



The confocal microscopy of the cornea in post-COVID syndrome; the clinical observation

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Abstract

One of the most important complications of the post-COVID-19 syndrome may be a small fiber neuropathy, which cannot be evaluated by a routine electroneuromyography. Confocal microscopy of the cornea (CCM) may be a promising method for early neuropathy verification. To study the possibility of the small fiber neuropathy evaluation using the CCM in a patient with the post-COVID-19 condition. The patient is a female of 61 years old, which suffered from a COVID-19 infection in October 2020. After a month, she noted the presence of myalgia and polyneuropathy. A year after the onset of the disease, in addition to myalgia, headaches, neuropathy pain, the patient also experienced bowel disorders and stomach aches, which cannot be explained with an alternative diagnosis. The patient underwent CCM to assess the structure of nerve fibers to prove the presence of the small fiber neuropathy as a complication of the COVID-19 infection. The presence of the small fiber neuropathy was evaluated in the patient as well as enlarged Langerhans cells in the cornea. Considering the pivotal role of these cells in the immunological processes, the "activated" dendritic cells in post-COVID-19 patients may serve as another evidence of the autoimmune nature of this complication and possibly, a method for the monitoring treatment effectiveness of this disease. In this clinical case of a 61-year-old patient with the post-COVID-19 syndrome, the possibility of application of the confocal microscopy of the cornea for the diagnosis of neuropathy of small fibers and monitoring of the patient's condition is shown.

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Introduction

Since 2019, a new disease has been emerged around the world - new coronavirus infection (COVID-19). The disease is caused by the SARS-Cov-2 adenovirus and is described with the presence of various, presumably, pulmonological manifestations; including cough, shortness of breath, anosmia, pyrexia, pneumonia and others (1). Among the laboratory data that allow to measure the severity of the disease's course, C-reactive protein (CRP) and ferritin are evaluated as the most informative ones. High titers of these acute-phase proteins may allude the presence of a so-called "cytokine storm", that is significantly worsening the prognosis of the patient (2-4). C-reactive protein increases in almost 90% of patients (5) and is applied not only as a criterion for assessing the severity of the acute disease, but as well may correlate with the development of the coronavirus complication that is called post-COVID-19 syndrome.

Key point

Post-COVID-19 syndrome may manifest with multiple autoimmune complications; one of the most important is small fiber neuropathy with pain syndrome and dysautonomia. Confocal microscopy of the cornea may be a promising method for the diagnostic of such disease.

Post-COVID-19 syndrome or long COVID-19 is a complication of the new coronavirus infection, characterized by a wide variety of symptoms, including brain fog (cognitive impairment), myalgia, headaches, fatigue, weakness and many others (6). On October 6, 2021, the World Health Organization (WHO) has published the clinical case definition of the post-COVID-19 syndrome by a Delfi consensus (7). In this document, the official position of WHO on this issue was introduced, including the preferred term - "post-COVID-19 condition", clinical characteristics and main symptoms, described by both patients



and healthcare professionals. The key message had the following statements; three months from the onset of acute COVID-19, two months or more of post-COVID-19 symptoms, especially fatigue, shortness of breath, cognitive dysfunction that may have fluctuating or relapsing course. The list of possible complications included immunological conditions (flue-like episodes, pyrexia, arthralgia and myalgia), autonomic dysfunction with the involvement of the cardiovascular system, gastrointestinal tract and other organs and also various neurological and psychiatric complications, among which a fibromyalgia-like condition takes one of the leading roles. Although the presence of a clinical definition of the post-COVID-19 condition is a very important step in defining a new syndrome, the pathophysiological explanation of such complications remains to be understudied.

At the moment, there are two basic theories explaining the pathogenesis of this condition. Those are; direct effect of the virus on tissues and cells and its subsequent persistence in the patient's body (8) and an indirect effect through the hyperactivation of immunity, as a result of which various autoimmune reactions occur in the body (9). Both theories agree on systemic effect of the condition with the involvement of the internal organs, blood vessels and nerve fibers. We postulated that, as a result of immunological inflammation, the nerve fibers of the smallest diameter that are widely spread in the skin, mucous membranes and internal organs are damaged. Their dysfunction causes pain syndrome and autonomic disorders in the internal organs. The cardiovascular system dysfunction leads to dysregulation of both small diameter vessels (microcirculatory dysfunction, cognitive impairment and hair loss) and large diameter vessels (postural tachycardia). The presence of autoantibodies, including ones to adrenergic and cholinergic receptors and other factors of immunological inflammation, cannot be excluded.

The skin biopsy is a "golden standard" of the small fiber neuropathy evaluation. The biopsy is most often taken from the lateral thigh, fixed with the Zamboni solution, after that the enzyme immunoassay is performed with the counting of the intraepidermal small nerve fiber density (10). Though the described method is reliable and undergone the international standardization, its application is difficult due to the need of histological laboratory, cryofixation and trained specialist for its interpretation. The method itself is interventional and cannot be conducted in the same place to evaluate the effectiveness of treatment (11, 12). Due to the described limitations, the new method for verifying small fiber neuropathy is presented – corneal confocal microscopy (CCM). This is a non-invasive, non-painful procedure, which requires only the confocal microscope with the retinal module, without purchasing any reagents or need for the histological laboratory (13). The obtained digital images can be kept without time limitations, since it was counted not just manually, but also with the help of

automatic and semi-automatic programs (14, 15). There is also a possibility of performing the confocal microscopy of the cornea analysis with a Heidelberg Engineering microscope and the HRT3 Rostock corneal module to assess the structure of nerve fibers.

Case Presentation

The patient is a female of 61 years old, has suffered from a new coronavirus infection in October 2020. The onset of the disease included pyrexia of 38-39°C, cough, anosmia and weakness. She had a positive PCR result for coronavirus, as well as undergo blood tests without significant changes. Paracetamol and new oral anticoagulants (apixaban) were prescribed. The course of the disease was easy and did not require hospitalization. As concomitant diseases, chronic bronchitis (smoking experience more than 20 years) and chronic type B gastritis in remission were evaluated. She did not have a positive allergic history or drug intolerance.

Two weeks after receiving a negative PCR result, the patient began to notice headaches, periods of fatigue and apathy. She was prescribed selective serotonin reuptake inhibitors. Finally, the patient noted an improvement in her condition, however, after a month, intermittent myalgias of the upper extremities and polyneuropathy of the lower extremities join the abovementioned symptoms. On this occasion, she took various non-steroidal anti-inflammatory drugs, but due to an exacerbation of chronic hyperacid gastritis, she could not have them for a longtime. Within six months, the patient was examined by various specialists, including a rheumatologist, immunologist, neurologist and psychiatrist. The patient could not note a pronounced positive effect from the prescribed therapy.

A year after the onset of the disease (October 2021), in addition to the myalgia, headaches, neuropathy pain, the patient also experienced bowel disorders (loose stools at least twice a week, regardless of the consumed food) and stomach ache, which she does not associate with her chronic disease. The patient underwent confocal microscopy of the cornea with a Heidelberg Engineering microscope and the HRT3 Rostock corneal module to assess the structure of nerve fibers. The following results were obtained.

In both eyes, thinning of nerve fibers, excessive branching and an increased tortuosity coefficient were observed in the center of the cornea (according to the CCM protocol program). Those are the typical results, illustrating the presence of small fiber neuropathy in the patient. However, more peculiar data were obtained from the analysis of the peripheral parts of the eyes. [Figure 1](#) shows hypertrophied Langerhans cells (marked with arrows). This is a subtype of dendritic cells (intraepidermal macrophages) responsible for the active capture and digestion of bacteria, the remains of dead cells and viral particles (16). In our patient, the cells are presented in a non-standard hypertrophied size, that is not a typical observation. [Figure 2](#) shows a healthy control patient with

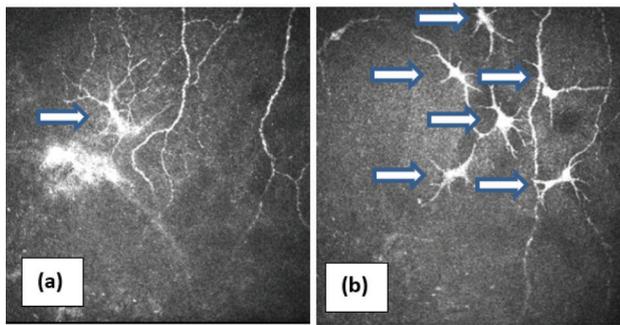


Figure 1. (a) and (b). Corneal confocal microscopy of the post-COVID-19 patient (female, 61 years). Arrows indicate hypertrophied Langerhans cells.

normal dendritic cells, marked with arrows. Considering the pivotal role of the Langerhans cells in the immunologic processes in the cornea, the enlarged and “activated” dendritic cells in post-COVID-19 patients may be another evidence of the autoimmune nature of this complication and, possibly, a promising method or developing better diagnostic and treatment options for these patients.

Discussion

There are no unified criteria for the laboratory and instrumental diagnosis of post-COVID-19 syndrome. Some studies support the small nerve fibers dysfunction in these patients (17-19). In that case, the routine electroneuromyography is not suitable, since the diameter of the nerve fibers is too small to make the significant impact on the result of the study. According to the criteria of small fiber neuropathy (20), the skin biopsy should be conducted, as well as validated clinical scales testing (e.g., COMPASS-31) and quantitative sensory testing (21). To establish the diagnosis, positive results should be found in two tests out of three (22). Post-COVID-19 condition rapidly turns into a complex problem of modern healthcare, primarily due to the novelty of this disease and the need for its in-debt study. Using the WHO clinical criteria, it is possible to determine the group of patients that needs active evaluation and is capable of developing and progression of COVID-19 complications.

Conclusion

Small fiber neuropathy – which can be diagnosed employing validated scales, skin biopsy and quantitative sensory testing – plays a significant role in the clinical manifestations of post-COVID-19 syndrome. A simpler and more reliable method for diagnosing neuropathy of small fibers can be confocal microscopy of the cornea due to the painlessness, non-invasiveness and technical simplicity of this method. Identifying neuronal fiber damage in patients with post-COVID-19 syndrome may be a promising tool in assessing disease progression and treatment efficacy. The identification of enlarged Langerhans cells as a factor of hyperactivation of the immune system may be evidence of the autoimmune nature of post-COVID-19 syndrome and requires further study.

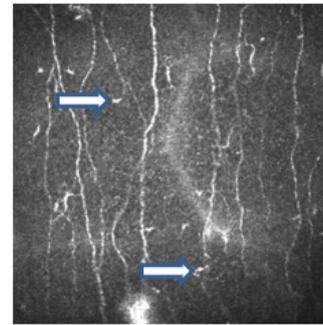


Figure 2. Corneal confocal microscopy of the healthy control (female, 30 years). Arrows indicate the examples of a normal-sized Langerhans cells.

Authors' contribution

BA and LM were the principal investigators of the study. GN and SL were included in preparing the concept and design. VU revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare no conflict of interest.

Ethical issues

This case report was conducted in accord with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from the patient for publication as a case report. The Local Ethical Committee of St. Petersburg State University (protocol No. 115-02-5 from 25.06.20) approved this study. Besides, ethical issues (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

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