

Discrepant Results of Molecular RT-PCR Tests in Patients with COVID-19 Infection

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TO THE EDITOR,

The reference diagnostic test for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the reverse transcription polymerase chain reaction (RT-PCR), a molecular assay performed on a rhinopharyngeal swab or broncho-alveolar lavage fluid. The viral kinetics show that the virus cannot be easily isolated in the respiratory tract until it reaches a detectable load (usually 24–48 hours before the onset of symptoms). Subsequently, positive results of RT-PCR tests can be found within the first 3 weeks of infection; from the 4th to 6th week the positivity of rhinopharyngeal swabs or deep materials progressively decreases [1].

A variety of viral RNA gene targets are used by different manufacturers of molecular tests: genes that code for envelope (E), nucleocapsid (N), spike proteins (S), RNA-dependent RNA polymerase (RdRp), and the open reading frame (ORF1a). Comparative studies have shown that the sensitivities of the tests to the individual genes between the different RT-PCR methods are similar, with the exception of the RdRp-SARSr primer probe, which has a slightly lower sensitivity. The protocol used for standard methods allows for searching E, N, and RdRp genes, a profile considered highly specific and more sensitive with respect to that of rapid methods, which use the S and/or ORF1 gene. A molecular test is considered positive when the amplification is detected at a threshold cycle

lower than 40 and, as indicated in the WHO recommendations, in a pandemic situation the positivity of a single gene is sufficient to classify the positive test.

The state-of-art on this topic is emerging and highlights different situations: the positivity of the RT-PCR test can persist 3 weeks after the onset of the disease, although after that period most cases demonstrate a negative result. In some cases, viral RNA can be detected by RT-PCR even beyond the 6th week after the first positive test [1]. In other cases, positivity is detected in samples taken 24 hours after two consecutive negative RT-PCR tests [2]. In a study of nine patients, the efforts in isolating the cultured virus were unsuccessful beyond the 8th day after the onset of the disease. This finding could be related to the decline in viral load [1]. However, it should be considered that a positive RT-PCR result only reflects detection of viral RNA and is not necessarily indicative of the presence of viable virus.

From 20 March to 20 April 2020, at the peak of the pandemic in Italy, we processed 15,664 swabs of 8864 patients. Positive RT-PCR test results were detected in 2009 (12.8%) swabs from 1155 patients (13%). Samples were taken in symptomatic patients referred to the emergency room or at the time of hospital admission and in asymptomatic patients (hospitalized patients without evidence of COVID-19 infection, individuals on quarantine for close contact with infected patients, guests and health workers of assisted healthcare residences) according to the surveillance protocols issued by the Italian Ministry of Health. Monitoring was stopped in most of the patients with positive test results due their negativization (two negative swabs performed 24 hours apart). However, in 35 cases (3% of positive patients) we observed discrepant results in the swabs performed in the same patients at different times. These were positive patients who underwent prolonged hospitalization or re-hospitalization or periodic checks for scheduled health surveillance. In these 35 cases, which were characterized by discrepant molecular test

results, the gene profile detected identified three different patterns:

- Re-positivization for a single gene
 We found 22 (63%) patients with a diagnosis of CoViD-19 infection confirmed by multiple positive RT-PCR tests who had negativized (two negative swabs performed 24 hours apart, absence of symptoms and/or resolution of the pulmonary picture) and who were repositivized after 15–20 days for a single gene, almost always the N gene. In this case the patients represented all the three study cohorts (prolonged hospitalization, re-hospitalization, supervised health workers). The fact that after two negative swabs only the N gene reappeared suggests that it could have a later clearance from the cells of the oropharynx and from the secretions of the respiratory tree. This situation has been widely described in Chinese healthcare workers discharged from hospitalization or quarantine after two negative results of the RT-PCR test and resolution of the clinical picture who, after 2 weeks, returned a positive test in the absence of symptoms [2]. Also, in our cases all re-positivized patients were asymptomatic; therefore, it cannot be excluded that RNA particles were non-viable.
- Re-positivization for multiple genes
 Eight patients (23%) consisting of supervised health workers and re-hospitalized patients who had negativized (two negative swabs after 24 hours) were found to be positive to at least two target genes (some to three) after 7–15 days. Re-positivization of the RT-PCR tests with at least two positive targets after multiple negative swabs in afebrile and asymptomatic patients suggests a new contact with the virus, as reported by Wang [3]. The results of these studies suggest the hypothesis of reappearance of SARS-CoV-2 without the specific clinical characteristics of primary infections [4].

- Re-positivization after a few days (for one or more genes)

This occurrence was found in five patients (14%) who tested positive for one or more target genes, who became negative after 24 hours and showed a positive gene profile at the next swab, also carried out after 24 hours, similar or identical to the initial one. In this case, given the very short interval between the different findings, the negativity of the intermediate swab could be due to: a defect in the sampling technique, in particular of the rhinopharyngeal swabs that must be performed in depth at the meeting point between the secretions coming from the posterior rhinopharynx and the respiratory secretions raised by the tracheobronchial tree; a fluctuating viral load because the viral cycle occurs in the alveolar lung cells where the virus binds to the angiotensin converting enzyme-2 (ACE-2) and dipeptidyl peptidase (DPP) receptors on ciliated bronchial cells and on type

II pneumocytes, respectively; or the absence of respiratory tract secretions that contain viral RNA.

From our data, which showed discrepant results in 3% in molecular tests of samples performed on the same patients after some time, the following considerations can be determined:

- Negative and declared healed patient should undergo further molecular investigation only in the presence of clinical symptoms
- A patient, even with a negative at the RT-PCR test who was declared cured, should adopt the same precautions and behaviors as the patient who never came in contact with the virus (possible re-infection/re-activation, state of healthy carrier)

In the next months, a post-pandemic scenario could be outlined by a higher frequency of cases with low viral load with a greater number of discrepant results due to the need to intercept positive

cases to limit the infection. Knowledge of the different possible patterns is important and it is desirable that the WHO may indicate common behavior guidelines to be followed by National Health Systems and by microbiology laboratories.

References

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In the end, it's not the years in your life that count. It's the life in your years.

Abraham Lincoln (1809-1865), American statesman and lawyer

who served as the 16th president of the United States from 1861 until his assassination in 1865

Capsule

Early high-titer plasma therapy to prevent severe COVID-19 in older adults

Therapies to interrupt the progression of early coronavirus disease-2019 (COVID-19) remain elusive. Among them, convalescent plasma administered to hospitalized patients has been unsuccessful, perhaps because antibodies should be administered earlier in the course of illness. Libster and associates conducted a randomized, double-blind, placebo-controlled trial of convalescent plasma with high IgG titers severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in older adult patients within 72 hours after the onset of mild COVID-19 symptoms. A total of 160 patients underwent randomization. In the intention-to-treat population, severe respiratory disease developed in 13 of 80 patients (16%) who received convalescent plasma and

25 of 80 patients (31%) who received placebo (relative risk 0.52, 95% confidence interval [95%CI] 0.29-0.94; $P=0.03$), with a relative risk reduction of 48%. A modified intention-to-treat analysis that excluded six patients who had a primary endpoint event before infusion of convalescent plasma or placebo showed a larger effect size (relative risk 0.40, 95%CI 0.20-0.81). No solicited adverse events were observed. The authors concluded that early administration of high-titer convalescent plasma against SARS-CoV-2 to mildly ill infected older adults reduced the progression of COVID-19.

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 Eitan Israeli