Pro-inflammatory sensitization of platelets after a mild course of COVID-19 infection

C. Tolksdorf¹, E. Moritz², A. Hafkemeyer², K. Lehmann³, K. Becker³, T. Thiele⁴, E. Schwedhelm⁵, M. Von Lucadou⁵, M.V. Tzvetkov², S. Engeli⁶, G. Jedlitschky², B.H. Rauch¹

¹The Carl von Ossietzky University of Oldenburg, Department of Human Medicine, Section of Pharmacology and Toxicology, Oldenburg, Germany
²Universitaetsmedizin Greifswald, Department of General Pharmacology, Greifswald, Germany
³Universitaetsmedizin Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany
⁴Rostock University Medical Centre Part of the Rostock University, Department of Transfusion Medicine, Rostock, Germany
⁵The University Medical Center Hamburg-Eppendorf, Institute of Clinical Pharmacology and Toxicology, Hamburg, Germany
⁶Universitaetsmedizin Greifswald, Department of Clinical Pharmacology, Greifswald, Germany

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Background: Coronavirus disease-2019 (COVID-19) is a viral respiratory illness caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) which can lead to an increased risk of thromboembolic events but also complications even after recovery from the disease. Multiple factors may enhance the risk of thrombotic events in COVID-19 patients including excessive inflammatory responses and increased platelet activity. To date there are hardly any data on how platelet function is affected after a mild course of infection.

Purpose: In this study, we investigated whether platelet function is affected after recovery from mild COVID-19 disease. Besides the response of platelets to platelet stimuli, expression of the transporter protein multidrug resistance protein 4 (MRP4) and one of its substrates, the inflammatory lipid mediator sphingosine-1-phosphate (S1P), were measured.

Methods: Venous blood samples were collected from patients within 2-15 weeks after recovery from a mild SARS-CoV-2 infection (recovered) and matched individuals (controls) after written informed consent. Participants were invited again at 6-9 months after infection to reexamine platelet function. Parameters of platelet aggregation were determined by light transmission aggregometry (LTA) in platelet-rich plasma (PRP). Expression of platelet activation markers were measured by Flow Cytometry (FACS). In addition, concentrations of S1P were determined by LC-MS/MS and MRP4-expression in platelets via Western blotting.

Results: Within 2-15 weeks after recovery, no alterations in platelet aggregation were observed after stimulation of PRP with various ADP (1, 2, and 10 µM) or collagen (1 and 2.5 µg/mL) concentrations. However, regarding FACS analysis, platelet surface expression of CD62P and PAC1 from convalescents prior activation by ADP (2 µM) and by CRP-XL (0.125 ng/mL) was significantly upregulated in comparison to samples from controls (p<0.05 for both). Intriguingly, S1P levels in platelet-poor plasma (PPP) from COVID-19 convalescents were significantly elevated compared to controls, i.e. 0.75±0.01 vs 0.65±0.02 nmol/mL, respectively (p<0.001). S1P in PRP and serum samples was not altered. Furthermore, platelet MRP4 expression was significantly elevated in COVID-19 convalescents (p<0.05). These differences in S1P and MRP4 levels were only present after 2-15 weeks of recovery, but not 6-9 months after infection.

Conclusions: While after a mild course of COVID-19 infection robust platelet functions such as aggregation determined by LTA were not affected, subtle differences in platelet activation markers occurred. Enhanced platelet MRP4 expression, which may correlate with the observed elevated S1P in PPP, are in agreement with a pro-inflammatory sensitization of platelets even after a mild COVID-19 infection. The implications of this observation concerning long-term effects of COVID-19 await further studies.