Chapter 21

Analysis of the quality of diagnostic tests for COVID-19 in a city in the northwestern region of Sao Paulo

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Geovana Caliente Marques

Heloísa Fusetto de Lima

Lidyane Bortoleto Garcia

Wagner Moneda Telini

Vera Lúcia Dorigão Guimarães

Daniele Cristina Bofo

ABSTRACT

In Brazil, the first cases of COVID-19, were diagnosed in February; on the 3rd of the same month, the country declared a Public Health Emergency of National Importance (ESPIN). The National Contingency Plan for COVID-19 was initiated, which in addition to the health measures, created TeleSUS. Given this scenario, the National Health Surveillance Agency (ANVISA), approves the expansion of testing. Most of the techniques used are: reverse transcriptase polymerase chain reaction (RT-PCR), loop-mediated isothermal amplification (LAMP), lateral flow technique, rapid individual test; and enzyme-linked immunosorbent assay (ELISA). According to the World Health Organization (WHO), the gold standard technique is from RT-PCR. Thus, this study aimed to analyze the quality of diagnostic tests in a city in the Northwestern region of the state of São Paulo. This was evaluated by means of a cross-sectional, observational and individual study, with simple random probabilistic sampling, with exclusion criteria for deaths and people with incomplete data in the list. It was analyzed, 215 residents, hospitalized, who performed the standard other test (RT-PCR) and TR, between June/2020-June/2021, in local and referral hospitals. The diagnostic tests, RT-PCR and TR, were analyzed categorically, from the development of 2x2 frequency tables. When analyzing the TR, it was observed that the sensitivity in 2020 was 95% and the specificity 17%; in 2021, the sensitivity was 100% and the specificity 37.5%. The PPV in 2020 was 71% and in 2021, 93%. The VPN in 2020 was 66% and in 2021 100%. It was possible to conclude that the TR proved to be a valid tool to follow up the notification strategy and monitoring of suspected cases in the midst of the COVID-19 Pandemic. Analyses like this could compose dynamic management strategies, as potential sentinel indicators.

1 INTRODUCTION

In the last month of 2019, in the city of Wuhan, located in Hubei Province (China), an outbreak of pneumonia of unknown cause emerged. In early 2020, Chinese researchers identified a new coronavirus (SARS-CoV-2), responsible for a Severe Acute Respiratory Syndrome, called COVID-19. At the beginning of the spread of the disease, all cases were related to a seafood and animal market, located in Wuhan (CALVACANTE et al, 2020).

This virus has characteristics that imply in a high basic reproduction number when compared to other coronaviruses, which directly implies in its transmissibility. In Brazil, the first cases were diagnosed in February; on the 3rd of the same month, the country declared a Public Health Emergency of National Importance (ESPIN). Several actions have already begun to be implemented in order to contain the advance

and dissemination of the disease in our territory. The first death was registered on March 17, 20 days after the appearance of the first case in the state of São Paulo (WERNECK, CARVALHO; 2020).

By March 22, in Brazil, all FHU had already notified cases of the disease. Since then, data on cases and deaths from COVID-19 have been collected and made available by the State and Municipal Health Secretariats, and consolidated by the Brazilian Ministry of Health. With this, it becomes possible to know the progress of the disease in the country, and furthermore, the policies that are being used and implemented to slow down the increase in the number of cases (CAVALCANTE et al, 2020).

The scientific community in the field of infectious diseases signals that the emergence of new pandemics is inevitable, and we just don't know when they will occur. In the 21st century, several epidemics could be contained on a temporal or geographical level, for example the two coronavirus epidemics (SARS-CoV and MERS), Ebola, and avian influenza. Together they all caused fewer deaths than COVID-19. In our country, this situation becomes even more uncertain and dramatic, given the contradictory recommendations coming from different spheres of government, with different suppression measures coming from each authority (OLIVEIRA et al, 2020)

In Brazil, the epidemic is facing a situation in which the population is extremely vulnerable, with high unemployment rates and cuts in social support policies; this situation worsens considerably the situation of individuals inserted in different social environments, further promoting the spread of the virus in the less favored who need to find alternative means of income and support, putting themselves at risk of exposure, often unnecessary (informal jobs, lack of supplies for personal hygiene, inadequate food, lack of resources to purchase medication) (WERNECK, CARVALHO; 2020)

Since the identification of the new virus in our country, the Ministry of Health has adopted specific strategies to face the epidemic. The number of cases and deaths are made available daily, in addition to epidemiological bulletins, containing information about the surveillance activities in this context. The MS has made available means to assist the population, including digital means, such as the application Coronavirus-SUS and the Whatsapp channel; it has implemented an on-call regime, including weekends (BRASIL, 2020).

At the beginning of the disease in our country, most cases were imported and the only strategies adopted were the search and isolation of cases and contacts to prevent community transmission of the virus; with the expansion of the disease, community transmission began, and other strategies needed to be adopted, seeking to prevent the occurrence of severe cases and deaths. These strategies included hospital admissions in the identification of a moderate/severe case and isolation guidelines for mild cases and contacts (BRASIL, 2020).

The strengthening of health care has also been sought, acting mainly in the search and training of human resources and expansion of coverage of the SUS, hiring new professionals, especially doctors who work in Intensive Care Centers (ICU). The safety of these professionals has become a priority, with special

attention to the production, acquisition, and distribution of personal protective equipment (PPE) (BRASIL, 2020).

In our country, besides the implementation of sanitary measures to contain the advance of the virus, the Ministry of Health started the creation of the National Contingency Plan for COVID-19, after it declared a Public Health Emergency of National Importance (ESPIN). Longo created the TeleSUS, which consists of a Telemedicine system, idealized to track, diagnose, treat, and monitor patients with Gripal Syndrome and COVID-19 (HARZHEIM et al., 2020).

Thus, the flexibility of digital technologies, which were adjusted to the health needs of the social context, proved to have great benefits, highlighting the reduction in care times and improvement in the quality of care, since there was aid in the detection, surveillance, and prevention of diseases, in addition to promoting health education, especially for cases positive for COVID-19 (CAETANO et al., 2020).

In this new model of care, there are two possibilities of using telehealth: (a) teleconsultations for monitoring clinically stable patients, and (b) teleconsultation as screening for severe cases. In addition, it enables direct triage, which classifies patients before they reach the health service, ensuring greater resoluteness of the case. Thus, telehealth is proving to be a critical component among the measures adopted by the Ministry of Health to increase the fight against coronavirus, and enable, at the same time, safer and more functional health services (CAETANO et al., 2020).

The trajectory and distribution of the effects of the pandemic in our country, made it possible to create social considerations as fundamental causes of health, in articulation with the notions of territory and social class. The main concept addressed, will be the Territorial, since it generates health implications by having influential factors to the health-disease process of a given population, because there is an association of the physical, social environment with sociocultural and historical characteristics (SANTOS,2020).

The first issue to be considered is the distribution of the incidence of coronavirus among groups and territories, because factors that generate risk situations, such as: poor sanitation, demographic density, distance between home and work, dependence on public transport and the internal limitations in space and support of housing. Thus, in our country, where social inequality is alarming, the fundamental strategy is the decentralization of diagnostic testing (SANTOS, 2020).

Considering these factors, some initiatives of the National Health Surveillance Agency (ANVISA) were created, aiming at the expansion of testing, such as the approval of rapid tests in pharmacies and strengthening primary health care, which in our health system is the gateway to SUS, and is present in the distant locations of the national territory, and has professionals trained in the application of other tests, such as syphilis and HIV (MAGNO et al, 2020).

In this sense, the population testing for COVID-19 must be coordinated in two fronts - priority access by primary health care and access to the test by the supplementary network, which are the private health services. Both places of care, must perform the notification of cases and these must be monitored

through the local epidemiological surveillance. In conclusion, the importance of the organization of testing strategies, allied to the organization of the health system, because the number of confirmed cases will allow the monitoring of the progression of the disease (MAGNO et al, 2020).

The diagnosis of COVID-19 is still a challenge worldwide, since there are aspects that make this practice difficult: the biological material to be used, such as naso and/or oropharyngeal swab, plasma, serum or whole blood; the definition of the biological marker with the highest chances of being detected; the type of methodology employed (virological methods, molecular biology and immunoassays); the ideal time of infection for sample collection and the ideal type of sample; and the accuracy of the diagnostic tests available (MAGNO et al, 2020).

Currently, most available tests use one of the following techniques: reverse transcriptase polymerase chain reaction (RT-PCR), loop-mediated isothermal amplification (LAMP), lateral flow technique, rapid individual test; and enzyme-linked immunosorbent assay (ELISA) (PACHITO et al., 2020).

The tests serve to identify the presence of the virus in activity, through the detection of its genome by the RT-PCR technique, and to detect a previous infection, through serological tests for the identification of specific antibodies (PILECCO et al., 2020).

According to the World Health Organization (WHO), the gold standard diagnosis for identification of the SARSCoV- 2 virus is performed through the techniques of polymerase chain reaction with reverse transcription with amplification in real time, or RT-PCR, and partial or total sequencing of the viral genome, since it amplifies virus RNA sequences, allowing its identification through samples obtained from nasopharyngeal aspirate (NPA), nasal and oral swab, and respiratory secretion from the lower tract, such as sputum, tracheal lavage or bronchoalveolar lavage, and the collection should be performed between the third and fifth after the onset of symptoms, up to a maximum of ten days after the occurrence (NOGUEIRA E SILVA, 2020).

The RT-PCR has limitations regarding the sensitivity of the test when samples with low viral load are used and disadvantages, such as the time required between the collection and availability of the result, as well as the need for specialized physical structure and qualified technical staff (PACHITO et al., 2020).

It is then a very sensitive technique with the highest specificity (100%) of all the tests available for the detection of this virus, but if collected incorrectly, a false negative can be obtained. (NOGUEIRA E SILVA, 2020) (RIBEIRO et al., 2020).

Therefore, despite being considered the most effective method of detection, a negative RT-PCR result does not rule out the diagnosis of virus infection, and it is recommended that the result be related to the patient's history and clinical conditions, as well as the epidemiological parameters of the region. Thus, if the patient has a high probability of being infected and the test is negative, it is recommended to perform it again with new samples (NOGUEIRA E SILVA, 2020).

Immunoenzymatic and immunochromatographic assays, on the other hand, will detect the infection indirectly, and should be performed as of the eighth day of the onset of symptoms, this being the time required for the immune system to produce antibodies in sufficient quantity for detection in the blood (LIMA et al, 2020).

However, laboratory testing is extremely important for the diagnosis of COVID-19 and to know the magnitude of the infection, its control and monitoring (PILECCO et al., 2020).

Given this context, we noticed the need to analyze the quality of the tests in a city in the Northwestern region of São Paulo, since this allows a more effective monitoring of the disease progression, thus ensuring greater resoluteness, improving the patient's health care. In addition, early diagnosis and reduction of false positives and negatives allows the reduction of the spread of the disease.

2 OBJECTIVES

2.1 PRIMARY

Analyze the quality of Diagnostic Tests for COVID-19: Test for Antigen Detection (TR) and Molecular Test (RT-PCR).

2.2 SECONDARY

To analyze the quality of the Test for Antigen Detection (TR), for COVID-19, for accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV);

To analyze the quality of the Molecular Test (RT-PCR), for COVID-19, regarding accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV);

Compare change in the pattern of diagnostic test characteristics between June/2020-June/2021;

3 STUDY CHARACTERISTIC

Type of sampling: simple random probability

Type of study: cross-sectional, observational, individual

Exclusion criteria: Deceased and people with incomplete data in the list

4 METHOD

4.1 PARTICIPANTS

Patients living in a city in Northwestern São Paulo, affected by COVID-19, admitted between June/2020-June/2021 in local and referral hospitals, totaling 215 individuals.

4.2 MATERIAL OR INSTRUMENTS

Rapid antigen detection tests are performed by immunochromatography technique, detecting the virus protein in samples collected from the oropharynx and nasopharynx during the acute phase of the disease, and should be performed between the first and eighth day of symptoms. All participants underwent the gold standard test (RT-PCR), developed by means of the reverse transcription polymerase chain reaction technique with real-time amplification and partial or total sequencing of the viral genome, through samples obtained from the nasopharyngeal aspirate (ANF) nasal and oral swabs, and by respiratory secretion from the lower tract, such as sputum, tracheal lavage, or bronchoalveolar lavage, and the collection should be done between the third and fifth day after the onset of symptoms, up to a maximum of ten days after the onset. Tests provided by Hilab and Basal laboratories, rapid tests, and Wama for RT-PCR were performed.

4.3 EXPECTED RESULTS

It is expected that the characteristic sensitivity will never vary, and that specificity will increase between the years 2020 and 2021, showing reproducibility of the tests. In addition, find an accuracy above 93%.

4.4 DATA ANALYSIS PROCEDURE

The diagnostic tests, RT-PCR and TR, were analyzed categorically. 2x2 frequency tables were prepared for data recording and subsequent analysis of sensitivity, specificity, PPV, and NPV.

In the year 2020, 108 patients diagnosed with COVID, were notified and submitted to the monitoring system, resulting in 74 individuals tested positive by RT-PCR; of these, 71 were considered true positives, however, 3 individuals were classified as false-negative. Furthermore, we obtained 34 negative results; of these, 28 were considered false-positive and 6 true-negative, as shown in table 1 below.

Table 1

YEAR 2020	PCR +	PCR -	TOTAL
TR +	71 (A)	28 (B)	99 (A+B)
TR -	03 (C)	06 (D)	09 (C+D)
TOTAL	74 (A+C)	34 (B+D)	108 (A+B+C+D)

In the year 2021, 107 patients diagnosed with COVID were reported and submitted to the monitoring system, resulting in 91 individuals tested positive by RT-PCR; all of these were considered true positives. Furthermore, we obtained 16 negative results; of these, 06 were considered false-positive and 10 true-negative, as shown in table 2 below.

Table 2

YEAR 2021	PCR +	PCR -	TOTAL
TR +	91 (A)	06 (B)	97 (A+B)
TR -	00 (C)	10 (D)	10 (C+D)
TOTAL	91 (A+C)	16 (B+D)	107 (A+B+C+D)

Rapid Diagnostic Test Quality Analysis

Sensitivity

Sensitivity analyzes the probability of the test having a positive result given that the individual is sick (REIS, E.; REIS, I., 2002). To calculate the sensitivity the formula was used:

Sensitivity: a/(a+c)

In this way, the results below were obtained:

Sensitivity 2020: 71/74 x 100 = 95%.
Sensitivity 2021: 91/91 x 100 = 100%.

Specificity

It is the probability of the test having a negative result given that the individual is not sick (REIS, E.; REIS, I., 2002). To calculate the specificity, the formula was used:

Specificity: d/(b+d)

Specificity 2020: 6/34 x 100 = 17%
Specificity 2021: 10/16 x 100 = 37.5

Positive Predictive Value (PPV)

It is the probability that the individual is sick because his test was positive (REIS, E.; REIS, I., 2002).

Positive predictive value: a/(a+b)

VPP 2020: 71/99 x 100 = 71%
VPP 2021: 91/ 97 x 100 = 93%

Negative Predictive Value (PPV)

It is the probability that the individual is not sick because his test result was negative (REIS, E.; REIS, I., 2002).

Negative predictive value: d/(c+d)

VPN 2020: 6/9 x 100 = 66%
VPN 2021: 10/10 x 100 = 100%.

5 DISCUSSION

The high values of sensitivity in the CA point to high accuracy of this screening strategy in the analyzed health territory. The increase in specificity of the CA in 2021, compared to the previous year, suggests improvement in the testing application process, considering that there was no change in the supplies used.

In 2020, according to the data analyzed, the rapid test showed PPV below satisfactory values, so it could not be considered an effective diagnostic method, justifying the performance of RT-PCR for all. However, in the year 2021, there was an increase in the PPV of the TR, which was associated with the increasing prevalence of the grievance. This resulted in an increase in the quality of this diagnostic test, which reached predictive values similar to the gold standard.

6 CONCLUSION

Diagnostic quality tests, such as sensitivity, specificity and PPV. They showed to be valid tools for the follow-up of the notification strategy and monitoring of suspected cases in the midst of the Pandemic COVID-19. Analyses like this, could compose dynamic management strategies, as potential sentinel indicators.

7 LIMITATIONS OF THE STUDY

The increase in VPN was a discrepant finding than expected in the face of the increased prevalence of COVID-19 cases. Prospective studies considering the TR and RT-PCR variables, could yield better results.

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