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Viral Replication and Antibody Kinetics in the Recognition of Asymptomatic COVID-19 Patients

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Abstract
Following the discovery of COVID-19 disease caused by the SARS-CoV-2 coronavirus, different studies have been carried out to recognize the stages of the disease and the methods of achieving correct diagnosis. Investigations of cases and their contacts have revealed different degrees in the symptomatology of the disease, with asymptomatic patients gaining relevance because of the controversy regarding their role in the spread of the disease. Recognition and assessment of asymptomatic patients is essential to carry out containment actions such as public health measures for affected patients and contacts. In this review, we assess the diagnostic aspects of asymptomatic patients according to the available evidence of people with COVID-19.

Introduction

Acute severe respiratory syndrome due to the new SARS-CoV-2 coronavirus has affected more than 133 million people and caused the death of >2.89 million individuals worldwide, by April 8, 2021 [1], becoming the largest epidemic/pandemic of the present century. With the discovery of COVID-19 disease, different studies have been carried out to recognize the different stages of the disease and the methods of achieving correct diagnosis of individuals with this disease [2, 3].

In addition, with the investigation of the cases and their contacts, different degrees of symptomatology have also been found, from patients with mild symptoms to those developing severe symptoms, severe acute respiratory failure requiring assisted ventilation, and even death. Asymptomatic patients have become relevant in the study of this disease. Their role in the spread of the disease was initially controversial in the scientific community; however, we now know that the role of these individuals in the transmission of the infection is important in magnitude but of shorter

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temporal duration compared to presymptomatic and symptomatic patients. The problem that we now face is differentiating the asymptomatic from the presymptomatic status of patients (also called the subclinical state) [4–9].

Periods to Consider in COVID-19

The average incubation period for SARS-CoV-2 has been estimated between 5 and 7 days. This range has been fairly constant among the populations studied, with a reported maximum of 19 days [6], although almost all the cases described do not exceed 14 days. Therefore, the isolation of contacts for 14 days has a pathophysiological basis, with transmission being unlikely to occur after this time [7, 8, 10, 11].

Virus detection by reverse transcriptase polymerase chain reaction (RT-PCR) is used to rapidly detect SARS-CoV-2/COVID-19 disease. The assay is based on multiple sets of primers and probes located in different regions of the SARS-CoV-2 genome and is able to discriminate SARS-CoV-2 from other human and animal coronaviruses with a potential detection limit of <10 genomic copies per reaction. All diagnostic tests are qualitative. However, cycle threshold (Ct) values have been used to indirectly quantify SARS-CoV-2 load in samples. A disadvantage of this type of detection is that it does not indicate whether the genetic material detected in the sample is still viable. Infectivity is the ability of the virus to infect cells and replicate in susceptible hosts and can be assessed by viral culture in human respiratory epithelial cells and several laboratory cells lines (VERO 6, VERO 81, VERO SLAM, MA104, and BGM cells) which can support viral replication and produce CPE after 48 h of infection with different SARS-CoV-2 strains, although to date we have no standardized cell systems.

An indirect way to estimate viral infectivity is by the measurement of Ct values, or the number of cycles required to obtain a positive signal demonstrating the presence of viral genetic material. At the beginning, a Ct result of ≤38 was associated with a high viral load and a higher probability of infectivity, while a Ct value of ≥40 was usually considered unviable, and although genetical material is found, it is limited and not infectious. Currently, a Ct ≥35 is considered as a mark of low viral load in the sample, and even this can vary depending on the molecular platform used. Therefore, at this time, a specific value of the Ct is not standardized to define infectivity.

There are also quantitative RT-PCR tests that measure the number of copies per sample volume, usually expressed per mL of secretion in respiratory samples. A copy number of 10^5 or more per mL is associated with a potential risk of disease transmission [11–15]. An RT-PCR test can remain positive for 14–21 days, but this does not indicate that the patient is spreading the disease during this period, especially if the patient is asymptomatic or has mild disease. In these two cases in particular, the transmission period would be an average of 7 and 9 days, respectively, with positive RT-PCR tests for 14–21 days [14–16].

Asymptomatic patients are defined as patients not presenting any symptoms during the window period after the development of infection, after the first positive RT-PCR test, and until the presentation of two negative RT-PCR tests separated by 24 h since the first sample taking. This period has an average duration of 14–21 days (Fig. 1). In patients considered asymptomatic, the number of copies reaches 10^5 per mL as in patients with mild disease. However, between the fifth and seventh day of what would be the supposed symptomatic period, this number of copies rapidly falls, being a much faster rate than in symptomatic patients [4, 15–20].

Asymptomatic patients are considered to have the same incubation period as those who develop symptoms, that is, of 6–7 days. As in symptomatic patients, asymptomatic patients can transmit the disease from two days before the theoretical time during which symptoms would develop to up to five days which would correspond to the presentation of symptoms. Therefore, asymptomatic patients could theoretically transmit the viral infection for up to seven days, as opposed to the nine days during which patients with mild disease could transmit infection (from two days before symptoms to seven days after the onset of symptoms). According to the results of population studies, up to 40% of asymptomatic patients have been identified, being double the 20% first assumed at the start of the pandemic as the total number of individuals infected by SARS-CoV-2 [17–20].

Antibody Kinetics for COVID-19

Three antibodies have been identified in SARS-CoV-2 infection: immunoglobulin (Ig) A, IgM, and IgG. IgA is rarely evaluated by chemiluminescence or ELISA tests and appears earlier in plasma, between days 3 and 5. Early detection and high IgA titers have been associated with a worse prognosis [7, 8, 13, 14].

For a rapid test to detect serum IgM, 7 days must elapse from the presentation of symptoms. This immunoglobu-

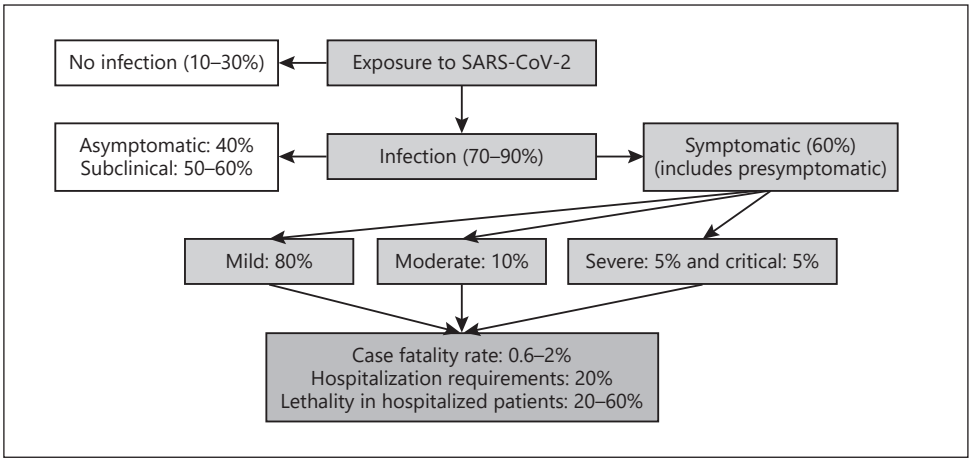

Fig. 1. Natural history of SARS-CoV-2 infection.

Incubation	Phase I Early infection	Phase II pulmonar IIA	 IIB	Phase III Hyperinflammation	Phase IV Recovery	Phase V Long COVID or PASCV
7 days (2–14)	7 days (4–14)	21–35 days			1–3 months	3–6 months
Asymptomatic and symptomatic	Asymptomatic	Does not apply				
	Symptomatic: 1–3 months and recovery: 1–3 months and PASC (post-acute sequelae of COVID-19 – before long COVID-19): 3–6 months					
Viral phase for asymptomatic 10 days and for symptomatic 14–21 days						
Seroconversion (transition from the point of viral infection to when any antibodies against the virus become present in the body) occurs after the 3rd week (between days 14–21) from the onset of symptoms. On day 21 all patients are positive for at least one antibody. Antibodies peak together between day 8–15. Antibodies have highly variable independent peaks but homogeneous persistence over time. Asymptomatic patients can also undergo seroconversion. The patients can have positive results for both antibodies for 5 and up to 7 weeks						
IgA begin to be synthesized from day 3–5 counted from the onset of symptoms. IgM begin to be synthesized from day 7 (6–8) counted from the onset of symptoms, reaching a maximum peak between weeks 2–3, reaching their minimum value at week 5 and disappear between weeks 7–12						
IgG begin to be synthesized from day 10 (8–14) counted from the onset of symptoms, reaching a maximum peak between days 21–35 and then decreasing from day 35 and remaining detectable between at 6 months and 1 year						

Fig. 2. Phases of COVID-19 and its association with antibody kinetics.

lin usually becomes positive from the 14th day after symptom onset, that is, from 14 to 21 days after infection. Serological tests can continue to give positive IgM results for 5–7 weeks without this meaning that the patient is in the "acute" phase of the disease. Early detection of IgM is not necessarily associated with greater clinical severity of the disease, and continued positivity is not necessarily related to the severity of the disease or the period of its transmission [2, 17–23].

Likewise, IgG is present in serum from day 10 of symptom onset, and on average on day 21, it is usually positive in serological tests. The presence of IgG does not mean cure or resolution of the disease. A patient may still be cured with a negative IgG serologic test. Serological tests are qualitative and require high serum or blood antibody titers or values for a positive result.

It is unusual for all patients to present a stable sequence of serological test results: first IgM, followed by IgM/IgG, and then IgG, and these changes do not occur every 7 days. The test most often shows a simultaneous positive IgM/IgG result in a patient than a positive IgM/IgG negative result. Almost all patients present IgM/IgG seroconversion only after the third week of the onset of symptoms and can have positive results for both antibodies for 5 and up to 7 weeks [20–24]. The serological results are not directly related to the clinical status of the patient, nor with the period in which they are ill, or with the probability of transmitting the disease [21, 23, 25, 26] (Fig. 2).

Asymptomatic Patients and Serological Tests

The follow-up and detection of asymptomatic patients are carried out with RT-PCR and not by serological tests. By the time a serological test is performed in an asymptomatic person with a positive result, both the period of disease transmission and the possibility of detecting a true asymptomatic patient have passed.

The results of studies of asymptomatic patients are discordant. While some studies have estimated 40% of real asymptomatic patients, cross-sectional studies have estimated this percentage to be of up to 80%. However, longitudinal studies have reported that up to 80% of asymptomatic patients were actually presymptomatic at the time of the first positive RT-PCR with the development of symptoms after an average of 4 days. In addition, 50–60% of patients classified as asymptomatic are actually in a subclinical period of the disease, presenting ground glass lung lesions compatible with COVID-19. In the follow-up of truly asymptomatic, albeit subclinical, patients, the resolution of COVID-19 has shown to be spontaneous within a period of 5–7 days. Theoretically, it would not be correct to classify a person as asymptomatic with the use of a positive serological test for 1 or 2 antibodies because this category has been assigned to those followed with RT-PCR [2, 4, 16–21].

While some patients are asymptomatic and can transmit the infection and >50% may have a subclinical COVID-19 presentation, the disease resolves on its own, and by the time these patients undergo a serological test and obtain a positive result, the transmission period of the infection has already passed. Taking into account the very large variation in antibody kinetics in SARS-CoV-2 infection, it may be irresponsible to use isolated results of serological tests to categorize patients, asymptomatic persons, and especially workers [25, 26].

Serological tests should be used in the study of the seroprevalence and seroincidence of COVID-19 disease in a specific population or should be used in patients with suspected infections that appear within 2 weeks of symptom onset. As in other diseases, in the medical history of the patient, anamnesis and the results of auxiliary studies are important complements to clinical judgment in patients with SARS-CoV-2 infection.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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