COVID-19 disease with persistently negative RT-PCR test for SARS-CoV-2

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Introduction

Disease cause by the novel Coronavirus SARS CoV-2 (COVID-19) has spread rapidly [1,2]. Coronavirus represent an heterogeneous group of large single chain RNA virus, widely distributed amongst mammals and birds and grouped in the Coronaviridae family [3]. The main sub-groups of human interest are the Alpha and Beta Coronavirus that conform the subdivision of the Coronavirinae along with the Gamma and Delta Coronavirus not recognized as human pathogens [4]. At the end of the second decade of the 21st Century the world witnessed the outbreak of a novel coronavirus, designated as SARS-CoV-2 and taxonomically assigned to the species of severe acute coronavirus related to the Severe Acute
Respiratory Syndrome (SARS-CoV). Sarbecovirus sub-genre, Betacoronavirus genre [5,6]. The coined term to refer to the disease related to the SARS-CoV-2 virus is COVID-19 [7]. Respiratory droplets and close contact have been recognized as the main routes of transmission of the COVID-19 virus, although the fecal-oral route cannot be excluded [8-10].

The clinical scenario of COVID-19 is very heterogeneous. The spectrum of the disease varies from very mild symptoms suggesting an upper respiratory infection to moderate and severe forms like Severe Acute Respiratory Syndrome (SARS) [11]. Moreover there are reports of asymptomatic cases which makes the containment of the infection very difficult.

Amongst infected patients with SARS-CoV-2, several conditions are related with a higher susceptibility to the virus and also with an elevated viral load of COVID-19. The highest rate of positive results for SARS-CoV-2 is obtained through combined samples of nasopharyngeal and oropharyngeal swabbing with proper processing following the guidelines of the Centers for Disease Control and Prevention (CDC) and when the samples is obtained during the first few days after the appearance of symptoms given the fact that the highest viral load has been documented during that period with an increased diagnostic yield of the Reverse Transcriptase Polymerase Chain Reaction Test (RT-q-PCR).

According to the World Health Organization (WHO), false negative results can be as high as in 32% of the samples tested. The main factors for this are related to the timing of the sample that should be close to the beginning of symptoms when the viral load is higher, inadequacy obtaining the sample and delays in the handling and transport of the sample [12].

It has been documented that during the course of the disease the rate of false negative results increases with time, being higher around the 9th day of symptoms and it changes again by day 21 of the start of symptoms (second wave) when the rate of false negatives reaches its peak according to an article published in Mayo Clinic Proceedings [13].

**Material and methods**

We conducted a descriptive study at the National Institute of Respiratory Diseases. We analyzed retrospectively the clinical, radiological and microbiological data of patients hospitalized with COVID-19 infection. For this study we classified infection of COVID-19 according to the epidemiological survey definition of COVID-19 updated in April 2020 plus typical tomographic findings suggestive of COVID-19 within the epidemiological context of the pandemic. We gathered all necessary information to document sociodemographic variables (age, residence, comorbidities and smoking history) as well as laboratory and tomographic data. We reviewed the clinical charts of all patient enrolled in the study and eliminated those who had incomplete charts. The information was stored in a database in Excel stratifying the data in function of the definition of variables. We evaluated de distribution of data with the Shapiro-Wilk Test of normality. The non-parametric data are reported with median. The nominal and ordinal variables are presented as percentages.

**Results**

A total of 38 hospitalized with COVID-19 disease with negative RT-PCR Test obtained through nasopharyngeal and oropharyngeal swabbing were analyzed during the study period. The average age of patients was 46 years and males represented 52.63% of the cases (n = 20). In regards to comorbidities Diabetes Mellitus was documented in 34.21% (n = 13) of the patients, Systemic Hypertension in 21.05% (n = 8), Obesity in 31.57% (n = 12) and Overweight in 42.10% (n = 16). Exposure to tobacco smoke was reported in 47.36% (n = 18) of the patients. No history of cancer, chronic renal failure or pulmonary disease was reported.

The RT-PCR Test for SARS-CoV-2 obtained through nasopharyngeal and oropharyngeal swabbing was initially negative in 100% (n = 38) of the cases. The median saturation of oxygen was 87% breathing room air. The severity of the disease on admission was: mild 71.05% (n = 27), moderate 21.05% (n = 8) and severe or critical in 7.89% (n = 3) of the cases respectively.

63.15% (n = 24) sought medical care after 6 or more days with symptoms. Lymphopenia was documented in 78.94% (n = 30). Median LDH at the time of admission was 300, being elevated in 63.15% (n = 24) of the cases. The initial tomographic imaging of the chest revealed predominantly ground glass pattern in 81.57% (n = 31) and predominantly consolidation in 18.42% (n = 7). The registered mortality was 15.78% (n = 6).

**Discussion**

The current recommendation by the Centers of Control and Prevention (CDC) for the diagnosis of the COVID-19 is the Reverse Transcriptase Polymerase Chain Reaction Test also known as RT-PCR Test (Gold Standard) [14-19] and it's also recommended that physicians coordinated and convey their findings with local authorities following current public health guidelines. It is recommended that the initial sample from the respiratory tract for the diagnosis and detection of patients with COVID-19 disease to be taken within the first 5 days of the appearance of symptoms suggestive of the disease since it has been demonstrated it is during this period that a high viral load is present in the upper and lower respiratory tract [20-24]. In our study the RT-PCR Test used for the detection of COVID-19 was the one recommended by the World Health Organization (WHO) and under the accreditation of the Institute of Epidemiological Diagnosis and Reference (InDRE).

The swabbing of the nasopharynx and oropharynx is frequently recommended for the diagnosis of the infection.
Although the use of only one swab has become the norm due to better patient tolerance, operator safety and lower cost, we know of potential inconveniences of this practice as realized in some patients that link this to a lower detection of the RNA SARS-CoV-2 and an increase in the false negative test results impacting negatively the diagnostic and therapeutic decisions.

Wang, et al. reported that swabbing of the oropharynx was used more frequently that the nasopharynx route in the China cohorts during the outbreak of the COVID-19 infection [25-27]. RNA SARS-CoV-2, however was detected in only 32% of the oropharyngeal swabs, a rate significantly lower than the one for the nasopharyngeal swabs that was of 63% [28]. Although it’s recommended that the collection of nasopharyngeal and oropharyngeal samples could be done independently, sending both samples together in just one aliquot for processing seems to be an attractive alternative without compromising results.

We now know that sometimes tests have to be repeated or samples have to be taken from the lower respiratory tract in patients with high suspicion of the disease by clinical and tomographic findings in which the RT-PCR Test has been negative in addition to rule out other possible viral pathogens like seasonal influenza and the Syncytial Respiratory virus [29].

In our study population, all the patients who met the definition of suspicious case underwent testing with RT-PCR through nasopharyngeal and oropharyngeal swabbing and Chest Tomography. Those patients with initial RT-PCR negative for SARS CoV-2 were tested for Influenza A & B, Influenza H1N1 and H5N3 and other respiratory viruses and a new control RT-PCR test was obtained at 48 hours if the history of exposure, clinical and radiographic data and clinical course continue to strongly suggest the diagnosis of COVID-19 as the main diagnostic probability.

Repetition of test is particularly important if a patient has a clinical suspicion of viral pneumonia, history of potential exposure and/or tomographic findings compatible with COVID-19 pneumonia. In our study 100% of patients had a least a second RT-PCR test 48 hours after the initial test and some had between 2 and 4 tests altogether depending on the clinical course and hospital length of stay. In addition to the second test for COVID-19 we tested patient for atypical agents including Mycoplasma Pneumonia and Chlamydia Pneumonia and in patients who developed Acute Respiratory Distress Syndrome (ARDS) we added bronchial cultures, blood and urine cultures as well as the urinary antigen for Legionella.

Some patients with COVID-19 Pneumonia have demonstrated high viral loads of RNA SARS-CoV-2 in feces as well as a delay in shedding the virus in the respiratory tract at the end of the clinical course [30-36]. The gastrointestinal manifestations have been reported previously in patients with severe infections due to coronavirus. Therefore, although the literature recommends taking samples directly from the respiratory tract as the method of choice to detect SARS-CoV-2 infections, in advanced cases of COVID-19 rectal swabbing can be performed. In our institution we did not perform rectal swabbing [37].

Seroconversion appears to happen after 7 days of a symptomatic infection in 50% of the cases (14 days in total), but this is not followed by a rapid decrease of the viral load [38,39]. Serology may play an important role in the epidemiology of COVID-19, however, even as serology could be useful in confirming an infection retrospectively, in our institution, as of today, it’s not considered a standard practice to do routine serologic testing in patients with an initial negative RT-PCR test [40,41].

We found that 63.15% of patients sought medical care 6 days or later after the beginning of symptoms, all of which could have contributed to the false negative results of the RT-PCR Test.

Patients who died of the disease with a persistent negative RT-PCR test presented with the following characteristics: 100% male with initial lypmphopenia, elevated D-Dimer and LDH on admission, history of smoking, high body mass index (BMI) and the presence of comorbidities like Systemic Hypertension or Type 2 Diabetes and they also presented with severe disease according to the tomographic evaluation of COVID-19 (Figure 1).

84.21% of the study patients were discharged due to clinical improvement.

**Conclusion**

Patients with COVID-19 and a persistently negative RT-PCR test with fatal outcomes did not differ from the rest of the COVID-19 population since they present with the same characteristics shared by the rest of characteristics patients like lymphopenia, comorbidities, elevation of D-Dimer and DHL on admission as well as a tomographic COVID-19 score of severe illness, however we could suggest that the percentage of patients with a mild form of the disease is higher in those with a persistently negative RT-PCR test.

**Limitations of the study**

Not a multicenter study

The management of patients was not homogeneous beyond supportive measures for COVID-19 symptoms. During the study period some of the patients were included in other research projects.

We did not performed serologic testing at discharge to document IgG immunity against SARS-CoV-2 as a proof that discharged patients had either truly presented with an infection by the novel coronavirus or had immunity to it.

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