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COVID-19 AND CLINIC LABORATORY DIAGNOSTICS

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The pandemic of a new coronavirus infection has caused an urgent need to develop, validate and put into practice effective methods of laboratory diagnostics that allow to verify the etiology of the disease, determine the presence of an immune response and its phase, as well as assess a wide range of pathophysiological disorders and complications arising from COVID-19. In the context of the COVID-19 pandemic, the important place of laboratory diagnostics is beyond doubt. It is the means and methods of laboratory diagnostics that are of fundamental importance for identifying those infected, including when the disease is asymptomatic or symptoms have not yet appeared, as well as for objectively determining the severity of the condition.

COVID-19 VA KLINIK LABORATOR DIAGNOSTIKA

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Yangi koronavirus infeksiyasi pandemiyasi kasallikning etiologiyasini tekshirish, immunitet reaktsiyasi va uning fazasi mavjudligini aniqlash, shuningdek, uning holatini baholash imkonini beruvchi laboratoriya diagnostikasining samarali usullarini ishlab chiqish, tasdiqlash va amaliyotga tatbiq etishning dolzarb zaruratini keltirib chiqardi. COVID-19 dan kelib chiqadigan patofiziologik kasalliklar va asoratlarning keng doirasi COVID-19 pandemiyasi sharoitida laboratoriya diagnostikasining muhim o'rni shubhasizdir. Aynan laboratoriya diagnostikasi vositalari va usullari infektsiyalanganlarni aniqlash uchun, shu jumladan kasallik asimptomatik yoki alomatlar hali paydo bo'lmaganda, shuningdek, vaziyatning og'irligini ob'ektiv aniqlash uchun muhim ahamiyatga ega.

COVID-19 И КЛИНИЧЕСКАЯ ЛАБОРАТОРНАЯ ДИАГНОСТИКА

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Пандемия новой коронавирусной инфекции вызвала экстренную потребность в разработке, валидации и внедрении в практику эффективных методов лабораторной диагностики, позволяющих верифицировать этиологию заболевания, определять наличие иммунного ответа и его фазу, а также оценивать широкий спектр патофизиологических нарушений, и осложнений, возникающих при COVID-19. В условиях пандемии новой коронавирусной инфекции COVID-19 важное место лабораторной диагностики не вызывает сомнений. Именно средства и методы лабораторной диагностики имеют основополагающее значение для выявления инфицированных, в том числе когда болезнь протекает бессимптомно или симптомы еще не проявились, а также для объективного определения степени тяжести состояния.

At the end of 2019, humanity encountered a new representative of the Coronaviridae family, SARS-CoV 2 (sub-genus Sarbecovirus, genus Betacoronavirus) [4], which, like SARS-CoV, is most closely related to the bat virus (88% similarity of nucleotide sequences), but at the same time has a lower degree of similarity with SARS-CoV - 79% [5-7]. The emergence of this virus led to serious consequences for humanity, causing a pandemic of severe respiratory disease COVID-19, which swept all countries and continents, claimed and continues to claim hundreds of thousands of human lives. In the course of effectively countering this unprecedented biological threat, the world medical community is striving to develop various strategies for the treatment and prevention of COVID-19, the success of which directly depends on the effectiveness of the applied approaches, methods and technologies for laboratory diagnosis of infection.

Laboratory diagnostic methods are a key component in diagnosing and monitoring the course of COVID-19. Reliable tests should be used to detect active infections with varying degrees of clinical symptoms, evaluate immune response and monitor cure, and diagnose and differentiate characteristic comorbid conditions and complications. In this regard, laboratory diagnostics for COVID-19 is complex and includes specific tests aimed at detecting the virus itself and the immune response to its invasion, markers used for the differential diagnosis of viral and bacterial infections, as well as general clinical studies that allow monitoring of the inflammatory reaction, organ dysfunction, the state of the blood coagulation system, etc. In the case of bacterial co- and superinfection, microbiological diagnostic methods are important. There is also the possibility of obtaining false positive responses when setting up PCR. Despite the decrease in the risks of DNA

(RNA) contamination when performing real-time PCR, compared with the electrophoretic format for recording results, this problem remains significant and requires a high level of organization of laboratory studies, especially with their significant volumes. It should be borne in mind that positive PCR responses do not mean the presence of a live virus in the sample, since the method detects only RNA fragments - SARS-CoV-2 markers. The issues of accuracy of laboratory research are inextricably linked with the peculiarities of performing the preanalytical and analytical stages of diagnostics. We can distinguish the following factors that largely determine the accuracy of diagnostic analysis, which must be taken into account when planning and performing the preanalytical stage of work [1,3,11].

The main method is real-time RT-PCR. The material for the study is the combined nasopharyngeal and oropharyngeal smears placed in 1 test tube with a transport medium. If the result is negative and at a later date for sampling, it is better to use sputum samples or bronchoalveolar lavage in patients with a severe course of the disease. The absence of a positive result in RT-PCR against the background of typical clinical signs of a new coronavirus infection does not allow us to reliably exclude the etiological role of SARS-CoV-2 coronavirus, and in this case, serodiagnostic methods are reasonable. When performing serodiagnostic studies, it is necessary to use the most sensitive and specific diagnostic tools, which primarily include tests for total (total) antiviral antibodies (IgM/IgG/IgA), as well as IgG (from 8–14 days after clinical manifestations). ny). Separate determination of IgM and IgG is considered less justified, since the efficiency of detection of total antibodies to SARS-CoV-2 exceeds that of detection of individual classes of antiviral immunoglobulins [14]. The detection of isolated IgM in patients is characterized by lower sensitivity [11] and may also lead to false positive results due to their lability and relatively lower specificity compared to other classes of antiviral antibodies. Rapid tests may have low sensitivity [10], are screening, and are not recommended for the etiological laboratory diagnosis of COVID 19.

The humoral immune response in COVID-19 is formed along a universal path and consists in the sequential synthesis of IgM, which appear on days 5–7, reach a peak by the 14th day of the disease and leave the circulation over the next two weeks, IgA with similar kinetics, and IgG, which begin be determined from 2–3 weeks of the disease and circulate indefinitely, presumably providing acquired immunity to this disease. To determine the presence and level of antibodies, test systems based on immunochromatographic, immunochemiluminescent, and enzyme immunoassay methods are used [6]. The most simple m is a high-quality immunochromatographic method, implemented in the form of test strips, allowing for 10-15 minutes. detect the presence or absence of antibodies in whole blood (venous or capillary), serum or plasma. Currently, a number of test systems using this method are registered in our country. All these systems are built on a universal principle using specific antibodies labeled with colloidal gold to the corresponding immunoglobulins and differ in the type of antibodies detected (only IgG, total antibodies, IgM and IgG separately), in terms of configuration, ease of use, and ease of reading the result (visibility).). Test systems using the immunochromatographic method are characterized by fast results, high specificity with satisfactory sensitivity, do not require high qualification of the personnel using them, do not impose special requirements on storage conditions and can be implemented everywhere for the purpose of primary screening, being true “point of care” tests. ". A relative disadvantage is the impossibility of obtaining a quantitative result that allows assessing the dynamics of changes in the level of immunoglobulins. Interest in the widespread use of serological tests is increasing, but there are still many questions and uncertainty regarding the extent and duration of immunity caused by SARS-CoV-2 infection, the frequency of false positive and false negative test results. According to WHO, laboratory tests that detect antibodies to SARS-CoV-2 in humans need further validation to determine their accuracy and reliability [11,12].

It occurs almost simultaneously (a similar feature of seroconversion was previously shown for the SARS-CoV coronavirus) or sequentially, with a short interval of 2–3 days [10]. Moreover, in some patients, IgM is first detected, in others - IgG, and after 17-23 days they are detected in 100%. Within 3 weeks of the onset of clinical symptoms, a gradual quantitative increase in IgM and Ig G is observed. After 3 weeks, there is a decrease in IgM titers, while IgG remain high. Taking into account these features, the detection of total antibodies in the blood provides the maximum diagnostic sensitivity [8]. In parallel with the study of the immune response to infection, studies aimed at studying the kinetics of virus release during the infectious process were carried out [7]. It has been established that seroconversion of IgM and IgG, which occurs almost simulta-

neously, is not associated with the cessation of virus isolation: in most patients whose blood contains IgM and IgG to the SARS-CoV-2 coronavirus, RNA of this pathogen is found in the respiratory tract [2].

In the first scientific publications on the course of COVID-19, an unprecedented prevalence of complications caused by a violation of the hemostasis system was noted almost immediately. Thrombotic complications (TO) and the development of consumption coagulopathy (DIC) often accompanied the severe course of the disease, and also caused the death of patients. Thus, according to a number of studies in ICU patients, even against the background of thromboprophylaxis, the frequency of TO ranged from 23% to 69%, while 71% of patients who developed DIC died [4]. It should be noted that in later publications such a high frequency of coagulopathy was no longer described, which may be due to the beginning of the routine use of heparins for the correction of hypercoagulable states. According to a meta-analysis, hemostasis parameters in hospitalized patients with COVID-19 predominantly demonstrate mild thrombocytopenia, an increase in D -dimer levels, a prolongation of prothrombin time, and an increase in fibrinogen levels. Statistically significant differences between surviving and deceased patients were observed in D-dimer levels (≈3 times) and fibrin degradation products (≈2 times), as well as a significant prolongation of prothrombin time (by 14%) [11]. Changes in various parts of the hemostasis system in COVID-19 are multidirectional, and therefore the diagnostic and prognostic significance of individual hemostasis tests may be unobvious and contradictory. Making clinical decisions based on changes in individual parameters can lead to the wrong choice of therapy. So, for example, with the aggravation of the course of the disease, as well as with the onset of coagulopathy of consumption, the level of fibrinogen decreases, as well as the level of antithrombin III, which is not measured routinely [9,12]. These changes affect the hemostasis system in different ways, so defining one parameter without the other can lead to false conclusions. That is why multifactorial changes in the hemostasis system that occur against the background of the course of coronavirus infection, especially during the development of critical conditions, are most effectively assessed using global tests that show the resulting state of the patient's hemostasis, taking into account all factors, including the influence of administered therapy. Thus, it has been shown that in patients with COVID-19 and acute respiratory failure, compared with the control group of healthy volunteers, hypercoagulation is recorded according to the parameters of thromboelastometry/-graphy, which may indicate a propensity for this group of patients to develop TO [8].

The data of scientific publications make it possible to quite fully characterize the indicators of the diagnostic accuracy of PCR analysis in detecting the SARS-CoV-2 virus and the factors influencing them. These are the timing of material sampling, with the maximum sensitivity of the test at 5-6 days after the onset of the first symptoms, the severity of the course of the disease, which correlates with the duration of detection of virus markers, the type of material being studied - a higher probability of finding the virus in bronchoalveolar lavage and sputum (during separation), compared with material from the nasopharynx and oropharynx, and low detectability in blood and urine. At the same time, even according to the most optimistic data, the diagnostic sensitivity of PCR does not exceed 90%. To date, the algorithm for diagnosing a new coronavirus infection includes instrumental (radiological) and laboratory research methods. From a clinical point of view, the results of CT, in combination with the relevant epidemiological history, can be used as a first and immediate guide for doctors to start treatment and take the necessary anti-epidemic measures, while PCR serves as a confirmation tool, its results can be used later to decide on the next steps (isolation, treatment). But at the same time, it should be noted that the health care of many countries is faced with a shortage of computed tomographs and qualified specialists, which makes this method inaccessible for full-scale research, in contrast to the laboratory molecular genetic test. PCR analysis is indispensable for examinations of contact persons, monitoring of morbidity. Thus, it is an integrated approach using PCR and CT, taking into account the factors affecting the accuracy of diagnosis, that makes it possible to obtain reliable results, correctly interpret them, which is necessary both for making a correct diagnosis for a particular patient and for obtaining objective data on the incidence of the population, timely decision-making on the necessary anti-epidemic and preventive measures.

The COVID-19 pandemic, which caused unprecedented changes in the way of life around the world, showed that the usual approach to assessing a case of a disease is insufficient and re-

quired the urgent development of effective diagnostic tests that allow to identify infected and sick people with high sensitivity and specificity, determine the stage of the disease, and also to confirm the cure, which is necessary both to limit the spread of infection and to conduct appropriate treatment of the diseased. Methods have been introduced into practice to detect the presence of coronavirus in various biological substrates, as well as to evaluate the immune response to infection. Each of these methods has its advantages and disadvantages, point of application, features of application and evaluation of results. There is no universal way to diagnose COVID-19. Physicians should carefully consider the pros and cons of each method and the results of studies should be interpreted taking into account the clinical picture of the disease and the epidemiological history. Further studies are required to assess the clinical relevance of the available methods. Of great importance is the use of a number of laboratory tests and biomarkers to objectively support the adoption of appropriate clinical decisions in the development of concomitant COVID-19 conditions and complications. First of all, this concerns methods for monitoring violations of the hemostasis system, as well as biomarkers of bacterial infection. The seasonal increase in the incidence due to acute respiratory infections in the autumn and winter period in the context of the COVID-19 pandemic will be expected to be associated with a certain contribution of the new coronavirus to the etiological structure of ARVI pathogens. In this regard, great hopes are placed on improving the methods of diagnosis and treatment of COVID-19, where laboratory tests will be of key importance.

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