J Bras Patol Med Lab. 2020; 56: 1-9.

10.5935/1676-2444.20200049

COVID-19: clinical and laboratory manifestations in novel coronavirus infection

COVID-19: manifestações clínicas e laboratoriais na infecção pelo novo coronavírus

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ABSTRACT

COVID-19 is a highly contagious disease caused by the coronavirus of severe acute respiratory syndrome 2 (SARS-CoV-2). In 2020, due to the outbreak, it was considered by the World Health Organization (WHO) as a pandemic. The infection caused by the novel coronavirus has high mortality in a small portion of the infected population, especially in elderly, immunosuppressed, diabetic, cardiac, and hypertensive individuals. Many infected are asymptomatic (and may be carriers) or present mild or moderate flu-like symptoms. The most severe clinical picture of COVID-19 is characterized by an inflammatory cytokine storm, with hematological changes and coagulation dysfunction, which can lead to tissue damage and death. Nonspecific laboratory biomarkers may be either increased or decreased as the course of the disease progresses and are often useful in predicting complications of the disease, such as the use of D-dimer and platelet/lymphocyte ratio. Specific laboratory diagnosis is based on the detection of viral ribonucleic acid (RNA) by real-time polymerase chain reaction (RT-PCR) of nasal and oropharyngeal swab samples; it is more effective when performed in the first days after symptom onset. Serological tests are useful in detecting the immune response, since both class M (IgM) and class G (IgG) immunoglobulin antibodies can be detected seven days after the onset of clinical symptoms, and may extend for more than 25 days, although not exempting the individual from remaining infectious, depending on their viral load and clinical presentation. The rational use of specific laboratory markers must respect the disease chronology, and the correct interpretation may provide subsidies for a better management of affected patients, as well as identifying asymptomatic carriers or those with mild symptoms.

Key words: coronavirus infections; SARS virus; immunologic tests; reverse transcriptase-polymerase chain reaction; biomarkers; clinical pathology.

RESUMO

COVID-19 é uma doença altamente contagiosa provocada pelo coronavírus da síndrome respiratória aguda grave 2 (SARS-CoV-2). Em 2020, devido ao surto, foi caracterizada pela Organização Mundial da Saúde (OMS) como pandemia. A infecção causada pelo novo coronavírus tem alta mortalidade em uma pequena parcela da população infectada, especialmente em indivíduos idosos, imunodeprimidos, diabéticos, cardiopatas e hipertensos. Muitos infectados são assintomáticos (e podem ser portadores) ou apresentam sintomas leves a moderados, semelhantes ao estado gripal. O quadro clínico da COVID-19 na forma mais severa é caracterizado por uma tempestade inflamatória de citocinas, com alterações hematológicas e da coagulação que podem levar ao dano tecidual e morte. Exames laboratoriais inespecíficos podem apresentar-se mais elevados ou diminuídos conforme o curso da doença, e muitas vezes são úteis na predição de complicações, como o uso do D-dímero e a razão plaqueta/linfócitos. O diagnóstico laboratorial específico se baseia na detecção do ácido ribonucleico (RNA) viral por reação em cadeia da polimerase em tempo real (RT-PCR) de amostras de suabe nasal e orofaríngeo; é mais efetivo nos primeiros dias após o início dos sintomas. Testes sorológicos são úteis na detecção da resposta imune, pois tanto os anticorpos da imunoglobulina da classe M (IgM) quanto da classe G (IgG) podem ser detectados após sete dias do início dos sintomas clínicos, podendo se estender por mais de 25 dias, embora não isente o indivíduo de continuar infectante, dependendo de sua carga viral e apresentação clínica. O uso racional

First submission on 05/15/20; last submission on 05/16/20; accepted for publication on 05/16/20; published on 06/09/20

dos marcadores laboratoriais específicos deve respeitar a cronologia da doença, e a interpretação correta pode fornecer subsídios para um melhor manejo dos pacientes acometidos, bem como identificar portadores assintomáticos ou com pouco sintomas.

Unitermos: *infecções por coronavirus; vírus da SARS; testes imunológicos; reação em cadeia da polimerase via transcriptase reversa; biomarcadores; patologia clínica.*

RESUMEN

La COVID-19 es una enfermedad altamente contagiosa causada por el coronavirus del síndrome respiratorio agudo grave (SARS-CoV-2). En 2002, a causa del brote, fue reconocida como una pandemia por la Organización Mundial de la Salud (OMS). La infección por el nuevo coronavirus provoca alta mortalidad en una pequeña parcela de la población infectada, especialmente en ancianos, pacientes inmunodeprimidos, diabéticos, cardiópatas e hipertensos. Muchos infectados son asintomáticos (y pueden ser portadores) o presentan síntomas leves a moderados, como en un estado gripal. El cuadro clínico de la COVID-19 en la forma más grave es caracterizado por una tormenta inflamatoria de citoquinas, con cambios hematológicos y de la coagulación que pueden llevar a daño tisular y muerte. Pruebas de laboratorio inespecíficas pueden presentar tasas más altas o bajas según el curso de la enfermedad, y muchas veces son útiles en la predicción de complicaciones, como el uso del dímero D y la ratio plaquetas/linfocitos. El diagnóstico de laboratorio específico se basa en la detección del ácido ribonucleico (ARN) viral por reacción en cadena de la polimerasa (PCR) en tiempo real de muestras de hisopado nasal y orofaríngeo; es más efectiva en los primeros días tras el inicio de los síntomas. Pruebas serológicas son útiles para detectar la respuesta inmune, pues tanto los anticuerpos de la inmunoglobulina M (IgM) como de la G (IgG) pueden se detectar siete días después del inicio de los síntomas clínicos, y pueden permanecer por más de 25 días, aunque no eximen al individuo de seguir infeccioso, dependiendo de su carga viral y presentación clínica. El uso racional de los marcadores de laboratorio específicos debe respetar la cronología de la enfermedad, y la interpretación correcta puede proporcionar recursos para un mejor manejo de los pacientes afectados, así como identificar portadores asintomáticos o con pocos síntomas.

Palabras clave: *infecciones por coronavirus; virus del SRAS; pruebas inmunológicas; reacción en cadena de la polimerasa de transcriptasa inversa; biomarcadores; patología clínica.*

INTRODUCTION AND DEFINITION

Coronaviruses (CoVs) are enveloped viruses with 60 to 130 nm diameter, that contain a positive sense, single-stranded ribonucleic acid (RNA) genome, with genome size ranging from 26 to 32 kilobases (Kb) in length[1, 2]. This virus can present pleomorphic capsids and have corona or crown-like surface projections, hence the name coronavirus[2].

The Novel Coronavirus, order *Nidovirales*, family *Coronaviridae*, subfamily *Orthocoronavirinae*, was named severe acute respiratory syndrome – coronaviruses-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV), and the World Health Organization (WHO) announced COVID-19 (coronavirus disease) as the name of this new disease on February 2020[1, 3, 4].

Coronaviruses can be divided into three groups according to genetic and antigenic criteria: α-CoVs, ß-CoVs, and γ-CoVs. Coronaviruses infect mainly birds e mammalian, being α- and ß-CoVs also capable of infecting mammalians and, therefore, may cause infection in humans[5]. The infection mainly affects the superior respiratory tract similar to a simple cold but it may compromise the inferior respiratory tract causing severe acute respiratory syndrome (SARS)[5].

Another coronaviruses species cause mild respiratory diseases in humans (HCoV.HKV1, HCoV-OC43, HCoV-NL63, and HCoV-229E)[4, 6]. Six coronaviruses are known to infect humans, but two of them are capable of causing SARS with worst prognosis: SARS-CoV and middle east respiratory syndrome coronaviruses (MERS-CoV), which have led to outbreaks in China and the Middle East, respectively[4, 7].

Some CoVs were originally discovered as a cause of limited enzootic infections only in animals but progressed to establish zoonotic disease in humans. Due to the similarity of 96.2% RNA with the coronavirus that infects Chinese horseshoe bats

(*Rhinolophus* sp.), this mammal is thought to be the host of origin of the novel coronavirus. In addition to having a 79.5% similarity with the genetic code of SARS-CoV and exerts the same molecular mechanism of infection observed in human COVID-19, which involves binding to the angiotensin-converting enzyme 2 (ACE-2), acting as a cell receptor for the virus[5, 7].

The first reports of SARS appeared in November 2002, in Southern China – Guangdong Province, but only in February of the following year, 305 cases and five deaths from the still unknown pneumonia, were reported. The disease spread mainly among health professionals, who disseminated it into their homes, and China's Ministry of Health reported that it was an unknown and atypical pneumonia outbreak. Countries such as Vietnam, Hong Kong, Singapore, and Canada had also been affected by this unknown syndrome. Due to the spread of this atypical syndrome, several countries and laboratories gathered for a multicentric research collaboration on search for the etiological agent of the infection, performing laboratory tests for its identification[8].

The Centers for Diseases Control on Prevention (CDC) from the United States of America (USA) and Hong Kong, in March 2003, isolated this coronavirus from patients with SARS. They observed that this new virus was phylogenetically different from the other organisms in the human disease-causing group. In April 2003, WHO proposed the implementation of a global surveillance system to prevent international dissemination. The tests used to identify this new causative agent of the disease included polymerase chain reaction (PCR), cell culture, and electron microscopy studies. After the description of this virus, it was called a new coronavirus, SARS-CoV, which causes SARS[9].

In December 2019 and January 2020, cases of atypical pneumonia (COVID-19) occurred in Wuhan, China[10]. These patients had tested negative for real-time PCR (RT-PCR) from bronchoalveolar lavage for SARS-CoV. However, the virus found in this samples underwent phylogenetic and recombination analyses, which revealed a 99.9% similarity to the *Coronaviruses* family[11], being a *β-Coronaviruses* of the subgenre *Sarbecoviruses*, in the SARS-like coronaviruses group (SL-CoVs), in whom the horseshoe bat (*Rhinolophus* sp.) is a reservoir[12].

It is hypothesized that the first cases of COVID-19 were associated with a free-seafood market in Wuhan, where contaminated patients used to consume food. It is assumed that the mechanism of transmission occurred from animal to human. However, the manifestation of other cases was due to human-to-human interactions[2], and that transmission may occur before the onset of symptoms of the disease by asymptomatic individuals[13].

It is crucial to notice that the risk of symptomatic infection increases with age; thus, young individuals and children may be asymptomatic carriers[14]. This reinforces the importance of self-isolation and measures of hygiene, even in the absence of clinical manifestations. The transmission of viral particles between individuals is related to the viral load in the upper respiratory tract[15], and it may occur through droplets from coughing, sneezing and saliva, handshake, or fomites, such as cellphones, door handles, cups, and keys, with subsequent contact with mucous membranes[12, 16, 17].

Some studies have shown that the temporal viral viability differs in different materials, such as plastic and metal. Viral particles may remain viable from a few hours up to nine days, and viral viability depends not only on the type of material but also on physicochemical factors, as temperature and pH. Thus, disinfection with 0.1% sodium hypochlorite or 70% alcohol (62%-91%) significantly reduces the number of infecting viral particles on such surfaces[17, 18].

Incubation period after exposure to infection can range from two to 14 days[10]. The first patients reported common prodromal symptoms of infection such as fever, cough, fatigue, myalgias. These may be accompanied by respiratory secretions, headache, hemoptysis, and diarrhea, and the complications of infection may lead to SARS and cardiac or renal impairment, secondary infection, and shock[19]. Mortality is significant in the elderly, mainly over the age of 80 years[20, 21]. Death rates are related to critical cases and the presence of comorbidities as heart diseases, hypertension, diabetes, chronic respiratory diseases, and neoplasia[20, 22].

After the detection of this new etiologic agent, named COVID-19/2019nCoV, the number of contaminated individuals in China grew exponentially, taking a large proportion in other countries, the disease was considered as a pandemic by the WHO in March 2020. More than four million cases and over 283 thousand related deaths have been reported worldwide as of May 13, 2020[16, 23, 24]. In Brazil, the first case was confirmed in São Paulo city on February 26, 2020, and the disease has spread across the country, reaching over 177 thousand notified cases and 12 thousand related deaths as of May 12, 2020, and, on March 26, 2020, the community transmission was declared in all national territory[16, 23-25]. The transmission rate has grown exponentially daily.

Based on the patterns of transmission visualized in China, after COVID-19 detection, dynamic epidemiological models have been proposed[26-28]. According to WHO, the number of secondary infections generated from an affected individual is between 2% and 2.5% for COVID-19, and the worldwide crude mortality rate was around 3%-4% (April 2020), reaching 6.3% (May 2020), and is related to quality access to health care[25, 29].

CLINICAL MANIFESTATIONS

SARS-CoV-2 infection may present clinically as one of these three main pictures: asymptomatic carriers, individuals with acute respiratory disease (ARD), or patients with pneumonia in different degrees of severity[30].

In the largest disease epicenters, the first cases arose from asymptomatic individuals with confirmed laboratory diagnosis, but such screening of asymptomatics through molecular tests proved complex since the conduct in suspected cases varies across different countries. This challenge becomes even more considerable in the observation of pediatric cases, which contribute to a large portion of the asymptomatic carriers, requiring heightened attention to avoid transmission[23, 31]. However, symptoms are more evident in patients with positive molecular tests and who have respiratory manifestations and imaging compatible with the diagnosis of pneumonia. Clinical records from patients at the beginning of the infection indicate that the most common symptoms of infection are fever, cough, myalgia, and fatigue, and may also be accompanied by respiratory secretion, headache, hemoptysis, and diarrhea[16, 19, 23].

Some of the initial symptoms may resemble those of other viral respiratory infections, such as *Noroviruses* and *Influenza*. Dyspnea and high fever are symptoms that define the main clinical difference between COVID-19 and the common cold, which is accompanied by nasal congestion, lacrimation, sneezing, and coryza, initially hyaline that over the days becomes greenish-yellow. On the other hand, when compared to *Influenza* infection, COVID-19 presents with similar clinic symptoms, but with a higher proportion of evolutions to severe and critical infections, demanding oxygen therapy and ventilatory support[32, 33].

Different studies show that about 86% of patients do not present disease severity, only about 14% require oxygen therapy in a hospital unit, and less than 5% of this group require intensive care[34]. Lu *et al*. (2020)[35] performed a meta-analysis involving the clinical presentation of patients from different studies. The main symptoms were: fever (88.3%); cough (68.6%); myalgia or fatigue (35.8%); expectoration (23.2%); dyspnea (21.9%); headache or dizziness (12.1%); diarrhea (4.8%); and vomiting or nausea (3.9%).

Most patients progress with a good prognosis, and it is essential to highlight that in the elderly or individuals with previous comorbidities, such as diabetes, cardiovascular and kidney diseases, COVID-19 can progress more aggressively, with pneumonia and acute respiratory distress syndrome (ARDS) being observed accompanied by cardiac, hepatic and kidney dysfunction[5, 36].

Patients with signs and symptoms such as high fever, tachypnea, and dyspnea, and clinical indications of the severity, require closer attention from the medical care team[23]. The progression of the first symptoms from COVID-19 to sepsis is slow, and extrapulmonary involvement is characterized mainly by refractory heart failure and kidney damage, leading about 25% of such critically ill patients to renal replacement therapy[28, 37]. The severe evolution to sepsis and septic shock is also cited in the literature, and it can reach an incidence rate 50% higher in critically ill patients, as described by Zhou *et al*. (2020)[38], demonstrating the intrinsic capacity of SARS-CoV-2 to lead to sepsis, especially when worsened by secondary infections.

Thus, the use of screening tools such as Sequential Organ Failure Assessment (SOFA) helps in the early diagnosis and management of patient's condition at hospital admission, favoring the improvement of clinical outcomes[38, 39]. In a previous study, the SOFA score on the first day of hospitalization of patients who did not survive was on average six points (range 4-8), while patients who survived presented a mean of four (range 3-4), evidencing the predictive potential of mortality of nonspecific, yet highly presumptive, clinical and laboratory criteria that compose this index[28].

Although less described in the reported cases, something that has been gaining prominence is the presence of hyposmia/anosmia and hypogeusia/ageusia in patients without rhinorrhea or nasal congestion, raising the possibility of direct neurological impairment by SARS-CoV-2, although more studies are necessary for such statement[40].

The estimated frequency of the symptoms and the evolution of COVID-19 in the general population is illustrated in **Figure 1**.

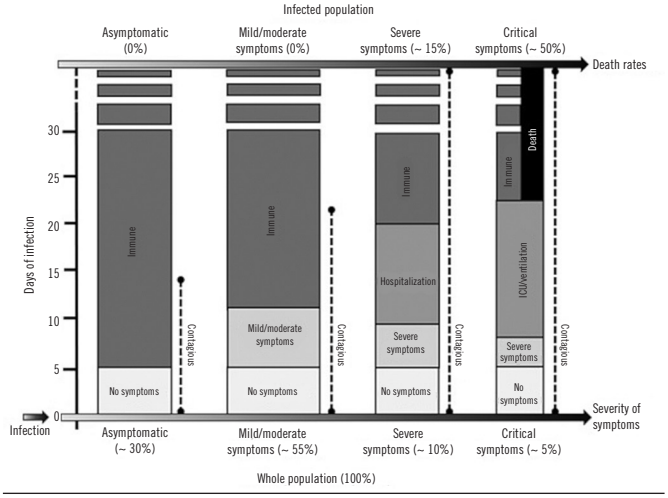

FIGURE 1 – *Symptoms and evolution of COVID-19*
Source: based in Guan WJ, et al.*, 2020[41]; adapted from DASA, 2020[42].*
ICU: intensive care units.

LABORATORY DIAGNOSIS – NONSPECIFIC AND SPECIFIC EXAMS

The clinical laboratory is an important and essential tool for the diagnosis, follow-up, and evolution, as well as in the prognosis of any pathology that is active or not. In the COVID-19 pandemic, the involvement of several biomarkers as indicators of the current state of the disease has been reported, while others have proved to be useful prognostic markers. General laboratory findings in SARS-CoV-2 infection sometimes indicate leukocytosis or leukopenia, with marked lymphopenia in the early stages of the disease, as well as the presence of neutrophilia, that has been related to an unfavorable prognosis[26, 41, 43].

The most frequent laboratory alterations in patients with COVID-19 are the 75%-93% increase in C-reactive protein (CRP) with 50%-98% decreased serum albumin, and total leukocytes count with considerable variation as reported in the literature, sometimes appearing increased, sometimes decreased, but with the evident presence of lymphopenia (35%-75%). There is also a decrease in hemoglobin, around 41% to 50%, and increase of erythrocyte sedimentation rate (ESR) by 15% to 85%, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) around 8% to 37%, and lactate dehydrogenase (LDH) in approximately 12%[44].

Infection-related biomarkers values, including CRP, procalcitonin (PCT) and ESR increase gradually as the clinical status deteriorates, as well as increased D-dimer, creatine kinase (CK), creatine kinase-MB fraction (CK-MB), LDH, ALT, AST, urea, creatinine, cardiac troponin, and serum amyloid A protein (SAA)[11, 44-48].

Because it is part of the inflammatory cascade involved in the pathogenesis of COVID-19, serum interleukin-6 (IL-6) is also increased according to the progression of SARS[49]. In the first week, the disease may progress to pneumonia, respiratory disease, and even death. This progression is associated with the extreme increase of inflammatory cytokines, including interleukins IL-2, IL-7, IL-10, granulocytes colony stimulating factor (GCSF), interferon gamma-induced protein of 10 KDa (IP-10), monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein 1α (MIP-1α), and transforming growth factor α (TGFα)[50]. In intensive care units (ICU), patients have shown high plasma levels of these cytokines (IL-2, IL-7, IL-10, GCSF, IP-10, MIP-1α, and TGFα)[5]. Laboratory characteristics of COVID-19 in ICU patients include lymphopenia with CD4 and CD8 lymphocyte depletion, prolonged prothrombin time (PT), LDH, D-dimer, PCR, transaminases, and increased cytokines – and such increases are higher than those found in patients not admitted to an ICU. Laboratory abnormalities were similar to those previously observed in patients with SARS-CoV and MERS-CoV infection[47].

Another proposed systemic marker is the platelet/lymphocyte ratio (PLR), whose prognostic value was associated with length of hospital stay and the case outcomes[51]. In severe cases, the reduction of partial oxygen pressure (PaO_2) and [PaO_2/oxygen inspired fraction (FiO_2)] ratio has also been observed[47, 48]. Besides, an innovative parameter called monocyte distribution width (MDW) was significantly increased in all patients with COVID-19, especially in those in worse clinical conditions. For prognostic purposes, it is observed that the values of LDH, AST, ALT, total bilirubin, creatinine, cardiac troponin, D-dimer, PT, PCT, and CRP are increased while serum albumin is decreased[44]. Serum PCT quantification with serial titrations has been used to indicate bacterial coinfection in patients with COVID-19. Coinfection leads to greater severity to the patient's condition, and PCT concentration may indicate the most appropriate therapeutic options early on, but more studies are needed to support this approach[52, 53].

Several articles have proposed possible correlations between laboratory findings and the potential severity of COVID-19, such as the relationship between hypoalbuminemia, lymphopenia, high levels of CRP, D-dimer, LDH and higher occurrence of SARS, as well as a higher viral load is related to greater disease severity, but these pieces of information and correlations are not uniform, requiring even more significant evidence for its clinical use[5, 35, 37, 38].

The viral nucleic acid detection test is the main technique for laboratory diagnosis and is essential in the current context of the pandemic by the novel coronavirus. The use of RT-PCR is considered the gold standard for viral identification in patients with clinical symptoms in the acute phase of the disease, being part of different screening protocols in suspected cases[3, 54-56]. The RT-PCR method is based on the amplification of nucleic acids, and the samples for this analysis need to come mainly from nasal and oropharyngeal swabs, or sputum, tracheal secretion, and bronchoalveolar lavage in critically ill patients[3]. However, implementing RT-PCR in the protocol requires an improved and adequate technological structure for its performance, besides having pre-analytical interfering factors such as sample collection, transportation, and storage, which, when performed inappropriately, may result in errors in the laboratory analysis[3, 54, 56].

Regarding the laboratory testing for the evaluation of suspected cases with a probability of infection or an epidemiological detection of individuals who have had contact with a confirmed case of COVID-19, RT-PCR should be used, even if the individuals are asymptomatic or oligosymptomatic. When

the results are conflicting, a new sample should be collected and from this material, performed either the sequencing of the viral RNA or the amplification of the genetic material using the nucleic acid amplification test (NAAT), thus providing a reliable result[3, 56].

The Brazilian Ministry of Health recommends the RT-PCR technique to be performed between days three and nine after the onset of the symptoms because, in this phase, the highest viral load can be found. Nevertheless, the sample collection can be acceptable if performed up until the 10th day. Studies show that after the 7th day, RT-PCR positivity begins to drop, reaching 45% between days 15 to 39 after the onset of the symptoms (**Figure 2**). Due to the high sensitivity and specificity of this technique, positive results confirm the infection in the epidemiological presence of the pandemic, requiring no further complementary investigations besides positive laboratory results in the presence of signs and symptoms of the disease. False-negative results are possible since due to several factors, such as sample collection at a very early stage, improper samples collection, storage, or transportation, as well as for reasons inherent to the test, such as viral mutations or the presence of inhibitory factors for the PCR method[57-59].

Immunological tests are still under development and validation to allow their insertion as a reliable diagnostic tool and easier to use dynamically as the pandemic goes on. Individual factors regarding immunological response depend on both the patient and the characteristics of the antigen used, and the surge of antibodies may be early or late, depending on the patient (Figure 2). The scientific literature discussing the immune response to the new coronavirus is still quite limited. Reports indicate that immunoglobulin class A (IgA) and class M (IgM) antibodies have an average serological detection around the 7th day after the onset of symptoms, followed by a rise in immunoglobulin class G (IgG) levels, concurrently. The negative predictive value of these tests is low when performed in the acute phase of the disease, which means that the negative results do not rule out the disease, and these individuals may be infected and are, therefore, a source of contamination to other people. In relation to positive test results, these show high predictive values[3, 16, 54, 57-59].

Several studies have indicated the role and characteristics of serological analysis in SARS-CoV-2 infection. According to some authors, the production of IgM and IgA against the antigen protein S (viral spicule protein) and N (structural helical nucleocapsid protein) occurs on average seven days after the onset of symptoms, and soon after it, IgG can be detected almost simultaneously to the IgM, but there is not necessarily a decrease in viral load. These individuals may still be infected and potentially spreading the virus[52, 54, 60].

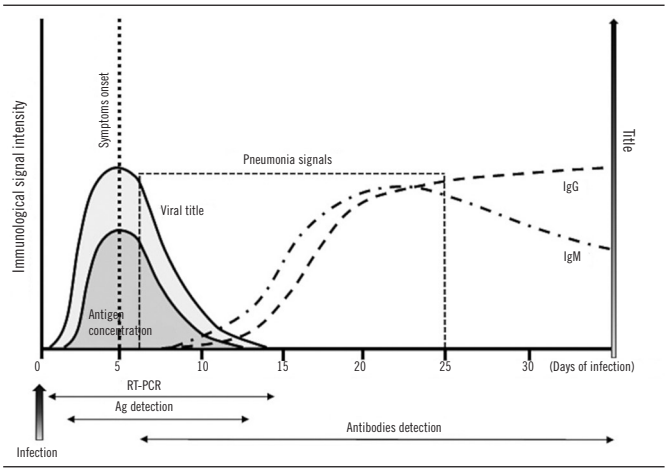

FIGURE 2 – *Clinical and laboratory evolution of COVID-19*
Source: adapted from Sethuraman N, et al.*, 2020[61].*
RT-PCR: real-time polymerase chain reaction; IgM: immunoglobulin class M; IgG: immunoglobulin class G; Ag: antigen.

In general, immunological tests should not play a role in the screening or diagnosis of patients with a recent clinical presentation, and their use is intended for hospitalized patients with a not so recent condition and the diagnosis of health professionals (symptomatic from the 7th day of symptom onset) to facilitate the dynamics of leaving and returning to the healthcare workforce. However, it should be clear that negative results do not exclude the disease[54, 60].

The **Table** below establishes a test sensitivity pattern after the onset of symptoms.

TABLE – Sensitivity tests in relation to the days after the onset of symptoms.

SARS-CoV-2 test	Day after the onset of symptoms		
	1-7	8-14	15-36
RNA by RT-PCR	67%	54%	45%
Total antibody	38%	90%	100%
IgM	29%	73%	94%
IgG	19%	54%	80%

Source: adapted from Zhao J, et al.*, 2020[59].*
SARS-CoV-2: acute respiratory syndrome – coronaviruses-2; RNA; ribonucleic acid; RT-PCR: real-time polymerase chain reaction; IgM: immunoglobulin class M; IgG: immunoglobulin class G.

FINAL CONSIDERATIONS

COVID-19 is a highly contagious disease caused by the novel coronavirus SARS-CoV-2, and it was considered a world pandemic by WHO in 2020. This disease can lead to potentially fatal consequences among susceptible individuals. The rate of fatal cases ranges globally and is near to 6.9% between diagnosed individuals as of early May 2020, and it varies depending on factors such as age, immunological status, social and hygiene conditions, and accessibility to health

care[62]. It is important to account for underdiagnosis and underreporting, especially in lower-income countries, which may inflate the mortality rate and skew the distribution curve according to how much testing a given country has performed.

SARS-CoV-2 spreading occurs most frequently from mild/moderated symptomatic or asymptomatic individuals, who may unknowingly transmit the virus[30]. A smaller proportion of infected patients has a severe presentation of COVID-19, but in this population, the mortality is higher than in the clinically milder counterparts[30, 62].

Initial clinical manifestations resemble *Norovirus* and *Influenza* infections, but pulmonary involvement is similar to that of complicated influenza H_1N_1, SARS, and MERS-CoV infections[23, 32, 33]. Many unspecific laboratory parameters are altered during the COVID-19 course of infection, but some have shown prognostic value to monitor the disease evolution, revealing additional value beyond the presence of SARS-CoV-2 or the clinical diagnosis or immunological status assessment in specific exams. Thus, laboratory analysis has a fundamental role in defining diagnosis, assessing development, and more accurately predicting prognosis of COVID-19 patients. Additionally and importantly, laboratory investigations are on the basis of epidemiological studies that drive effective healthcare-related governmental strategies and evidence-based medical decisions.

RT-PCR positivity and seroconversion may vary in different groups of infected individuals, including the large population of asymptomatic individuals, where there can be underdiagnosed and underreported cases. Despite several and timely articles about COVID-19 and its pathophysiological mechanisms, many questions remain unanswered, particularly about the long-term potential immunity patterns in individuals with different clinical and laboratory presentations, including symptomatic or asymptomatic cases, and about the applicability of laboratory tests according to the phase of infection.

For epidemiological surveillance, it would be necessary to collect clinical specimens for viral detection or immune response in mass scale to define a real situation of this disease worldwide. Because of the elevated costs of mass people testing, the rational use of laboratory tools is essential. Using available evidence from international literature, a clinically useful approach for suspected individuals, and a toolbox for interpretation of diagnostic markers used for detection of COVID-19 has been devised to guide, in part, the rational use of specific exams by the clinicians. The diagram of laboratory biomarkers' applicability is detailed in **Figure 3**.

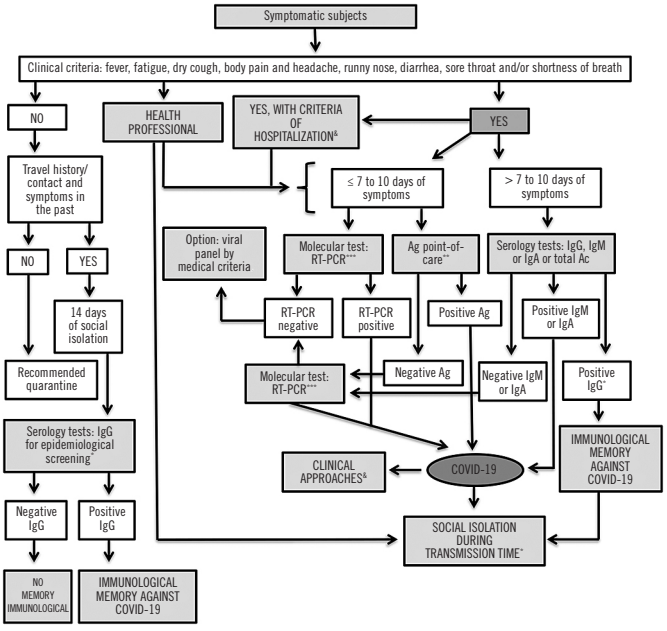

FIGURE 3 – *Laboratory approach of individuals suspected of COVID-19*
Source: By the authors.
*IgM: immunoglobulin class M; IgA: immunoglobulin class A; IgG: immunoglobulin class G; Ac: antibodies; Ag: antigen; RT-PCR: real-time polymerase chain reaction; *transmission time: five to more than 25 days – medical criteria; **rapid test – point-of-care; ***gold-standard test; #with negative RT-PCR; &: local and medical criteria.*

REFERENCES

1. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virology Journal. 2019; 16: 69. DOI: 10.1186/s12985-019-1182-0.

2. Cascella M, Rajnik M, Cuomo A, et al. Features, evaluation and treatment coronavirus (COVID-19) [Updated 2020 Mar 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan.

3. World Health Organization (WHO). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Interim guidance. Reference: WHO/COVID-19/laboratory/2020.5, 19 March 2020. Available at: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 [accessed on: 1 Apr, 2019].

4. Gorbalenya AE, Baker SC, Barik RS, et al. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiol. 2020, 5: 536-44. Available at: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 [accessed on: 1 Apr, 2019].

5. Guo Y, Cao Q, Hong Z, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Military Med Res. 2020, 7: 11. DOI: 10.1186/s40779-020-00240-0.

6. Mäkelä MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. J Clin Microbiol. 1998; 36(2): 539-42.

7. Zhu NA, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020; published online Feb 24. DOI: 10.1056/NEJMoa2001017.

8. World Health Organization (WHO). Update 49 – SARS case fatality ratio, incubation period. Reference: emergencies preparedness, response, 7 May 2003. Available at: https://www.who.int/csr/sars/archive/2003_05_07a/en/ [accessed on: 7 Apr, 2019].

9. World Health Organization (WHO). New case of laboratory-confirmed SARS in Guangdong, China – update 5. Reference: emergencies preparedness, response, 31 January 2004. Available at: https://www.who.int/csr/don/2004_01_31/en/ [accessed on: 7 Apr, 2019].

10. Li Q, Guan X, Wu P, et al. Original early transmission dynamics in Wuhan, China, of novel coronavirus – infected pneumonia. N Engl J Med. 2020. Published online Jan 29. DOI: 10.1056/NEJMoa2001316.

11. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020. Published online Jan 29; 395: 507-13. DOI: 10.1016/S0140-6736(20)30211-7.

12. G X-Y, Li J-L, Yang X-L, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013; 503: 535-38. DOI: 10.1038/nature12711.

13. Du Z, Xu X, Wu Y, et al. Serial interval of COVID-19 among publicly reported confirmed cases. Emerg Infect Dis. 2020; 26(6). DOI: 10.3201/eid2606.200357.

14. Wu JT, Leung K, Bushman M, et al. Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. Nature Med. 2020. Published online March 19. DOI: 10.1038/s41591020-0822-7.

15. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(7798): 270-73. DOI: 10.1038/s41586-020-2012-7.

16. Boletim epidemiológico no. 5. Secretaria de Vigilância em Saúde. Ministério da Saúde. Boletim Epidemiológico – COE COVID-19, 13/03/2020. Available at: https://www.saude.gov.br/images/pdf/2020/marco/21/2020-03-13-Boletim-Epidemiologico-05.pdf [accessed on: 7 Apr, 2019].

17. van Doremalen N, Bushmaker T, Morris DH. Aerosol and surface stability of ARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020. Published online March 17. DOI: 10.1056/NEJMc2004973.

18. Kampf G, Todt D, Pfaender S. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect. 2020; 104: 246-51. DOI: 10.1016/j.jhin.2020.01.022.

19. Huang C, Wang Y, Li X. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223): 497-506. DOI: 10.1016/S0140-6736(20)30183-5.

20. Chinese Center for Disease Control and Prevention. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) — China, 2020. CCDC Weekly; 2(x): 1-10.

21. Dowd JB, Rotondi V, Andriano L, et al. Demographic science aids in understanding the spread and fatality rates of COVID-19. MedRXiv. 2020; p. 1-17. Published online March 15. DOI: 10.1101/2020.03.15.20036293.

22. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China – summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020. DOI: 10.1001/jama.2020.264.

23. Organização Pan Americana de Saúde (OPAS). Folha informativa – COVID-19 (doença causada pelo novo coronavírus). 2020. Available at: https://www.paho.org/bra/index.php?option=com_content&view=article&id=6101:COVID19&Itemid=875 [accessed on: 7 Apr, 2019].

24. World Health Organization (WHO). WHO coronavirus disease (COVID-19). Reference: situation report – 113. 2020. Available at: https://COVID19.who.int/ [accessed on: 14 May, 2020].

25. Secretaria de Vigilância em Saúde. Ministério da Saúde. COVID-19 – painel coronavírus 13/05/2020. Available at: https://www.COVID.saude.gov.br [accessed on: 14 May, 2020].

26. He S, Tang S, Rong L. A discrete stochastic model of the COVID-19 outbreak: forecast and control. Math Biosci Eng. 2020; 17(4): 2792-804. DOI: 10.3934/mbe.2020153.

27. Liu Q, Liu Z, Zhu J, et al. Assessing the global tendency of COVID-19 outbreak. MedRXiv. 2020; 1-18. Published online March 29. DOI: 10.1101/2020.03.18.20038224.

28. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet. 2020. Published online February 21. DOI: 10.1016/S2213-2600(20)30079-5.

29. World Health Organization (WHO). Coronavirus disease 2019 (COVID-19). Reference: situation report – 46, 6 March 2020. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports [accessed on: 7 Apr, 2020].

30. Lai CC, Liu YH, Wang YC, et al. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): facts and myths. J Microbiol Immunol Infect. 2020. DOI: 10.1016/j.jmii.2020.02.012.

31. Lu X, Zhang L, Du H, et al. SARS-CoV-2 infection in children. N Engl J Med. 2020. Published online March 18: 1-3. DOI: 10.1056/NEJMc2005073.

32. World Health Organization (WHO). Q&A: similarities and differences – COVID-19 and influenza. Reference: Newsroom/Q&A Detail, 17 March 2020. Available at: https://www.who.int/news-room/q-a-detail/q-a-similarities-and-differences-COVID-19-and-influenza. [accessed on: 15 Apr, 2020].

33. Ministério da Saúde (Brazil). Quais são os sintomas. Reference: coronavírus – COVID-19. Available at: https://coronavirus.saude.gov.br/. [accessed on: 15 Apr, 2020].

34. World Health Organization (WHO). Clinical management of severe acute respiratory infection when COVID-19 is suspected. Reference: interim guidance, 13 March 2020. Available at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. [accessed on: 15 Apr, 2020].

35. Li L-Q, Huang T, Wang Y-Q, et al. 2019 novel coronavirus patients' clinical characteristics, discharge rate and fatality rate of meta-analysis. J Med Virol. 2020; 1: 1-12. DOI: 0000-0002-6373-2263.

36. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China: life sciences. 2020; 63(3): 364-74. DOI: 10.1007/s11427-020-1643-8.

37. Thomas-Rüddel D, Winning J, Dickmann P, et al. Coronavirus disease 2019 (COVID-19): update for anesthesiologists and intensivists March 2020 [Coronavirus disease 2019" (COVID-19): update für Anästhesisten und Intensivmediziner März 2020] [published online ahead of print, 2020 Mar 24]. Anaesthesist. 2020; 1-10. DOI: 10.1007/s00101-020-00760-3.

38. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. [published online ahead of print, 2020 Mar 9]. Lancet. 2020; 395: 1054-62. DOI: 10.1016/ S0140-6736(20)30566-3.

39. Napolitano LM. Sepsis 2018: definitions and guideline changes. Surg Infect. 2018; 19(2): 117-25. DOI: 10.1089/sur.2017.278.

40. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol. [Published online April 10, 2020]. DOI: 10.1001/jamaneurol.2020.1127.

41. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020; published online Feb 28. DOI: 10.1056/NEJMoa2002032.

42. Campana GA, Levi JE, Chebabo A, et al. COVID-19 – recomendações de uso de exames laboratoriais. Versão 1.0 de 28/03/2020. Available at: https://www.dasa.com.br/informes. [accessed on: 1 Apr, 2019].

43. Yi Y, Lagniton PNP, Ye S, et al. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci. 2020; 16(10): 1753-66. DOI: 10.7150/ijbs.45134.

44. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020. [Published online ahead of print, 2020 March 3] DOI: 10.1515/cclm-2020-0198.

45. Chen W, Lan Y, Yuan X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect. 2020; 9(1): 469-73. DOI: 10.1080/22221751.2020.1732837.

46. Li H, Xiang X, Ren H, et al. Serum amyloid A is a biomarker of severe coronavirus disease and poor prognosis. J Infect. 2020; 80(6): 646-55. DOI: 10.1016/j.jinf.2020.03.035.

47. Wang D, Hu B, Hu C, et al. Clinical caracteristics of 138 hospitalized patients with 2019 novel coronavirus – infected pneumonia in Wuhan, China. JAMA. 2020; E1-E9. DOI: 10.1001/jama.2020.1585.

48. Wu J, Li W, Shi X, et al. Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). J Internal Med. 2020. [Published online March 27, 2020]. DOI: 10.1111/joim.13063.

49. Ulhaq ZS, Soraya GV. Interleukin-6 as a potential biomarker of COVID-19 progression. Med Mal Infect. 2020. [Published online on April 3, 2020]. DOI: 10.1016/j.medmal.2020.04.002.

50. Singhal T. A review of coronavirus disease-2019 (COVID-19). Indian J Pediatrics. 2020; 87(4): 2810-86. DOI: 10.1007/s12098-020-03263-6.

51. Qu R, Ling Y, Zhang Y-H-Z, et al. Platelet-to-lymphocyte ratio is associated with prognosis in patients with coronavirus disease-19. J Med Virol. 2020; 1-9. DOI: 10.1002/jmv.25767.

52. Galli C, Plebani M. Clinical laboratory and SARS-CoV-2 infection: where do we stand? Clin Chem Lab Med. 2020. [Published online ahead of print, 2020 March 24]. DOI: 10.1515/cclm-2020-0372.

53. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chim Acta. 2020; 505: 190-91. DOI: 10.1016/j.cca.2020.03.004.

54. Sociedade Brasileira de Patologia Clínica e Medicina Laboratorial – SBPC/ML. Métodos laboratoriais para diagnóstico da infecção pelo SARS-CoV-2. Reference: recomendações da Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial, 3 April 2020. Available at: http://www.sbpc.org.br/wp-content/uploads/2020/04/MetodosLaboratoriaisDiagnosticoSARS-CoV-2.pdf. [accessed on: 23 Apr, 2020].

55. Ahn D-G, Shin H-J, Kim M-H, et al. Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 (COVID-19). J Microbiol Biotechnol. 2020; 30(3): 313-24. DOI: 10.4014/jmb.2003.03011.

56. World Health Organization (WHO). Laboratory testing strategy recommendations for COVID-19. Interim guidance. Reference: WHO/2019-nCoV/lab_testing/2020.1, 21 March 2020. Available at: https://apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf. [accessed on: 24 Apr, 2019].

57. Ministério da Saúde (Brazil). Vigilância integrada de síndromes respiratórias agudas doença pelo coronavírus 2019, influenza e outros vírus respiratórios. Reference: Guia de Vigilância Epidemiológica: emergência de saúde pública de importância nacional pela doença pelo coronavírus 2019. Available at: https://www.saude.gov.br/images/pdf/2020/April/06/GuiaDeVigiEp-final.pdf. [accessed on: 23 Apr, 2020].

58. Loeffelholz MJ, Tang Y-W. Laboratory diagnosis of emerging human coronavirus infections – the state of the art. Emerg Microbes Infect. 2020; 9(1): 747-56. DOI: 10.1080/22221751.2020.1745095.

59. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. 2020; ciaa344. [Epub ahead of print]. DOI: 10.1093/cid/ciaa344.

60. Tang Y-W, Schmitz JE, Persing DH, et al. The laboratory diagnosis of COVID-19 infection: current issues and challenges. J Clin Microbiol. 2020. [JCM accepted manuscript posted online, 3 April 2020]. DOI: 10.1128/JCM.00512-20.

61. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA. 2020. [Published online May 6, 2020]. E1-E3. DOI: 10.1001/jama.2020.8259.

62. World Health Organization (WHO). WHO coronavirus disease (COVID-19) dashboard. Reference: WHO (COVID-19) homepage, 12 May 2020. Available at: https://COVID19.who.int/. [accessed on: 12 May, 2020].

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