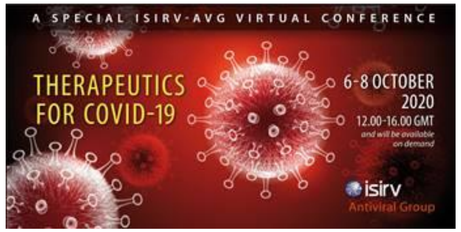
The role of serological diagnosis in the study of the COVID-19 pathogenesis



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The role of serological diagnosis in the study of the COVID-19 pathogenesis

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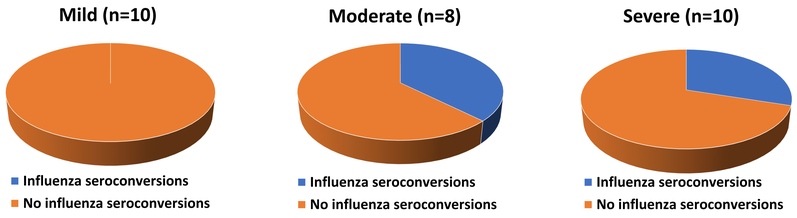
Introduction. COVID-19 caused by novel coronavirus SARS-CoV-2 is spreading globally around the world. Preclinical and clinical trials are underway on a number of vaccines prepared using various platforms. However, the mechanisms of the immunopathogenesis of severe coronavirus infections remain poorly understood, despite the fact that cases of severe acute respiratory syndrome caused by coronavirus was observed in 2002-2003 (SARS-CoV), as well as Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. A number of questions remain unclear about pathogenesis of severe coronavirus infection in relation to coinfection and factors of adaptive immunity.

Methods. In this study, we used archival sera of patients remaining after current laboratory studies of patients with different severity of COVID-19. The patients were hospitalized in February-April in 2020 Vsevolozhsk Clinical Interdistrict Hospital, Leningrad region, Russian Federation, Saint Petersburg, Russian Federation. The COVID-19 severity was estimated according to Interim guidelines for the prevention, diagnosis and treatment of new coronavirus infection (COVID-19), Version 8. The study was approved by the Local Ethics committee of the FSBSI "IEM". At the first stage of the study, 110 blood sera were studied, including 28 pairs of samples obtained upon admission to the hospital and after 5-6 days. Blood sera from patients obtained before the spread of COVID-19 in early 2019 were used as control samples, including 18 paired sera obtained from patients with confirmed influenza infection. First, sera were tested for antibodies to coronavirus using the "SARS-Cov-2 IgG Screen" kit (Imbian Lab, Novosibirsk, Russia). The sera were studied in ELISA-test for the presence of IgG and IgM antibodies specific to the recombinant S and N proteins of SARS-CoV-2 (AtaGenix, Wuhan, Hubei). The HRP-Linked Goat Anti-Mouse IgG Antibody (Sigma, St. Louis, USA) and rabbit-anti-human IgM (Cloud-Clone Corp., Wuhan, Hubei) were used as conjugate.
Paired sera from patients with COVID-19 were also tested for hemagglutination inhibition (HI) antibodies against A/New York/61/2015 (H1N1)pdm09 and A/Hong Kong/4801/2014(H3N2) influenza viruses, virus-specific ELISA IgG and IgM were evaluated using purified A/California/07/2009(H1N1)pdm09 and A/Hong Kong/4801/2014(H3N2) influenza viruses. Influenza viruses were provided from Federal State Budgetary Scientific Institution "Institute of Experimental Medicine" collection of viruses.
For statistical analysis the antibody titers were expressed as log2 of the inversed final dilution. Non-parametric measure of statistical dependence between 2 variables was done using Spearman's rank correlation coefficient. The p-value < 0.05 was considered to be statistically significant.

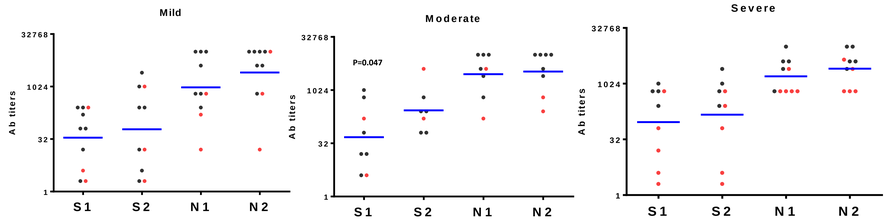
Results. In patients with moderate and severe forms of infection in six cases at least 4-fold increases in antibodies to influenza A/H1N1 and in one to influenza A/H3N2 were detected (Fig. 1). At the same time, these patients had a positive PCR result for SARS-CoV-2 or an increase in serum antibodies to one of the SARS-CoV-2 proteins.

Fig 1. The proportion of patients with ≥ 4-fold increase in antibodies to influenza A/H1N1 and A/H3N2 in paired sera



In the sera of patients with COVID-19, antibodies were detected in most cases to the S and N proteins of SARS-CoV-2. These patients were both with and without isolation of coronavirus by PCR. In the analysis of paired sera, it was shown that in the case of severe infection, higher mean initial titers of serum IgG antibodies to the S-protein were observed compared to mild forms (Fig. 2). Statistically significant increases in antibodies to the S-protein of SARS-CoV-2 were noted in the case of moderate infection (Figs. 2, 3). A high level of correlation was shown when serum IgG were detected using "SARS-Cov-2 IgG Screen" kit or recombinant S-protein, Spearmen r=0.83. The positive value according to the screen data corresponded to 1:256 antibody titers obtained using S-protein.

Fig 2. Serum IgG antibody titers in patients with COVID-19



The red color indicates patients whose screening did not reveal SARS-Cov-2

The level of serum IgG to the S-protein was higher than the level of IgM, with respect to the N-protein it was the opposite, and the levels of IgM to the N-protein began to decrease, while the IgG increased (Fig. 4).

Fig 3. The ≥4- fold IgG increase in paired sera

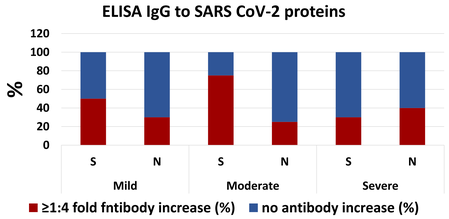
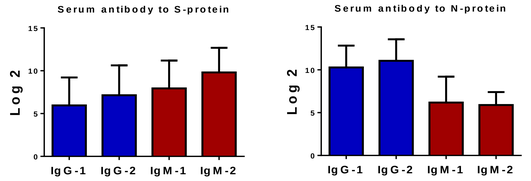


Fig. 4. Serum IgG and IgM



Conclusions. Serological diagnostics additionally confirmed coronavirus infection if the virus was not detected by PCR. Screening for influenza viruses can significantly improve the clinical management of patients as there are antiviral drugs available for influenza. The study of adaptive immunity factors, such as the formation of virus-specific antibodies to SARS-CoV-2, is important in light of understanding the need for vaccination, including in individuals who have had natural COVID-19 infection. A comprehensive study of antibodies to COVID in recovered patients can be useful in the development of vaccines, identification of contingents for vaccination and the use of plasma preparations.
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Conflict of interest. The Authors have nothing to declare.