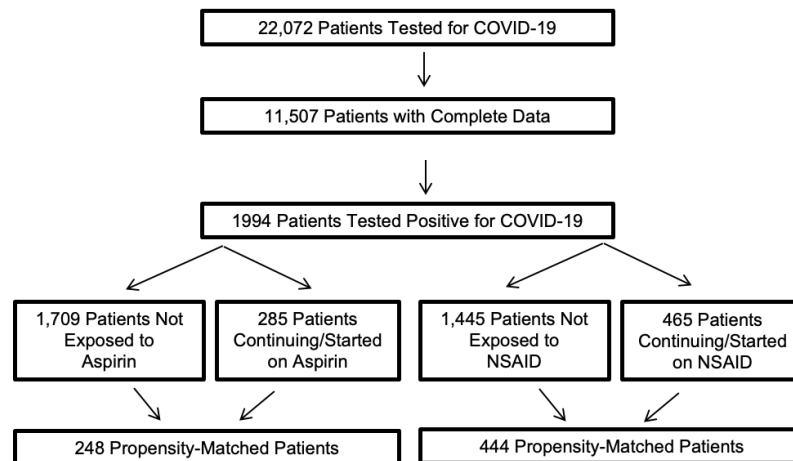**Figure 3. Patients Testing Positive for SARS-CoV-2 taking Aspirin or NSAIDs.** Patients
testing positive for a SARS-CoV-2 amplicon at two Cleveland Clinic hospitals were evaluated.
Patients initiated with aspirin or NSAID therapy or continuing aspirin or NSAID if admitted to the
hospital were included in this study. Clinical variables in each group where then re-evaluated
following careful propensity matching

209

210

211

212

213 Table 1 shows the unadjusted characteristics of each comparative cohort for aspirin.

Factor	N	No Aspirin (N=1,709) Statistics	N	Aspirin Use (N=285) Statistics	p-value
Medications					
CLOPID	1,709	9 (0.53)	285	27 (9.5)	<0.001[c]
Ticag	1,709	1 (0.06)	285	6 (2.1)	<0.001[d]
Prasug	1,709	0 (0.00)	285	0 (0.00)	
Cangr	1,709	0 (0.00)	285	0 (0.00)	
Cilost	1,709	0 (0.00)	285	0 (0.00)	
Pentox	1,709	0 (0.00)	285	1 (0.35)	0.14[d]
AntiPlt	1,709	10 (0.59)	285	285 (100.0)	<0.001[c]
Multiple Therapy	1,709	0 (0.00)	285	34 (11.9)	<0.001[d]
AC_therputic	1,709	94 (5.5)	285	56 (19.6)	<0.001[c]
AC_prophylct	1,709	355 (20.8)	285	215 (75.4)	<0.001[c]
NSAIDs	1,650	294 (17.8)	260	171 (65.8)	<0.001[c]
Covariates					
Age	1,709	50.6 ± 17.5	285	70.0 ± 13.6	<0.001[a2]
Platelets	689	217.4 ± 79.3	253	208.7 ± 85.3	0.14[a1]
Gender	1,651		285		<0.001[c]
Male		804 (48.7)		172 (60.4)	
Female		847 (51.3)		113 (39.6)	
Race	1,564		280		<0.001[c]
White		948 (60.6)		144 (51.4)	
Black		506 (32.4)		124 (44.3)	
Other		110 (7.0)		12 (4.3)	
Ethnicity	1,480		277		<0.001[c]
Hispanic		204 (13.8)		7 (2.5)	
Non-Hispanic		1,276 (86.2)		270 (97.5)	
Smoking	1,417		268		<0.001[c]
No		924 (65.2)		123 (45.9)	
Former		362 (25.5)		124 (46.3)	
Current		131 (9.2)		21 (7.8)	
RespSuprt	1,709	191 (11.2)	285	117 (41.1)	<0.001[c]
OnPressors	1,709	81 (4.7)	285	47 (16.5)	<0.001[c]
HemodInstab	1,709	85 (5.0)	285	48 (16.8)	<0.001[c]
COPD_emphysema	1,399	82 (5.9)	274	53 (19.3)	<0.001[c]
Asthma	1,410	243 (17.2)	273	66 (24.2)	0.007[c]
Diabetes	1,424	318 (22.3)	278	147 (52.9)	<0.001[c]
Hypertension	1,447	659 (45.5)	281	244 (86.8)	<0.001[c]
Coronary_artery_disease	1,405	116 (8.3)	275	100 (36.4)	<0.001[c]
Heart_Failure	1,404	108 (7.7)	274	78 (28.5)	<0.001[c]
Cancer	1,447	184 (12.7)	280	63 (22.5)	<0.001[c]
On_immunosuppressive_treatment	1,456	144 (9.9)	277	36 (13.0)	0.12[c]

Statistics presented as Mean ± SD, N (column %). p-values: a1=t test, a2=Satterthwaite t-test, c=Pearson's chi square test, d=Fisher's Exact test.

214

215   Table 2 shows the unadjusted characteristics of each comparative cohort for NSAIDs.

Factor	No NSAIDs (N=1,445)		NSAIDs (N=465)		p-value
	N	Statistics	N	Statistics	
Medications					
CLOPID	1,445	12 (0.83)	465	21 (4.5)	<0.001[c]
Ticag	1,445	0 (0.00)	465	7 (1.5)	<0.001[d]
Prasug	1,445	0 (0.00)	465	0 (0.00)	
Cangr	1,445	0 (0.00)	465	0 (0.00)	
Cilost	1,445	0 (0.00)	465	0 (0.00)	
Pentox	1,445	0 (0.00)	465	1 (0.22)	0.24[d]
AntiPlt	1,445	96 (6.6)	465	174 (37.4)	<0.001[c]
Multiple Therapy	1,445	5 (0.35)	465	26 (5.6)	<0.001[c]
AC_therputic	1,445	95 (6.6)	465	45 (9.7)	0.026[c]
AC_prophylct	1,445	328 (22.7)	465	203 (43.7)	<0.001[c]
Covariates					
Age	1,445	51.5 ± 18.2	465	58.9 ± 17.1	<0.001[a]
Platelets	574	213.1 ± 80.8	314	212.8 ± 78.0	0.97[a]
Gender	1,390		462		0.36[c]
Male		688 (49.5)		240 (51.9)	
Female		702 (50.5)		222 (48.1)	
Race	1,310		451		0.005[c]
White		801 (61.1)		243 (53.9)	
Black		416 (31.8)		181 (40.1)	
Other		93 (7.1)		27 (6.0)	
Ethnicity	1,228		454		<0.001[c]
Hispanic		178 (14.5)		31 (6.8)	
Non-Hispanic		1,050 (85.5)		423 (93.2)	
Smoking	1,162		452		<0.001[c]
No		763 (65.7)		247 (54.6)	
Former		303 (26.1)		160 (35.4)	
Current		96 (8.3)		45 (10.0)	
RespSuprt	1,445	200 (13.8)	465	85 (18.3)	0.019[c]
OnPressors	1,445	91 (6.3)	465	27 (5.8)	0.70[c]
HemodInstab	1,445	94 (6.5)	465	29 (6.2)	0.84[c]
COPD_emphysema	1,161	74 (6.4)	440	54 (12.3)	<0.001[c]
Asthma	1,168	199 (17.0)	442	100 (22.6)	0.010[c]
Diabetes	1,179	278 (23.6)	450	164 (36.4)	<0.001[c]
Hypertension	1,198	571 (47.7)	453	292 (64.5)	<0.001[c]
Coronary_artery_disease	1,162	107 (9.2)	445	98 (22.0)	<0.001[c]
Heart_Failure	1,162	105 (9.0)	444	70 (15.8)	<0.001[c]
Cancer	1,202	164 (13.6)	447	75 (16.8)	0.11[c]
On_immunosuppressive_treatment	1,209	119 (9.8)	446	55 (12.3)	0.14[c]
History_of_transplant	1,159	9 (0.78)	443	10 (2.3)	0.014[c]

Statistics presented as Mean ± SD, N (column %).

p-values: a=t-test, c=Pearson's chi-square test, d=Fisher's Exact test.

216

217   The 248 propensity-matched patients either treated with aspirin or not demonstrated no significant

218   group differences in demographics or clinical covariates. Aspirin therapy did not alter mortality (13.3%

219   vs 15.3%, p=0.53). The 444 propensity-matched patients either exposed or not to NSAIDs demonstrated

220   no significant group differences in demographics or clinical covariates. NSAID therapy did not alter

221   mortality (7.0% vs 7.2%, p=0.90). In propensity-matched patients treated with aspirin, the incidence of

222   MI (2.0% vs 0.81%, p=0.27) and VTE (4.0% vs 1.6%, p=0.12) were not significantly different, but aspirin

223   therapy was associated with an increased risk of thrombotic stroke (3.6% vs 0.40%, p=0.036). In

224   propensity-matched patients treated with NSAIDs, the incidence of MI (0.68% vs 0.23%, p=0.34), VTE

225   (2.0% vs 0.90%, p=0.17), and thrombotic stroke (1.1% vs 0.45%, p=0.27) was not affected. Using the

226   composite thrombotic endpoint of MI, VTE, and thrombotic stroke, both aspirin (9.3% aspirin vs 2.8% no

227   aspirin, p=0.005) and NSAID therapy (3.8% NSAIDs vs 1.6% no NSAIDs, p=0.046) were associated with

228   signals for thrombosis (Supplemental Figure 1). Overall, there was no change in mortality in COVID-19

229   for patient treated with either aspirin (OR 0.52, 95% CI: 0.51-1.41; p=0.52) or NSAIDs (OR 0.97, 95% CI:

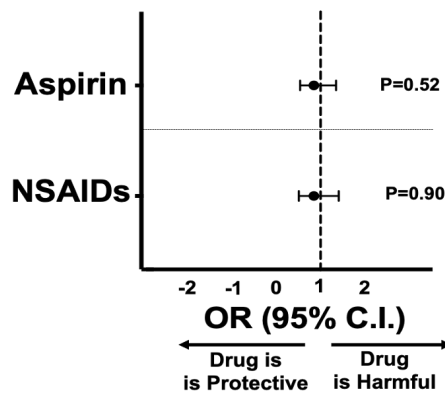230   0.58-1.62; p=0.90) (Figure 4).

Figure 4. Mortality for Propensity-matched patients: Propensity-matched data for patients testing positive for COVD-19 and outcomes taking either 81 mg aspirin (n=248 in each group) or NSAIDs (n=444 in each group) at the time of diagnosis. Forest plot representation of data as Odds Ratio (OR) with 95% confidence interval (C.I.) for the primary endpoint of death

231

232   However, both aspirin and NSAID use in COVID-19 show signals for harm with increased thrombotic risk

233 with aspirin (OR 3.52, 95% CI: 1.48-8.40; p=0.005) and NSAIDs (OR 2.49, 95% CI: 0.58-1.62; p=0.046) for

234 the composite endpoint of MI, thrombotic stroke, and VTE (Supplemental Figure 2).

235

236 **Discussion**

237 In this study, we make the observation that both ACE2 and TMPRSS2 proteins which bind and ligate

238 SARS-CoV-2 are expressed in healthy human platelets. The expression of these receptors in platelets

239 does not vary significantly with age and, while numerically higher, are not strikingly different in patients

240 with CAD compared to healthy controls. The presence of known SARS-CoV-2 receptors on platelets

241 suggests the possibility that SARS-CoV-2 may directly activate platelets and contribute to thrombosis or

242 promote thrombosis indirectly by mediators secreted from platelets .

243

244 A recent investigation revealed platelet reactivity is enhanced in COVID-19 patients[20,32-34] and appears to

245 be suppressed by the presence of high dose aspirin *in vitro*[33]. In the absence of randomized controlled

246 data for aspirin in patients with COVID-19, we conducted a propensity-matched analysis of patients

247 showing aspirin has no mortality benefit in patients with COVID-19, and, in fact, displays a slightly

248 increased signal for harm driven mostly by thrombotic stroke. Platelet reactivity data *in vitro* is often

249 extrapolated to suggest a risk for harm, but it is important to acknowledge that the behavior of anti-

250 platelet medications *in vivo* can be markedly different from *in vitro* studies. Our goal was to clarify this

251 concern by using real-life data with both mortality and thrombotic end points.

252

253 The failure to show a protective effect of the antiplatelet medication aspirin in patients with COVID-19

254 may be related to the dose administered, an insensitivity to aspirin's mechanism of platelet inhibition in

255 COVID-19, or an altered platelet phenotype as was clearly demonstrated by Manne *et al.* comparing

256 healthy platelets to platelets from patients with COVID-19[33]. Cameron *et al.* previously demonstrated a

14

257 divergent platelet phenotype in patients with chronic arterial disease and diabetes with resistance to

258 aspirin and clopidogrel therapy in diseased but not healthy platelets[21]. Similarly, Liang *et al*

259 demonstrated in platelets from patients with diabetes, surface $P2Y_{12}$ receptors are arranged in a

260 different conformation and are impressively resistant to inhibition by clopidogrel [35].

261

262 Elbadawi *et al.* reported the absolute neutrophil count and not D-dimer, a traditional biomarker

263 associated with thrombosis, is an independent predictor of thrombotic events in patients with COVID-

264 19[36]. The mortality benefit of dexamethasone, an immunosuppressant and anti-inflammatory

265 medication, in hospitalized patients with COVID-19[37] and recent reports of immunothrombosis[17,38-41] and

266 microvascular occlusion[18,42-44] by multiple independent groups suggest platelets may be indirect

267 mediators of thrombosis and perhaps not the best direct targets for pharmacological intervention.

268 Contemporaneous with submission of this manuscript, a smaller, non-propensity matched study has

269 shown aspirin treatment decreased mortality that was driven by reduced ICU level care and mechanical

270 ventilatory needs but not thrombosis in patients with COVID-19. This report suggests a protective effect

271 of aspirin that is distinct from altering end-organ thrombosis [45], and possibly from immune-mediated

272 acute respiratory distress syndrome (ARDS) as previously demonstrated[46,47]. By evaluating another anti-

273 inflammatory mechanism using patients treated with NSAIDs in parallel with aspirin in the same hospital

274 and locations in the U.S., we similarly show no effect on mortality, with all statistical models accounting

275 for any contribution of prophylactic and therapeutic heparin use in hospitalized patients and subsequent

276 outcomes.

277

278 The signal for increased composite thrombotic events in COVID-19 patients treated with aspirin was

279 surprising and driven mostly by stroke. Recent observational studies show mixed results for COVID-19-

280 related stroke risk with one small study suggesting an increased risk in younger patients[48], one large

281   study showing an overall low risk[49], and one very large study paradoxically showing that COVID-19

282   infection is associated with a decreased risk of thrombotic cerebrovascular stroke[50]. A mechanistic

283   explanation for our finding may be related to the known neuroprotective effect of interleukin-6 (IL-6)[51]

284   which is greatly elevated in systemic SARS-CoV-2 infection[52] and reported to be reduced by aspirin[53].

285

286   We show quite clearly in our study with investigators working independently of each other in different

287   regions of the U.S. that human platelets contain the SARS-CoV-2 receptors ACE2 and TMPRSS2. The

288   inter-individual expression difference of platelet ACE2 and TMPRSS2 was striking. Our overall

289   observation is consistent with the findings of Zaid *et al.* who identified SARS-CoV-2 mRNA in human

290   platelets implying a mechanism of entry must exist, and then a report by Zhang *et al.* who identified

291   ACE2 on human platelets[20,54]. Our data are at odds with Manne *et al.* who failed to detect ACE2 protein

292   in platelets by immunoblotting using white blood cells (WBC) as a positive control [33]. Notably, Manne *et*

293   *al.* employed a CD45 depletion step on isolated platelets to eliminate the possibility of WBC

294   contamination prior to immunoblotting. CD45 is also present on platelets, and we previously

295   demonstrated this step decreases the platelet yield available for immunoblotting [22]. Lastly, Nassa *et al.*

296   have very elegantly shown that the platelet transcriptome and proteome are dynamic and often mRNA

297   to protein concordance is not observed but, rather, dependent on external platelet cues[55]. Overall, our

298   data are congruent with Koupenova *et al*. suggesting that the ssRNA virus SARS-CoV-2 may behave

299   similarly to the ssRNA influenza virus by utilizing platelets to modulate immune function that ultimately

300   may lead to immunothrombosis[16].

301

302

303

304

305 **Study Limitations**

306 The observational nature of this study from just two hospitals has intrinsic limitations, and the small

307 patient sample to allow for propensity matching limits generalizability of our findings. A few patients

308 testing positive for SARS-CoV-2 were ambulatory and we relied on physician prescriptions making it

309 impossible to confirm compliance to aspirin therapy.

310

311 **Conclusions**

312 SARS-CoV-2 high affinity receptors are present in platelets from healthy individuals. This finding crucially

313 suggests platelets may be involved in COVID-19 pathogenesis and the observed thrombotic phenotype.

314 However, our real-world clinical data suggests regular intake of low dose aspirin does not protect

315 against adverse thrombotic events or death in COVID-19 patients. Platelets are fastidious components

316 of the circulatory system with a wide range of critical functions, including contributing to

317 immunoinflammatory host responses. Thus, targeting platelet thrombotic function may alter its roles in

318 other domains. The nuanced mechanisms of thrombosis in COVID-19 may be unique and deserves

319 further investigation. The use of traditional antiplatelet agents may not protect against thrombotic

320 events or mortality in COVID-19, but, in fact, cause harm. The awareness of this potential harm and role

321 of randomized controlled drug trials in assessing the suitability of antiplatelet agents in COVID-19 is

322 critical.

323

324

325

326

327

328

329 **Contributors**

330 Study design (AS, SJC, RB, AE, AK, JF, LGS, MKC, HK, JPI, MK, SK, TM, KRM)

331 Data collection (AS, SJC, RB, MKC, HK, JPI, MG, MK, JEF, JRB, EH, AA)

332 Data analysis (AS, SJC, AE, MKC, HK, JPI, MK, JEF, JRB, EH, SK)

333 Data interpretation (AE, AK, JF, LGS, MKC, HK, JPI, MG, MK, JEF, JRB, EH, AA, SK)

334 Figures (AS, SJC, MG, MK, JEF, JRB, EH, AA, SK)

335 Literature (AS, SJC, RB, AE, AK, JF, LGS, MKC, HK, MK, JEF, JRB, EH, SK, TM KRM)

336 Writing (AS, SJC, RB, AE AK, ,JF, MKC, HK, JPI, MG, MK, JEF, SK, TM, KRM)

337 All authors gave final approval for this version of the manuscript to be published.

338

339 **Acknowledgements**

343

344 **Declaration of Interests**

345 None of the authors have any relevant conflicting financial, personal, or professional relationships.

346
347
348
349
350
351
352
353
354
355
356
357

References

1   Klok, F. A. *et al.* Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* **191**, 145-147, doi:10.1016/j.thromres.2020.04.013 (2020).

2   Merkler, A. E. *et al.* Risk of Ischemic Stroke in Patients With Coronavirus Disease 2019 (COVID-19) vs Patients With Influenza. *JAMA Neurol*, doi:10.1001/jamaneurol.2020.2730 (2020).

3   Helms, J. *et al.* High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med* **46**, 1089-1098, doi:10.1007/s00134-020-06062-x (2020).

4   Shang, J. *et al.* Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* **117**, 11727-11734, doi:10.1073/pnas.2003138117 (2020).

5   Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271-280.e278, doi:10.1016/j.cell.2020.02.052 (2020).

6   Saheb Sharif-Askari, N. *et al.* Airways Expression of SARS-CoV-2 Receptor, ACE2, and TMPRSS2 Is Lower in Children Than Adults and Increases with Smoking and COPD. *Mol Ther Methods Clin Dev* **18**, 1-6, doi:10.1016/j.omtm.2020.05.013 (2020).

7   Sungnak, W. *et al.* SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med* **26**, 681-687, doi:10.1038/s41591-020-0868-6 (2020).

8   Qi, F., Qian, S., Zhang, S. & Zhang, Z. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem Biophys Res Commun* **526**, 135-140, doi:10.1016/j.bbrc.2020.03.044 (2020).

9   Zhao, Y. *et al.* Single-Cell RNA Expression Profiling of ACE2, the Receptor of SARS-CoV-2. *Am J Respir Crit Care Med* **202**, 756-759, doi:10.1164/rccm.202001-0179LE (2020).

10   Hamming, I. *et al.* Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* **203**, 631-637, doi:10.1002/path.1570 (2004).

11   Bertram, S. *et al.* Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts. *PLoS One* **7**, e35876, doi:10.1371/journal.pone.0035876 (2012).

12   He, L. *et al.* Pericyte-specific vascular expression of SARS-CoV-2 receptor ACE2 – implications for microvascular inflammation and hypercoagulopathy in COVID-19 patients. *bioRxiv*, 2020.2005.2011.088500, doi:10.1101/2020.05.11.088500 (2020).

13   Harmer, D., Gilbert, M., Borman, R. & Clark, K. L. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett* **532**, 107-110, doi:10.1016/s0014-5793(02)03640-2 (2002).

14   Hikmet, F. *et al.* The protein expression profile of ACE2 in human tissues. *Mol Syst Biol* **16**, e9610-e9610, doi:10.15252/msb.20209610 (2020).

15   Liang, W. *et al.* Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. *Gut* **69**, 1141-1143, doi:10.1136/gutjnl-2020-320832 (2020).

16   Koupenova, M. *et al.* The role of platelets in mediating a response to human influenza infection. *Nat Commun* **10**, 1780, doi:10.1038/s41467-019-09607-x (2019).

17   Zuo, Y. *et al.* Neutrophil extracellular traps in COVID-19. *JCI Insight* **5**, doi:10.1172/jci.insight.138999 (2020).

18   Goshua, G. *et al.* Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol* **7**, e575-e582, doi:10.1016/S2352-3026(20)30216-7 (2020).

406 19 Manne, B. K. *et al.* Platelet Gene Expression and Function in COVID-19 Patients. *Blood*,
407   doi:10.1182/blood.2020007214 (2020).
408 20 Zaid, Y. *et al.* Platelets Can Associate with SARS-Cov-2 RNA and Are Hyperactivated in COVID-19.
409   *Circ Res*, doi:10.1161/CIRCRESAHA.120.317703 (2020).
410 21 Cameron, S. J. *et al.* Platelet Extracellular Regulated Protein Kinase 5 Is a Redox Switch and
411   Triggers Maladaptive Platelet Responses and Myocardial Infarct Expansion. *Circulation* **132**, 47-
412   58, doi:10.1161/CIRCULATIONAHA.115.015656 (2015).
413 22 Schmidt, R. A. *et al.* The platelet phenotype in patients with ST-segment elevation myocardial
414   infarction is different from non-ST-segment elevation myocardial infarction. *Transl Res* **195**, 1-
415   12, doi:10.1016/j.trsl.2017.11.006 (2018).
416 23 Lemme, M. *et al.* Atrial-like Engineered Heart Tissue: An In Vitro Model of the Human Atrium.
417   *Stem Cell Reports* **11**, 1378-1390, doi:10.1016/j.stemcr.2018.10.008 (2018).
418 24 Li, F., Thomas, L. E. & Li, F. Addressing Extreme Propensity Scores via the Overlap Weights. *Am J
419   Epidemiol* **188**, 250-257, doi:10.1093/aje/kwy201 (2019).
420 25 Ding, P. & VanderWeele, T. J. Sensitivity Analysis Without Assumptions. *Epidemiology* **27**, 368-
421   377, doi:10.1097/EDE.0000000000000457 (2016).
422 26 Towler, P. *et al.* ACE2 X-ray structures reveal a large hinge-bending motion important for
423   inhibitor binding and catalysis. *J Biol Chem* **279**, 17996-18007, doi:10.1074/jbc.M311191200
424   (2004).
425 27 Chen, R. *et al.* Glycoproteomics analysis of human liver tissue by combination of multiple
426   enzyme digestion and hydrazide chemistry. *J Proteome Res* **8**, 651-661, doi:10.1021/pr8008012
427   (2009).
428 28 Glasgow, A. *et al.* Engineered ACE2 receptor traps potently neutralize SARS-CoV-2. *Proc Natl
429   Acad Sci U S A*, doi:10.1073/pnas.2016093117 (2020).
430 29 Alenina, N. & Bader, M. ACE2 in Brain Physiology and Pathophysiology: Evidence from
431   Transgenic Animal Models. *Neurochem Res* **44**, 1323-1329, doi:10.1007/s11064-018-2679-4
432   (2019).
433 30 Li, M., Chen, L., Zhang, J., Xiong, C. & Li, X. The SARS-CoV-2 receptor ACE2 expression of
434   maternal-fetal interface and fetal organs by single-cell transcriptome study. *PLoS One* **15**,
435   e0230295, doi:10.1371/journal.pone.0230295 (2020).
436 31 Monteil, V. *et al.* Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-
437   Grade Soluble Human ACE2. *Cell* **181**, 905-913 e907, doi:10.1016/j.cell.2020.04.004 (2020).
438 32 Hottz, E. D. *et al.* Platelet activation and platelet-monocyte aggregate formation trigger tissue
439   factor expression in patients with severe COVID-19. *Blood* **136**, 1330-1341,
440   doi:10.1182/blood.2020007252 (2020).
441 33 Manne, B. K. *et al.* Platelet gene expression and function in patients with COVID-19. *Blood* **136**,
442   1317-1329, doi:10.1182/blood.2020007214 (2020).
443 34 Barrett, T. J. *et al.* Platelet and Vascular Biomarkers Associate With Thrombosis and Death in
444   Coronavirus Disease. *Circ Res* **127**, 945-947, doi:10.1161/CIRCRESAHA.120.317803 (2020).
445 35 Hu, L. *et al.* Platelets Express Activated P2Y12 Receptor in Patients With Diabetes Mellitus.
446   *Circulation* **136**, 817-833, doi:10.1161/CIRCULATIONAHA.116.026995 (2017).
447 36 Elbadawi, A. *et al.* Incidence and Outcomes of Thrombotic Events in Symptomatic Patients With
448   COVID-19. *Arteriosclerosis, thrombosis, and vascular biology*, ATVBAHA120315304,
449   doi:10.1161/ATVBAHA.120.315304 (2020).
450 37 Group, R. C. *et al.* Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. *N
451   Engl J Med*, doi:10.1056/NEJMoa2021436 (2020).
452 38 Barnes, B. J. *et al.* Targeting potential drivers of COVID-19: Neutrophil extracellular traps. *J Exp
453   Med* **217**, doi:10.1084/jem.20200652 (2020).

454 39 Wang, J. *et al.* Excessive Neutrophils and Neutrophil Extracellular Traps in COVID-19. *Front*
455   *Immunol* **11**, 2063, doi:10.3389/fimmu.2020.02063 (2020).
456 40 Veras, F. P. *et al.* SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19
457   pathology. *J Exp Med* **217**, doi:10.1084/jem.20201129 (2020).
458 41 Skendros, P. *et al.* Complement and tissue factor-enriched neutrophil extracellular traps are key
459   drivers in COVID-19 immunothrombosis. *J Clin Invest* **130**, 6151-6157, doi:10.1172/JCI141374
460   (2020).
461 42 Nicolai, L. *et al.* Immunothrombotic Dysregulation in COVID-19 Pneumonia Is Associated With
462   Respiratory Failure and Coagulopathy. *Circulation* **142**, 1176-1189,
463   doi:10.1161/CIRCULATIONAHA.120.048488 (2020).
464 43 von der Thusen, J. H. *et al.* Case report: a fatal combination of hemophagocytic
465   lymphohistiocytosis with extensive pulmonary microvascular damage in COVID-19 pneumonia. *J*
466   *Hematop*, 1-5, doi:10.1007/s12308-020-00423-7 (2020).
467 44 Rovas, A. *et al.* Microvascular dysfunction in COVID-19: the MYSTIC study. *Angiogenesis*,
468   doi:10.1007/s10456-020-09753-7 (2020).
469 45 Chow, J. H. *et al.* Aspirin Use is Associated with Decreased Mechanical Ventilation, ICU
470   Admission, and In-Hospital Mortality in Hospitalized Patients with COVID-19. *Anesth Analg*,
471   doi:10.1213/ANE.0000000000005292 (2020).
472 46 Erlich, J. M., Talmor, D. S., Cartin-Ceba, R., Gajic, O. & Kor, D. J. Prehospitalization antiplatelet
473   therapy is associated with a reduced incidence of acute lung injury: a population-based cohort
474   study. *Chest* **139**, 289-295, doi:10.1378/chest.10-0891 (2011).
475 47 Kor, D. J. *et al.* Effect of Aspirin on Development of ARDS in At-Risk Patients Presenting to the
476   Emergency Department: The LIPS-A Randomized Clinical Trial. *JAMA* **315**, 2406-2414,
477   doi:10.1001/jama.2016.6330 (2016).
478 48 Ellul, M. A. *et al.* Neurological associations of COVID-19. *Lancet Neurol* **19**, 767-783,
479   doi:10.1016/S1474-4422(20)30221-0 (2020).
480 49 Shahjouei, S. *et al.* Risk of stroke in hospitalized SARS-CoV-2 infected patients: A multinational
481   study. *EBioMedicine* **59**, 102939, doi:10.1016/j.ebiom.2020.102939 (2020).
482 50 Bekelis, K. *et al.* Ischemic Stroke Occurs Less Frequently in Patients With COVID-19: A
483   Multicenter Cross-Sectional Study. *Stroke*, STROKEAHA120031217,
484   doi:10.1161/STROKEAHA.120.031217 (2020).
485 51 Smith, C. J. *et al.* Peak plasma interleukin-6 and other peripheral markers of inflammation in the
486   first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term
487   outcome. *BMC Neurol* **4**, 2, doi:10.1186/1471-2377-4-2 (2004).
488 52 Leisman, D. E. *et al.* Cytokine elevation in severe and critical COVID-19: a rapid systematic
489   review, meta-analysis, and comparison with other inflammatory syndromes. *Lancet Respir Med*,
490   doi:10.1016/S2213-2600(20)30404-5 (2020).
491 53 Haynes, D. R., Wright, P. F., Gadd, S. J., Whitehouse, M. W. & Vernon-Roberts, B. Is aspirin a
492   prodrug for antioxidant and cytokine-modulating oxymetabolites? *Agents Actions* **39**, 49-58,
493   doi:10.1007/BF01975714 (1993).
494 54 Zhang, S. *et al.* SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. *J Hematol*
495   *Oncol* **13**, 120, doi:10.1186/s13045-020-00954-7 (2020).
496 55 Nassa, G. *et al.* Splicing of platelet resident pre-mRNAs upon activation by physiological stimuli
497   results in functionally relevant proteome modifications. *Sci Rep* **8**, 498, doi:10.1038/s41598-017-
498   18985-5 (2018).
499
500

Figures

Figure 1. Expression of ACE2 and TMPRSS2 in Platelets by Confocal Microscopy. Platelets isolated from venous blood of healthy individuals was stained for 1h with the following antibodies: CD41 (platelet-specific marker), ACE2, TMPRSS2, and DAPI to eliminate any DNA components. Mounted slides were resolved by confocal fluorescent microscopy using a 100x objective lens. Images are representative of n=6 donors for ACE2 and n=3 for TMPRSS2. Each image represents a different donor. The scale bar is noted.

Figure 2. A. Expression of TMPRSS2 in Platelets: Washed platelets from healthy individuals (mean age 40.1 ± 2.8 years, n=20) were isolated and proteins separate by SDS-PAGE with molecular weight shown in KiloDaltons (KDa). Immunoblotting was conducted an using an anti-TMPRSS2 antibody or anti-tubulin immunoblotting as a loading control. The ratio of protein to loading control is expressed as a function of age and the correlation coefficient is noted (r ± 95% CI, P=0.30). Human brain lysate served as a positive control for TMPRSS2 migrating at the expected molecular weight (~50 KDa). Data shown are representative of 20 healthy individuals (10 male and 10 female) and 10 patients with coronary artery disease (CAD). The mean ratio of TMPRSS2/Tubulin ± SEM is noted, P=0.145 between healthy and CAD by Mann Whitney *U*). **B. Expression of ACE2 in Platelets:** Washed platelets from healthy individuals (mean age 40.1 ± 2.8 years, n=20) were isolated and proteins separate by SDS-PAGE with molecular weight shown in KiloDaltons (KDa). Lane 1 is human platelet lysate, lane 2 is human brain lysate, lane 3 is human placenta lysate, lane 4 is lysate from engineered human heart tissue. Immunoblotting was conducted using an using anti-ACE2 antibody. Anti-tubulin and anti-GAPDH are loading controls. ACE2 migrates at the expected molecular weight (~100 KDa) shown by an arrowhead with glycosylated forms indicated by *. The ratio of ACE2 protein to loading control is expressed as a function of age and the correlation coefficient is noted (r $\pm$ 95% CI, P=0.79). Data shown are representative of 20 healthy individuals (10 male and 10 female) and 10 patients with coronary artery disease (CAD). The mean ratio of ACE2/Tubulin $\pm$ SEM is noted, P=0.112 between healthy and CAD by Mann Whitney *U*).

Figure 3. Patients Testing Positive for SARS-CoV-2 taking Aspirin or NSAIDs. Patients testing positive for a SARS-CoV-2 amplicon at two Cleveland Clinic hospitals were evaluated. Patients initiated with aspirin or NSAID therapy or continuing aspirin or NSAID if admitted to the hospital were included in this study. Clinical variables in each group where then re-evaluated following careful propensity matching .

Table 1. Characteristics of Population taking Aspirin Therapy: Unadjusted data are for patients testing positive for SARS-CoV-2 not taking aspirin or with established aspirin therapy or initiated with low dose aspirin at the time of diagnosis.

Table 2. Characteristics of Population taking NSAID Therapy: Unadjusted data are for patients testing positive for SARS-CoV-2 not taking NSAID or with established NSAID therapy or initiated with NSAID at the time of diagnosis.

Figure 4. Mortality for Propensity-matched patients: Propensity-matched data for patients testing positive for COVD-19 and outcomes taking either 81 mg aspirin (n=248 in each group) or NSAIDs (n=444 in each group) at the time of diagnosis. Forest plot representation of data as Odds Ratio (OR) with 95% confidence interval (C.I.) for the primary endpoint of death.