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Case report

Circulating microaggregates as biomarkers for the Post-COVID syndrome

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ABSTRACT

CoVID-19 can develop into Post-COVID syndrome of potentially high morbidity, with procoagulation and reactivation of dormant viral infections being hypothesized pathophysiological mechanisms. We report on a patient suffering from fatigue, post exertional malaise, pain and neurological symptoms as a consequence of the second CoVID infection. Using live confocal microscopy on native whole blood samples we detected microaggregates of thrombocytes, leukocytes and plasma proteins in peripheral blood. In addition, there was specific cellular immunological reactivity to EBV. Upon anticoagulatory and virustatic pharmacological therapy we observed dissolution of microaggregates and significant stable clinical remission. We suggest to consider circulating microaggregates as a morphological indicator of chronic post-COVID syndrome.

Introduction

COVID-19 can develop into Post-COVID syndrome with potentially high morbidity, with activation of coagulation and reactivation of dormant viral infections being two potential pathomechanisms [1]. Due to the heterogeneity of symptoms and disease course, finding a diagnostic strategy with routine laboratory tests only is challenging. Therefore, we have applied real time live confocal microscopy for the analysis of blood samples of a patient suffering from Post-COVID syndrome and have evaluated its suitability for a better characterization of underlying pathomechanisms.

Case presentation

One month after a second COVID-19 infection a 35-year old female patient, previously fully vaccinated against SARS-COV-2, developed fatigue, post exertional malaise, pain, neurological symptoms, with other causes for the symptoms excluded. There is a positive patient and familial anamnesis of autoimmune diseases. Urine was positive for protein and leukocytes. Besides leukopenia with relative dominance of neutrophils over lymphocytes, we noted IgG2 and IgG3 deficiency, and secondary hypogonadism. While anti-EBV antibodies were negative, there was a significant elevation of specific T cells reacting against EBV peptides. Indirect immunofluorescence on mouse tissue showed weak reactivity of *bona fide* autoantibodies against autonomic myenteric nerves *(data not shown)*.

In order to evaluate the presence of microaggregates in circulating blood with potential rheological consequences, we applied real time live confocal microscopy on native whole blood samples (Fig. 1) [2]. We detected formation of globular microaggregates measuring 100 μm in diameter in average consisting of cellular and acellular plasmatic components. Besides thrombocytes, cells in close proximity to the microaggregates were identified as mononuclear and polymorphonuclear leukocytes. There was a variable degree of cell lysis around a core of glycoproteins that were labeled with wheat germ agglutinin. Parallel thrombocytic function analysis pointed towards an increased platelet aggregation (data not shown).

The patient received enoxaparin 1 × 40 mg daily plus 100 mg acetylsalicylic acid twice daily in parallel to gastric protection with famotidine for four weeks. We observed stable 50 % improvement of sleep disorders, pain, fatigue, neurological symptoms including autonomous disorders, and post exertional malaise. In a secondary approach, we added 1,000 mg valaciclovir three times per day, which led to a further reduction of fatigue symptoms. Overall, Bell Score 1995 increased from 40 to 90 with further improvement until present. After completion of this treatment cycle, we again imaged the blood *via* real time live

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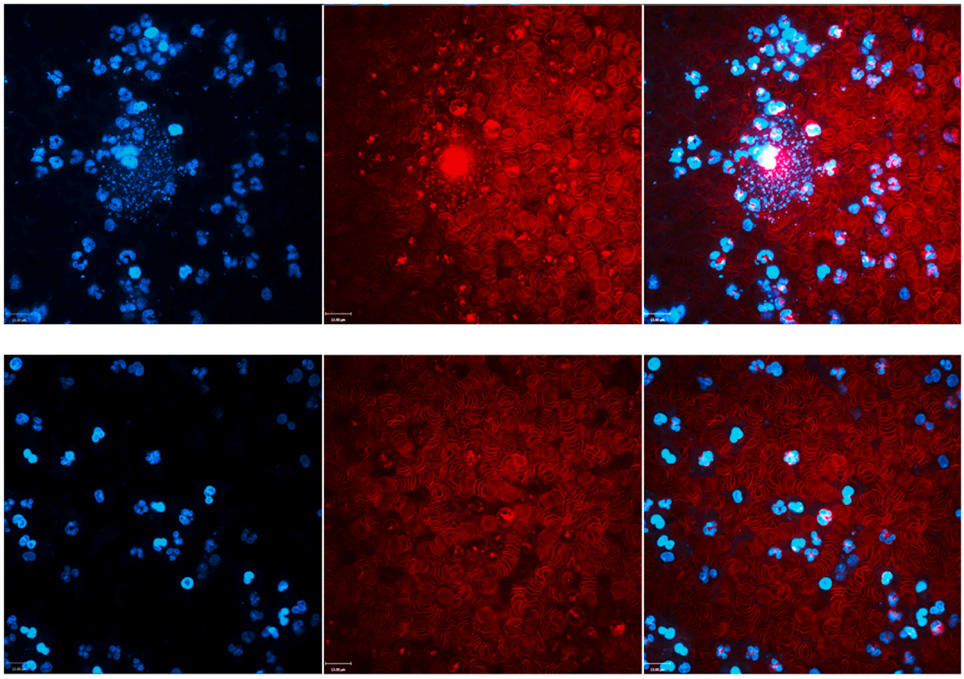

Fig. 1. Real-time live confocal microscopy detects circulating microaggregates as a marker for Post-COVID associated symptoms. Circulating blood microaggregates (upper row, arrow) of a patient suffering from Post-COVID resolved after an anticoagulatory treatment step (lower row). Staining of DNA (blue, left panel) shows typical morphology of polymorphonuclear granulocytes and mononuclear lymphocytes and monocytes. The fine dust-like reactivity (blue) in the top row, especially in the center of aggregate, is mitochondrial DNA of thrombocytes. In red (center) wheat germ agglutinin (WGA) detects N-acetyl-d-glucosamine and N-acetyl-d-neuraminic acid residues on the surface of erythrocytes and within precipitates of proteins. Overlay is on the right, scale bar 15 μm.

confocal microscopy and did not find any microaggregates.

Discussion

Real time live confocal microscopy is a versatile tool, which enables us to analyze solid, as well as liquid biopsies in great detail, without the need of fixation. The stained samples may be imaged immediately allowing the analysis of single cells and even molecules such as fibrin [2]. We could visualize circulating aggregates consisting of thrombocytes and granulocytes. Additional analyses should confirm whether thrombocytes within the aggregates are activated and whether granulocytes are of neutrophilic or eosinophilic type. Given the reversibility of these aggregates on pharmacological inhibition of platelet aggregation and the lack of mature fibrin within the aggregates, we speculate that the driving force lies within the primary hemostasis with little contribution of plasmatic coagulation factors. Beyond this morphological observation there is now need for proof of the pathophysiological relevance for clinical symptom development and resolution on a larger patient collective. Considering the size of the microaggregates, problems in the microcirculation might be at least one of the causes for the Post-COVID symptoms. Consequently, an elimination of these microaggregates might be an explanation for the clinical improvement of the Post-COVID symptoms.

Conclusion

We propose therefore, that microaggregates should be analyzed in more detail as they might contribute to post infectious syndromes such as the Post-COVID syndrome. An ideal method for such an analysis is real time live confocal microscopy.

Ethical approval

The case reported is a patient under therapy at private practices of Dr. Lisch and Dr. Wick.

Funding

Self-funding.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Declaration of Competing Interest

We declare no competing interests.

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