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Research letter

Increased protease-activated receptor 1 autoantibodies are associated with severe COVID-19

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Increased protease-activated receptor 1 autoantibodies are associated with severe COVID-19

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Key words

Severe COVID-19, disease outcome prediction, GPCR autoantibodies, coagulation, Protease-activated receptor 1

Plain language summary

Antibodies targeting a receptor on platelets (protease-activated receptor 1, PAR1) were increased in patients with severe/fatal COVID-19 disease course. PAR1 antibody levels correlate with blood markers for blood clotting (D-dimers), a hallmark of COVID-19. These results suggest a role for antibodies against PAR1 in the development of blood clotting observed in the setting of COVID-19.

Contributions

F.T., H.H., T.B., P.R., K.S., Y.S., G.H., G.R. and S.S. conceived study concept and design. F.T, D.H., A.S., H.G., T.B., A.R., P.R., H.H., G.R. and S.S contributed to literature search, data interpretation and writing the initial manuscript. All authors contributed to reviewing and editing of the manuscript. F.T., A.S., H.G., K.S., S.S., A.G., J.F., M.G., A.O., A.K., D.F., C.L.,

G.R., N.K., J.R., J.H., H.A., G.H., J.Y.H., K.I.G., K.S.-F. participated in data and sample collection and processing. FT., D.H., A.S., H.G., O.C.-M., J.P.B., N.M., H.H., G.R. participated in data analysis. F.T., D.H., A.S., H.G., H.H. and G.R. made figures and tables.

Competing interests

The authors declare no competing interests.

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Dear Editor,

Immune perturbation is a hallmark of Coronavirus Disease 2019 (COVID-19) with ambiguous roles of various immune cell compartments. Plasma cells, responsible for antibody production, have a two-pronged response while mounting an immune defence with (1) physiological immune response producing neutralizing antibodies against protein structures of SARS-CoV-2 and (2) potentially deleterious autoantibody generation. Growing evidence hints towards broad activation of plasma cells and the presence of pathologic autoantibodies (abs) that mediate immune perturbation in acute COVID-19 [1]. Recently, a systematic screening for abs confirmed induction of diverse functional abs in SARS-CoV-2 infection, targeting several immunomodulatory proteins, including cytokines/chemokines and their respective G-protein coupled receptors (GPCR) [1]. Abs against GPCR act as agonistic and allosteric receptor modulators and are linked to chronic inflammatory diseases [2] and, as we recently demonstrated, disease severity in acute COVID-19 [3].

Immune-mediated thrombosis is a key pathogenic mechanism in COVID-19 linked to morbidity and mortality [4]. Peripheral blood megakaryocytes are potential biomarkers of severe COVID-19 [5], displaying prothrombogenic metabolic programs and type I interferon signatures. Activated megakaryocytes and sequestering platelets might contribute to immune-mediated microthrombosis in COVID-19 [6]. Thrombin is another key factor in plasmatic coagulation, but it also induces platelet aggregation via GPCR protease-activated receptor 1 (PAR1), expressed on the plasma membrane of megakaryocytes, platelets, and endothelial cells. Thrombin activation is linked to acute respiratory distress syndrome and fatal outcome of COVID-19 [7], but it is (pre-)treatment with platelet inhibitors, not high-dose heparin therapy that reduces mortality and occurrence of thrombotic events in COVID-19 [8, 9]. Tackling platelet activation/PAR1-mediated coagulation could therefore be a therapeutic target preventing microthrombotic complications in severe COVID-19 [10]. Additionally,

thrombin mediates endothelial dysfunction in severe COVID-19 through PAR1 signalling [11]. Given these considerations, we hypothesized that anti-PAR1 abs are altered in COVID-19 and skew the coagulation system towards pro-thrombogenic states.

To investigate this, blood samples from 74 patients tested positive for SARS-CoV-2 infection by RT-PCR detection (S gene) on nasopharyngeal swabs were collected after informed consent and ethical board review* of the respective source studies from three hospitals (University Hospitals Schleswig-Holstein Kiel [* °D466/20] and Lübeck [* °13-003], Medical Clinic Research-Center-Borstel [* EK HL AZ 14-225]). 29 patients required intensive care unit (ICU) treatment and 14 died during hospital stay. Time of first sampling was within 48h of hospital or ICU admission while follow-up samples from 18 individuals were collected at random timepoints. In total, we collected 111 patient serum samples. Occurrence of thrombotic events within the COVID-19 related hospital stay was ascertained depending on the clinically suspected location of thrombosis by MRI/CT scan with contrasting agents or duplex sonography. The most common manifestations in our cohort were lung embolism, stroke and intestinal embolic ischemia. 29 single time point samples from healthy controls age-matched to the patients admitted to ICU were collected in Lübeck [* AZ16-199]. All serum samples were subjected to duplicate quantification of anti-PAR1 abs by IgG-specific indirect sandwich ELISA (CellTrend GmbH Luckenwalde, Germany) as described previously [12]. We use linear mixed models (LMM) to evaluate differences between anti-PAR1 abs, disease severity and outcome (survival and thrombotic events), accounting for repeated measures via inclusion of patient-specific random intercepts. Lab analytes (including anti-PAR1 abs) were natural log transformed and residual plots visually inspected for deviations from normality or homoscedasticity. LMM are reported with *p*-values (obtained using Satterthwaite's method) and 95% confidence intervals for log-transformed coefficient estimates. We assessed the power of anti-PAR1 abs in the prediction of survival and thrombotic events relative to established markers alone and in combination (D-dimers, CRP and IL-6) using logistic regression, and evaluated the resultant models using receiver operating characteristic (ROC) analysis. Analyses were run in R version 4.2.1 (R Core Team, Vienna, Austria) with the base package stats and packages LmerTest and pROC (versions 3.1-3 and 1.18.0, respectively).

Disease severity varied within the cohort from hospitalized moderate to severe COVID-19 following World Health Organization criteria. The median age (63 years) was identical in the COVID-19 cohort (range: 20-93 years) and the controls (range: 19-90 years). Circulating anti-PAR1 abs were markedly increased in COVID-19 patients that required ICU treatment (CI: 0.60 - 1.59; $p = 3.36 \times 10^{-5}$ in LMM, ICU escalation as fixed effect) in comparison to age-matched controls, but not in hospitalized patients without ICU treatment (CI: -0.06 - 0.84; *p* =

0.092 in LMM, figure 1a). Importantly, increased levels of anti-PAR1 abs within the ICU-treated sub-cohort were associated with fatal outcome (CI: 0.12 - 1.71; p = 0.0319 in LMM, mortality as fixed effect, figure 1b) and occurrence of thromboembolic events (CI: 0.42 - 2.00; p = 0.0062 in LMM, thrombotic events as fixed effect). Circulating anti-PAR1 abs correlated with D-dimers (CI: 0.32 - 1.14; p = 0.0010 in LMM, D-dimers as fixed effect, figure 1c) further underscoring that anti-PAR1 abs are linked to coagulation processes in acute COVID-19, while significant correlation was neither found with platelet counts nor inflammatory markers like IL-6 (p = 0.3467 in LMM, data not shown). Anti-PAR1 abs and D-dimers has similar area under ROC curves (AUROCs) for ICU patients for the endpoint survival (AUROC = 0.7095 vs. 0.7115) while anti-PAR1 abs performed better in discrimination of thromboembolism (AUROC = 0.7692 vs. 0.5992, figure 1d). Combination of both markers further increased the AUROC for both endpoints survival (AUROC = 0.7846) and thromboembolism (AUROC = 0.8347). Anti-PAR1 abs do not have better predictive value compared to IL-6 (AUROC = 0.7652/0.7972 for the endpoints thrombotic events/survival) and CRP (AUROC = 0.8132/0.6619), however, anti-PAR1 does improve the predictive power of IL-6 when the analytes are combined in logistic regression (anti-PAR1 abs + IL-6: AUROC = 0.8485/0.8182, the highest AUROC of all combined analyses of two analytes).

Our data reveal an association between ICU treatment in severe COVID-19 and generation of anti-PAR1 abs, which are associated with poor outcome. Intriguingly, we found no association between anti-PAR1 abs and systemic IL-6, despite the suggested agonistic role of anti-PAR1 abs on PAR1/p70S6K/ERK-dependent IL-6 expression in endothelial cells [13]. This suggests that endothelium-derived IL-6 does not impact systemic IL-6 levels. Indeed, monocytes have been described as the main source of IL-6 in COVID-19 [14], which may explain why endothelial cell and serum IL-6 appear to be uncoupled. This would explain not only the lack of correlation between serum IL-6 and anti-PAR1 abs, but also the improved predictive power that results from the combination of the two analytes. Additionally, increased PAR1-dependent platelet activation in COVID-19 leads to elevated aggregation of circulating cells and collagen [15]. We hypothesize that anti-PAR1 abs in combination with dysregulated coagulation proteases like activated protein C or matrix-metalloprotease-1 could activate PAR1-dependent signals in endothelial cells and platelets, thus contributing to immune-mediated microthrombosis as suggested by the correlation of anti-PAR1 abs with D-dimers. Stimulating platelets and endothelial cells with isolated IgG from COVID-19 patients with PAR1 inhibitors (e.g., voropaxar) could provide evidence of anti-PAR1 ab involvement in COVID-19-related coagulopathy.

Our study has several limitations. For most ICU patients, first sampling was upon ICU admission, making prediction of ICU necessity impossible. Although we did not observe sex effects on anti-PAR1 abs in either COVID-19 (p = 0.60 in LMM, sex as fixed effect) or control cohorts (p = 0.90 in Student's t-test), the sex ratios in the cohorts were skewed.

Our data suggest an association of anti-PAR1 abs with thrombotic complications and fatal outcome in COVID-19, warranting verification in larger cohorts. Functional assessment of anti-PAR1 abs is important to understand their molecular properties and pathophysiological role in thrombosis and endothelial dysfunction in COVID-19.

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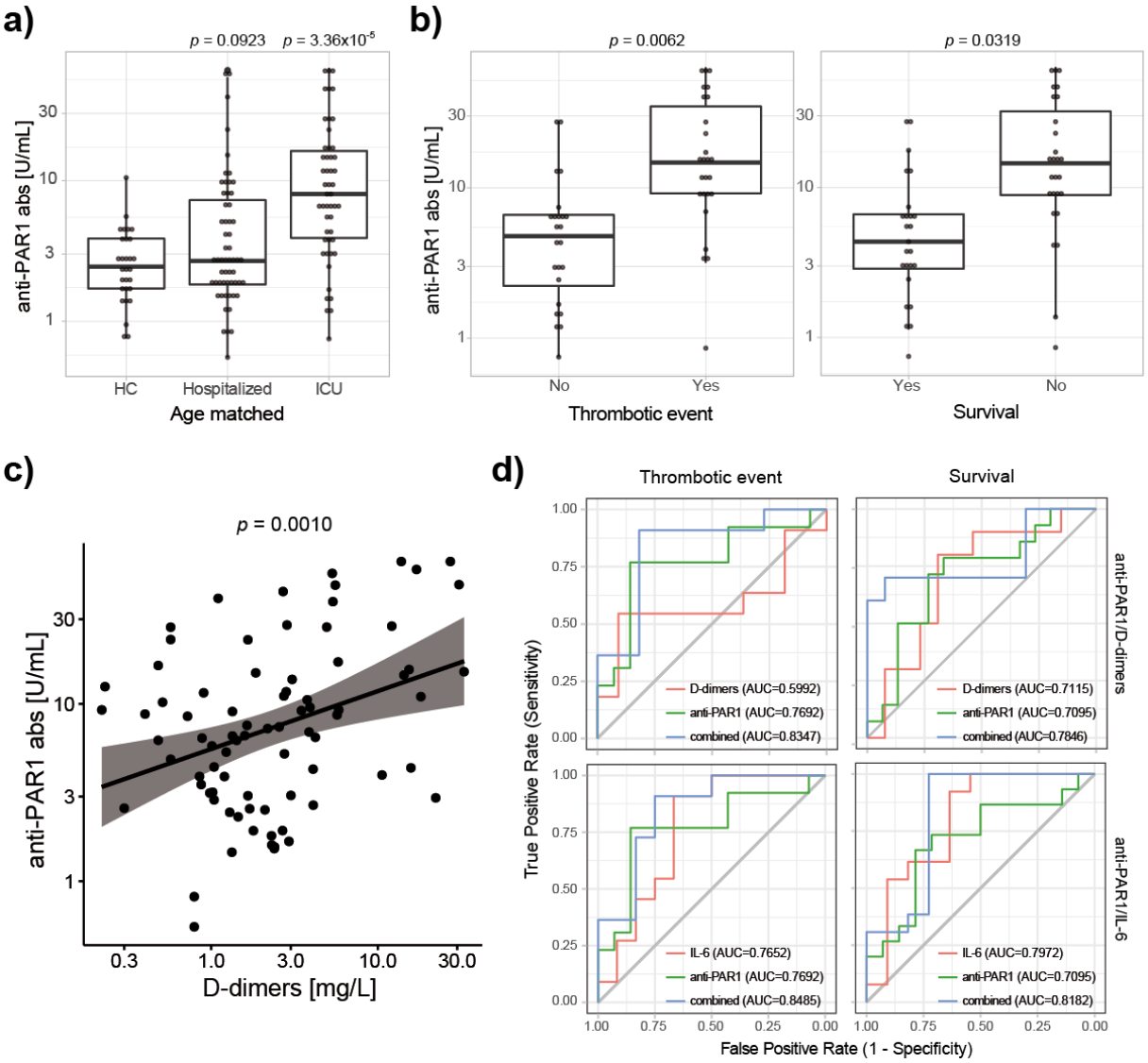
Figure Legends

Figure 1: anti-PAR1 abs correlated with disease severity and survival

a) anti-PAR1 ab levels in serum samples from healthy controls (29:29 samples:individuals) and patients with COVID-19, either hospitalized/non-ICU (59:45) or ICU treatment (52:29) ;LMM: $p = 0.092$ and 3.36×10^{-5}, respectively. HC = healthy controls. **b)** anti-PAR1 ab levels in serum samples from ICU-treated COVID-19 patients in the cohort (29 patients), stratified by the outcome thrombotic events and survival (LMM $p = 0.0062$ and 0.0319, respectively). **c)** Correlation analysis for anti-PAR1 abs in COVID-19 patients against D-dimers. Linear regression line with confidence interval is displayed; statistical analysis is based on LMM ($p = 0.0010$). **d)** Receiver operating characteristic (ROC) analysis of anti-PAR1 abs, D-dimers and combined (upper panels) as well as anti-PAR1 abs, IL-6 and combined (lower panels) for the clinical outcomes “thrombotic events” (left panels) and “survival” (right panels). AUROCs are indicated in the panels.


a)
p = 0.0923
p = 3.36x10-5
anti-PAR1 abs [U/mL]
30
10
3
1
HC
Hospitalized
ICU
Age matched
b)
p = 0.0062
p = 0.0319
anti-PAR1 abs [U/mL]
No
Yes
Thrombotic event
Yes
No
Survival
c)
p = 0.0010
anti-PAR1 abs [U/mL]
0.3
1.0
3.0
10.0
30.0
D-dimers [mg/L]
d)
Thrombotic event
Survival
True Positive Rate (Sensitivity)
False Positive Rate (1 - Specificity)
anti-PAR1/D-dimers
anti-PAR1/IL-6
D-dimers (AUC=0.5992)
anti-PAR1 (AUC=0.7692)
combined (AUC=0.8347)
D-dimers (AUC=0.7115)
anti-PAR1 (AUC=0.7095)
combined (AUC=0.7846)
IL-6 (AUC=0.7652)
anti-PAR1 (AUC=0.7692)
combined (AUC=0.8485)
IL-6 (AUC=0.7972)
anti-PAR1 (AUC=0.7095)
combined (AUC=0.8182)