Serology in SARS-CoV-2 Infection

Subjects: Others

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SARS-CoV-2/Coronavirus 2019 (COVID-19) is responsible for the pandemic, which started in December 2019. In addition to the typical respiratory symptoms, this virus also causes other severe complications, including neurological ones. In diagnostics, serological and polymerase chain reaction tests are useful not only in detecting past infections but can also predict the response to vaccination. It is now believed that an immune mechanism rather than direct viral neuroinvasion is responsible for neurological symptoms. For this reason, it is important to assess the presence of antibodies not only in the serum but also in the cerebrospinal fluid (CSF), especially in the case of neuro-COVID. A particular group of patients are people with multiple sclerosis (MS) whose disease-modifying drugs weaken the immune system and lead to an unpredictable serological response to SARS-CoV-2 infection. Based on available data, the article summarizes the current serological information concerning COVID-19 in CSF in patients with severe neurological complications and in those with MS.

Keywords: COVID-19 ; SARS-CoV-2 ; neuro-COVID

1. Introduction

Coronavirus 2019 (COVID-19) is a newly emerging disease, which has caused a global pandemic as announced by the World Health Organization (WHO) in March 2020 [1]. Severe acute respiratory virus-2 (SARS-CoV-2), the virus responsible for COVID-19, has affected over 151 million people and contributed to over 3 million deaths [2]. SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus and is composed mainly of N (nucleocapsid), S (spike), M (membrane) and E (envelope) proteins [3][4]. Entrance into a host cell is induced by a connection between a spike protein and angiotensin-converting enzyme 2 (ACE2) receptor. The same mechanism was responsible for the SARS pandemic in 2002/2003 [5]. The incubation period of the disease is 2–14 days, and its main symptoms include fever, cough and shortness of breath [6]. A SARS infection can lead to pneumonia and acute respiratory distress syndrome that can result in death [7]. According to present data 99.6% cases are mild, and 0.4% are serious or critical [2]. The severe course mainly involves the elderly and patients with comorbidities [8][9]. However, recently, some new variants (such as B.1.1.7 [or VOC 202012/0], B.1.351 [or 501.V2], B.1.617) with many new mutations have emerged and are potentially more virulent and infectious, and more importantly, cause severe disease in young people in addition to the elderly [2][10]. Due to the presence of asymptomatic infections, the number of infected people remains underestimated [9]. For routine diagnostic processes, molecular tests, such as polymerase chain reaction in real time (qPCR), which indicates the acute phase of the disease, and serological tests that can detect specific antibodies are used [11]. The second type of test can determine whether the patient has had contact with the virus, determines its serological status and is used, among others, in epidemiological studies assessing the incidence of SARS-CoV-2 infection in the population [12]. A crucial issue is the body's immune response to SARS-CoV-2 infection. During infection, an increase in the production of numerous proinflammatory cytokines, such as tumor necrosis alpha and interleukins-2 and -6 (TNF-α and IL-2 and -6, respectively) is observed [13]. A severe course of SARS-CoV-2 infection is undeniably connected with dysregulation of immune system and cytokine release syndrome [8]. Part of the immune response also involves the production of antibodies, mainly against S and N proteins, also known as neutralizing antibodies. The crucial role of these antibodies is to block virus entrance into host cell and activation of antibody-dependent cell cytotoxity (ADCC). As a result, the disease could be defeated, or the immune system's overactivity could induce a cytokine storm [12]. In addition, patients showed a reduction in B- and T-lymphocytes and natural killer (NK) cells. Both the increase in inflammatory cytokines and the decrease in lymphocyte counts are associated with the severity of the disease [13]. Due to the fact that entry into the human cell is associated with the ACE2 receptor and these receptors also exist on neurons and glial cells, speculations about the neurotropism of SARS-CoV-2 began [14]. From the early stages of the pandemic, neurological symptoms have been described, of which the most common are anosmia, ageusia, headaches and dizziness (Table 1) [15]. In addition, the course of COVID-19 may be associated with much more severe neurological complications, such as encephalopathy, Guillain-Barre syndrome, meningitis, encephalitis and/or necrotizing hemorrhagic encephalopathy [15][16]. Moreover, the relationship between SARS-CoV-2 infection and acute cerebrovascular diseases, such as acute ischemic stroke, cerebral venous sinus thrombosis,

cerebral hemorrhage and subarachnoid hemorrhage, were sought [15]. During studies on the neuroinvasiveness of SARS-CoV-2, the presence of anti-SARS-Cov-2 antibodies in the cerebrospinal fluid (CSF) and intrathecal synthesis were found; interestingly, in these cases, the PCR results from nasopharyngeal swabs remained negative [17]. Reports of possible cross-reactions with human proteins and formation of autoantibodies and as a consequence, development of autoimmune encephalitis have been published [18]. As previously mentioned, the severe course of COVID-19 is associated with the presence of comorbidities in people. It can be assumed that such comorbidities are neurological disorders, such as multiple sclerosis (MS). An interesting issue is the serological status of people with MS, especially those undergoing treatment with disease-modifying therapy. The current data suggest that even in the presence of a altered immune system, the risk of severe course and serological status are similar to those in the general population [19]. This entry summarizes the current knowledge of severe neurological complications in COVID-19 and the serological status of those with neurological diseases.

Table 1. Neurological symptoms and possible complications related to severe acute respiratory coronavirus -2 (SAR-CoV-2) infection.

Coronavirus-2019 (COVID-19) and Neurological Disturbances		
Neurological Symptoms	**Severe Neurological Complications**	**COVID-19 Complicates Course of Neurological Diseases**
Headaches **Dizziness** **Seizures** **Anosmia/hyposmia** **Ageusia/hypogeusia** **Hypoesthesia** **Paresis and paralysis** **Disturbances of consciousness** **Urination disorders**	**Guillain-Barre Syndrome and Miller-Fisher Syndrome** **Acute Transverse Myelitis** **Encephalopathy** **Demyelination** **Encephalitis/meningoencephalitis** **Autoimmune encephalitis** **Necrotizing hemorrhagic Encephalopathy** **Ischemic stroke** **Cerebral hemorrhage** **Cerebral venous sinus thrombosis** **Subarachnoid hemorrhage**	**Multiple sclerosis** **Neuromyelitis optica spectrum disorders** **Epilepsy** **Amyotrophic lateral sclerosis,** **Parkinson disease** **Dementia**

2. Serology in SARS-CoV-2 Infection

Currently, qPCR remains the gold standard for diagnosing an active SARS-CoV-2 infection. Despite the high sensitivity and specificity of the test, a risk of obtaining a false positive (sample contamination) or a false negative (incorrect storage of the collected material) results exists [2][20]. Serological tests are a valuable supplement to the diagnosis of SARS-CoV-2 as they enable retrospective detection of infection and remain irreplaceable in population studies. Moreover, they are cheaper, easier to make and the collected material is less complicated to store [3]. These types of tests are also invaluable in assessing the risk of infection in risk groups. To understand the mechanism of serological diagnostics, it is worth reviewing the structure of the SARS-CoV-2 virus. This virus consists of structural proteins, the most important of which in serological terms are the nucleocapsid protein (N) and the spike protein (S) as almost all serological methods detect the IgG and IgM produced against these proteins. The S protein is a homotrimer whose key element is the S1 subunit and the receptor binding domain (RBD) against which the neutralizing antibodies are being produced [3]. The S1 subunit has been shown to be the most specific for SARS-CoV-2, while the N protein is produced in the greatest amount [20][21]. It was shown that the antibodies against the N protein correspond more closely to the previous SARS-CoV-2 infection, while the antibodies against the S protein are part of the humoral response and have a greater neutralizing potential [22]. Coronaviruses are respiratory tract viruses, and because they come into contact with mucous membranes in the respiratory tract, they induce the formation of IgA antibodies, while at present, the diagnostics of IgM and IgG antibodies is of the greatest clinical importance [12]. Researchers disagree about the order in which seroconversion occurs in the SARS-CoV-2 infection. It has been reported that the typical order of production is IgM first after which IgG is absent [23]. However, other studies have shown that seroconversion occurs in a manner similar to other infections. A study of 173 patients with SARS-CoV-2 infections showed that conversion of total antibodies, IgM and IgG was 93.1%, 82.7% and 64.7%, respectively. The mean seroconversion times in this study were 11, 12 and 14 days, respectively [3]. Similarly, in a study on 214 patients, it was shown that the highest sensitivity involved detection of IgM antibodies to the S protein. In this study, the sensitivity in the IgM class was 77.1%, and IgG was 74.3%. For comparison, the sensitivity to the N protein was 68.2% in the IgM class and 70% in the IgG class [3]. The median detection of IgA of 5 days and IgG of 14 days were also compared. Comparing the IgM and IgA responses, the corresponding peak response was obtained on days 10–12 and 20–22 of the disease, respectively [24]. The IgA response turned out to be stronger and more durable than the response associated with IgM. A meta-analysis of 3856 confirmed cases showed that the positive index of IgM, IgG and their

combined detection amounted to 61.2%, 58.8% and 62.1%, respectively. Furthermore, it turned out that IgM/IgG was detected in 19% of asymptomatic cases [25]. In addition to the enzyme-linked immunosorbent and chemiluminescent assay (ELISA and CLIA, respectively) methods that are most commonly used in serological diagnostics, it is worth mentioning the lateral flow immunoassay, which can detect total IgG or IgM even in 15 min [26]. The meta-analysis showed a sensitivity for these tests ranging from 72.2% to 100% and a specificity from 98.9% to 100% [26]. Another serological method is the magnetic chemiluminescence test, which is based on the double antibody sandwich method. This test demonstrates concurrent seroconversion for IgG and IgM. After 19 days of illness, 100% of patients had positive IgG. The antibodies reached a plateau after six days of seroconversion [27]. However, the ELISA test is still the most frequently used technique. The sensitivity, specificity and positive and negative predictive values for IgM were assessed as 87.5%, 100%, 100%, 95.2%, respectively, and for IgG, these values were 70.8%, 96.6%, 85%, 89% and 1%, respectively [3]. The mechanism of action of the antibodies produced by plasma cells occurs via prevention of virus entry into the cell by shielding the N and S proteins and activating antibody-dependent cytotoxicity. Interestingly, it was shown that patients with a more severe disease course had an earlier and higher antibody response. This phenomenon, called antibody-dependent enhancement (ADE), can be explained by the fact that anti-S antibodies inhibit viral entry into the cell, thereby enhancing the binding of the virus to B-cells and macrophages via the Fcγ-II receptor after which these cells are activated, and the cytokine storm intensifies [28]. Serological tests have their advantages, but their limitations should be kept into consideration. Researchers should be aware of the possibility of false-negative results in cases in which the patient has not yet developed antibodies. It is worth noting that despite the time of seroconversion determined in many studies, individual variability also exists. False positive results may be the result of a cross-reaction between other human coronaviruses. Determining antibodies also requires skillful interpretation of the selected test. Due to the above factors, serological tests remain additional tools in the diagnosis of the SARS-CoV-2 virus [25].

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