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COVID-19 – A BRIEF REVIEW ON DIAGNOSIS, TREATMENT AND VACCINATION

COVID-19 - O SCURTĂ RECENZIE PRIVIND DIAGNOSTICUL, TRATAMENTUL ȘI VACCINAREA

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ABSTRACT | REZUMAT

COVID-19 is an infectious respiratory disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease is often characterized by flu-like symptoms, while severe cases are dominated by pulmonary complications. Additionally, the virus may cause diverse symptoms involving multiple organ systems. The main global focus of researchers is finding an effective therapeutic and prophylactic option. Current drugs are being repurposed for COVID-19 and new investigational agents are being studied as potential treatments. There are more than 100 vaccine candidates under development for COVID-19, some of them being in the final phase of the clinical trial. The aim of this literature review is to briefly summarize the currently available data on COVID-19 regarding the diagnosis, treatment and vaccination.

Keywords: COVID-19, SARS-CoV-2, review, pandemics

COVID-19 este o boală infecțioasă respiratorie cauzată de coronavirusul sindromului respirator acut sever 2 (SARS-CoV-2). Afecțiunea este deseori caracterizată de simptome asemănătoare gripei, în timp ce în cazurile severe sunt predominate complicațiile pulmonare. În plus, virusul poate cauza diverse simptome implicând diverse sisteme de organe. La nivel global, cercetătorii pun accent pe găsirea unui tratament eficace și a unei opțiuni profilactice. Medicamentele actuale sunt utilizate pentru COVID-19 și noi medicamente investigaționale sunt studiate ca și potențiale tratamente. Există mai mult de 100 de vaccinuri candidate contra COVID-19 care sunt în proces de dezvoltare, unele dintre ele fiind în faza finală a testelor clinice. Scopul acestei recenzii este de a rezuma informațiile disponibile în prezent privind diagnosticul, tratamentul și vaccinarea în cazul COVID-19.

Cuvinte cheie: COVID-19, SARS-CoV-2, recenzie, pandemii

In December 2019, healthcare workers from Wuhan, China reported a cluster of patients with pneumonia symptoms of unknown origins. A week later, SARS -CoV-2 was isolated from these patients and the disease called was officially named COVID-19 (13).

SARS-CoV-2 is the third coronavirus, together with SARS-CoV and MERS-CoV, which became a global health issue in the last twenty years. Compared to SARS-CoV and MERS-CoV, SARS-CoV 2 has a lower case fatality rate, but the infectivity and the speed of spread exceeds those of the other two coronaviruses (16). COVID-19 is characterized by the heterogeneous clinical manifestations ranging from mild, which are the most common, to critical. The disease poses a great challenge for the healthcare workers because of the lack of standard treatment, effective prophylaxis and pressure that severe cases put on the intensive care units.

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DIAGNOSIS

Physical findings

The clinical spectrum in most of COVID-19 cases is respiratory and varies from asymptomatic and paucisymptomatic forms to clinical severe conditions characterized by respiratory failure, to multiorgan and systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes (MODS) (17,31,36). From the first symptom to a possible death, there can be between 6 to 40 days, with an average of 14 days and the evolution is more severe for patients with a compromised immune system (28).

The proportion of individuals infected by SARS-CoV-2 who remain asymptomatic throughout the course of infection has not yet been definitely assessed (36). Individuals of all ages are involved in the SARS-CoV-2 asymptomatic infection.

In symptomatic patients, symptoms usually start after less than a week, consisting of fever, cough and dyspnoea (17,18,31),myalgia, fatigue and other signs of upper respiratory tract infections such as nasal congestion, sputum production, headache and haemopty-

sis may occur (17). The infection can progress to severe chest symptoms corresponding to pneumonia and pulmonary oedema. Pneumonia mostly occurs in the second or third week of a symptomatic infection in approximately 75% of patients (31,36). Gastrointestinal symptoms are present in some cases sometimes with severe distress (17).

WHO divided the clinical manifestations of COVID-19 by mild disease, moderate disease, severe disease and critical disease (46).

About 80% of patients develop mild, uncomplicated disease with no signs of pneumonia or moderate disease with clinical signs of mild pneumonia (6).

Severe disease with clinical signs of pneumonia was recorded in about 15 % of the patients and about 5% have critical disease with complications which can lead to death (6).

The most frequent symptom is fever which is associated with cough, severe dyspnoea, tachypnoea and hypoxia in adolescents and adults (4,31). Sometimes the fever symptom can be moderate or even absent even in severe forms of the disease (4). Cyanosis, cough and difficulty in breathing occur in children (4).

In critical cases with clinical signs of severe pneumonia the complications are such as: Acute Respiratory Distress Syndrome (ARDS), sepsis, which can be followed by septic shock and/or multiple organ dysfunction (MOD) or failure (MOF) after about a week (4,31). Different forms of ARDS are distinguished based on the degree of hypoxia (4,32). The diagnosis is made on clinical grounds and chest imaging (radiograph, CT scan or lung ultrasound) that show bilateral opacities, not fully explained by volume overload, lobar or lung collapse or nodules (4,32).

The clinical manifestation of patients with COVID-19 with sepsis is characterized by signs of organ dysfunction including altered mental status, severe dyspnoea, hypoxemia, renal impairment with reduced urine output, tachycardia, weak pulse, low blood pressure, skin mottling. Septic Shock is associated with increased mortality (27).

A growing number of clinical evidences showed that digestive system may serve as an alternative route of infection (diarrhoea, nausea, vomiting and abdominal discomfort).

Patients with severe COVID-19 seem to have higher rates of liver dysfunction. Mild to moderate liver injury including elevated aminotransferases, hypoproteinaemia and prothrombin time prolongation has been reported in the existing clinical investigations of COVID-19 (8). Recent single cell RNA sequencing data revealed a significant enrichment of ACE2 expression in cholangiocytes instead of hepatocytes, suggesting that SARS-CoV-2 might lead to direct damage of intrahepatic bile ducts (8).

SARS-CoV-2 and MERS-CoV had similar pathogenicity, causing myocarditis and heart failure increasing the difficulty of patient treatment (17).

Among the patients with severe symptoms of COVID-19, hypertension, heart disease and arrhythmia were reported. Patients with acute coronary syndrome (ACS) and COVID-19 have a poor prognosis (37). COVID-19 is also associated with mental and neurological manifestations, including encephalopathy, delirium, agitation, stroke, meningoencephalitis, impaired sense of smell or taste anxiety, depression and insomnia. In many cases, neurological manifestations have been reported even without respiratory symptoms (14).

Histological examination

Histological examination showed bilateral diffuse alveolar damage with cellular fibromyxoid exudates (39). The lung showed evident desquamation of pneumocytes and hyaline membrane formation, pulmonary oedema with hyaline membrane formation, indicating acute respiratory distress syndrome (ARDS) (39). Interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes, were observed. Multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were identified in the intra alveolar spaces, showing viral cytopathic-like changes (39). The liver biopsy specimens of the patient with COVID-19 showed moderate microvesicular steatosis and mild lobular and portal activity, indicating the injury could have been caused by either SARS -CoV-2 infection or drug-induced liver injury. In the heart tis-sue there were a few interstitial mononuclear inflammatory infiltrates (39).

Paraclinical examination

Prominent signs of viral pneumonia include decreased oxygen saturation, blood gas deviations, increasing and significant changes visible through chest X-rays and other imaging techniques, with ground glass abnormalities, patchy consolidation, alveolar exudates and interlobular involvement (36).

CT scans showed interstitial pneumonia, multiple, peripheral, patchy, and bilateral ground-glass opacities involving especially the lower lobes and consolidation in the bronchovascular and subpleural areas. The reticular pattern, due to lymphocyte infiltration in interstitial space (4), the paving pattern, due to interstitial inflammation of the lungs, multifocal or diffuse consolidation, due to fibrous exudate and pulmonary fibrosis appeared quite often in COVID-19 infection.

Laboratory findings of patients with COVID-19 show leukopenia with lymphopenia, decrease of CD4+ T-cell levels, and elevated inflammatory markers such as C-reactive protein or proinflammatory cytokines (36, 42). In sepsis, functional alterations of organs expressed by hyperbilirubinemia, acidosis, coagulopathy

and thrombocytopenia were reported. This scenario was associated with increased mortality (4).

METHODS OF DIAGNOSIS

Molecular diagnosis

The molecular technique is up-to-now worldwide applied, and it was nominated as nucleic acid amplification tests (NAAT) for COVID-19 virus. Real-time reverse transcription-polymerase chain reaction (rRT-PCR) represented the standard molecular assay used for SARS-CoV-2 detection. The standardized protocol had been documented by WHO and it was available online since the 17th of January 2020 (46).

The molecular protocol is based on identification of unique sequences of SARS-CoV-2 RNA using rRT-PCR and, when necessary, the conformation by nucleic acid sequencing. The targeted sequences of viral genome include the N, E, S and RdRP genes, according to WHO recommendations (46).

The rRT-PCR was selected by WHO as a unique reliable technique for SARS-CoV-2 virus determination because it presents a series of advantages, among them the most important being the possibility of quantitative determination and amplification of the DNA transcript obtained even in the presence of virus traces in the respiratory tract. More than over rRT-PCR presents a high sensitivity, being thus useful in the diagnosis of incipient infections (46).

Based on recommendations of the WHO on SARS and MERS there are several categories of patients candidates for SARS-CoV-2 testing by rRT-PCR, being enframed in different categories, such as: patients with one exposure history and two clinical conditions is considered as suspected case; patients with no clear exposure history in this case the suspected patients should meet 3 clinical conditions; patients with chest CT findings of viral pneumonia.

The molecular detection techniques are mainly applied on respiratory samples. According to WHO protocols the samples are upper respiratory specimens (nasopharyngeal and oropharyngeal swab or wash in ambulatory patients) (46) and/or lower respiratory specimens, such as: sputum (if produced) and/or endotracheal aspirate or broncho-alveolar lavage in patients with more severe respiratory disease (46). In some cases, stools, whole blood, urine, and if diseased, material from autopsy should be also considered and included in molecular testing (46).

One or more negative results at RT-PCR do not rule out the possibility of SARS-CoV-2 infection. RT-PCR technique can present false negative results in infected individuals due to the poor quality of the specimen, the sampling technique (not enough experienced personnel qualified for sampling), and the timing (the sample was collected too late or too early) according to the infectious status (31,38). The disadvantages of molecular technique are represented by the limited number of samples which can be processed per each batch and the relatively long time until the results are obtained (24-48 hours) (46).

In order to overcome the current time consuming and laborious RT-qPCR technique there are several new proposals of alternative molecular amplification techniques. LAMP reaction - loop-mediated isothermal amplification, amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions (24). The proposed method uses four specially designed primers and a DNA polymerase with strand displacement activity. The targeted DNA is synthetized in up to 109 copies in less than 1 hour at a constant temperature of 65°C. The final product is having a cauliflower appearance being formed by stem-loop DNAs with multiple inverted repeats of the target (24).

Serological diagnosis

There are serological assays available on the market and in use in different laboratories, but according to WHO recommendations these types of tests can be applied for surveillance and research (46).

The serological tests used for determinations of antibody levels against the SARS-CoV-2 are EIA (enzyme immunoassay) (34) and ELISA (enzyme-linked immunosorbent assay) (43). EIA kits are used for detection of IgM and IgG anti-SARS-CoV-2 antibodies internal nucleoprotein (NP) and surface spike protein receptor binding domain (RBD) (34). ELISA kits are developed to detect IgM and IgG anti-SARS-CoV antibodies SARS-CoV Rp3 NP (43).

Radiological diagnosis

CT scan examinations were also proposed for COVID-19 diagnosis (19,30,45). They found out that COVID-19 pneumonia has nonspecific and various chest CT imaging features. The typical chest CT includes multifocal bilateral GGOs with various patchy consolidations, peripherally subpleural distribution mainly in posterior part or lower lobe. The lesions were unilateral in the beginning and then evolved bilaterally. Due to these findings, the chest CT is used as a support diagnosis, combined with molecular techniques for confirmation of SARS-CoV-2 infection.

Differential diagnosis

The symptoms of the early stages of the disease are nonspecific. Differential diagnosis should include the possibility of a wide range of infectious and non-infectious (e.g., vasculitis, dermatomyositis) common respiratory disorders such as Adenovirus, Influenza, Human metapneumovirus (HmPV), Parainfluenza, Respiratory syncytial virus (RSV) or Rhinovirus (common cold). For suspected cases, rapid antigen detec-

tion, and other investigations should be adopted for evaluating common respiratory pathogens and non-infectious conditions (4).

TREATMENT

According to European Medicines Agency (EMA) (12), there is only one drug authorized for the treatment of COVID-19 infected patients. On the other hand, the U.S. Food and Drug Administration (FDA) (5) has not yet approved any drugs for treatment or prevention. However, there are numerous ongoing clinical trials that are evaluating potential treatments. As a result, healthcare professionals focus on symptomatic treatment and meticulous supportive care in order to provide a better outcome for patients infected with COVID-19.

Nucleotide analogs - remdesivir

Remdesivir (or GS-5734) is a nucleoside analogue which acts on RNA viruses by cancelling the viral transcription through inhibition of RNA polymerase (20). It has shown broad-spectrum antiviral activity in vitro against coronaviruses, including SARS-CoV, MERS-CoV and bat coronaviruses. Early administration of remdesivir was proved to reduce the viral load in lungs and improve the clinical signs in SARS-CoV mouse models (29). One double-blind, randomized, placebo-controlled trial conducted on 1062 patients infected with COVID-19, showed that treatment with remdesivir shortened the time to recovery in hospitalized patients (2). Remdesivir is currently the only drug authorized by EMA for the treatment of COVID-19 (12).

Protease inhibitors - lopinavir/ritonavir

Lopinavir / ritonavir is a drug combination currently used in the treatment and prevention of HIV. Lopinavir acts as an antiretroviral protease inhibitor, while ritonavir, a CYP3A4 inhibitor, increases the concentrations of lopinavir (23). A previous study showed that combination of lopinavir/ritonavir and interferon β1b administered to MERS-CoV non-human primate models improved the clinical outcomes and lowered the mean viral loads in necropsied lungs and extrapulmonary tissues (9). According to a clinical trial conducted on 1616 COVID-19 patients, the treatment with lopinavir / ritonavir showed no significant results in the duration of hospital stay, reduction of 28-day mortality and risk of progressing to invasive mechanical ventilation (15).

Antimalarials – Chloroquine, Hydroxychloroquine

Chloroquine and its analogue, hydroxychloroquine, are drugs mainly used for the treatment of malaria, but they also showed benefits in extraintestinal amoebiasis, immunological and dermatological diseases (1). Chloroquine was proven to possess antiviral activity against both RNA and DNA viruses (11). Hydroxychloroquine is preferred to chloroquine as it is less toxic and produces better inhibition of SARS-CoV-2 (26). Based on a clinical trial conducted in the United Kingdom on approximately 15% of the patients who were hospitalized with COVID-19, hydroxychloroquine treatment did not show a significant difference in lowering the incidence of death at 28 days, compared to patients who received usual care (33).

Convalescent plasma therapy

Convalescent plasma therapy has been viewed as a potential treatment since the beginning of the SARS-CoV-2 pandemic. This type of therapy involves drawing blood from patients who were sick and recovered from COVID-19, and identifying high titters of neutralizing antibodies. The plasma is administered after the onset of clinical signs, when it is most effective. Convalescent plasma may also be used for prophylaxis, to prevent infection in high-risk cases and medical staff (3). There are also risks that come with this type of therapy, such as immune-mediated reactions, microbial transmission and citrate toxicity (21).

Convalescent plasma has previously been tested in patients suffering from severe acute respiratory infections. More specifically, a systematic review revealed that convalescent plasma may had reduced mortality when administered to patients suffering from SARS-CoV or severe influenza (22). Current results on the use of convalescent patients in COVID-19 infected patients are inconclusive in regards to the improvement of clinical symptoms and decrease of mortality (7).

VACCINATION

Vaccines are the most effective tool in fighting infectious diseases. They are a cost-effective solution and they successfully reduce morbidity and mortality while having a safer profile than conventional drugs (40). Whole virus vaccines are a classic approach when it comes to creating a solid immunity against microbes. Such vaccines are being developed by Johnson & Johnson, University of Hong Kong and Codagenix, but they require additional safety testing compared to other vaccine types (10).

Another type of vaccine is the mRNA vaccine. As this is a relatively novel technology there are no mRNA vaccines on the market, although they are characterized by low-cost manufacturing, high potency and safe administration (25). Several pharmaceutical companies are researching this type of vaccine against SARS-CoV-2, including Stemirna Therapeutics Inc, eTheRNA Immunotherapies NV, Curevac AG and others. Moderna Inc began the phase 3 study on mRNA-

1273 vaccine in July, in order to assess its efficacy (47). Several institutions have started research on subunit vaccines against SARS-CoV-2 and they mainly aim to use the S protein as antigen. Although subunit vaccines are generally easy to produce and safe, they require adjuvants to provide a strong immune response (41). Viral vectored vaccines are also the focus of researchers. Compared to the subunit vaccines, which induce a humoral response, viral vectored vaccines induce a cytotoxic T lymphocyte response with potential for therapeutic use (35). The first results of a phase 2 trial which tested recombinant adenovirus type-5 vectored COVID-19 vaccine shows that it is safe and it induces significant immune response (44).

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