به نام خدا
Reactivation and Re-infection in Covid-19

ارائه دهنده: مجتبی راستی

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Possible reasons for re-positive SARS-CoV-2

- Multiple positive results in a patients after recovery, is a problem in Covid-19 pandemic.
- Re-positive result can put high pressure on patients psychologically and financially.
- Re-positive results might probably be due to reactivation, reinfection, viral shedding, or testing errors.
- Does patients really recovered?
- Standard criteria for patient discharge: Normal temperature (<38°C) for more than 72 h, A notable improvement in respiratory symptoms, Clear acute exudative lesions of chest computed tomography, Two consecutive negative results for RT-PCR carried out at least 24-h apart, Hospital care no longer required, Specific IgG appearance by a serological test.
highest percentage of positive rate has been detected from bronchoalveolar lavage (BAL) specimen (93%), followed by sputum (72%), nasal swab (63%), fibrobronchoscope brush biopsy (46%), pharyngeal swab (32%), feces (29%), and blood (1%).

the results of swab testing depend on many factors including time of swab sampling after initial symptom onset, site of the swab, transport of swab, and techniques used in swab collection, clinicians must not rule out COVID-19 in a highly suspected patient coming from an epidemic zone.

Between days 1 to 4 of disease (before symptoms) false negative is 100% in first day to 67% in fourth day. In fifth day and after symptoms appears, false negative is about 38% and in eighth and ninth day it reaches to 20% and 21% respectively. False negative reaches to 66% in day 21 after virus exposure.

CT scan findings have aided in diagnosing many such initial false-negative RT-PCR patients. Ai et al. in their retrospective study on 1014 patients who had RT-PCR from throat swab and CT chest done showed a sensitivity of 97% for chest CT in those having initial positive PCR results (95%CI, 95-98%; 580/601 patients), and it also showed a positive CT findings in 75% patients with initial negative PCR result.
Traditional Fiber Swab
Sample diffuses and becomes trapped in the fiber mattress

Flocked Swab
Liquid sample stays close to the surface and elutes out rapidly and spontaneously

Flocked Swab
Velvet brush-like texture efficiently dislodges and collects infected respiratory epithelial cells
COVID-19 Swab Sample Locations:

- Anterior Nares
- Mid-turbinate
- Nasopharyngeal
- Oropharyngeal
Before symptom onset

Detection unlikely

After symptom onset

PCR - Likely positive

PCR - Likely negative

Antibody detection

Week -2  Week -1  Week 1  Week 2  Week 3  Week 4  Week 5  Week 6

Symptom onset

Nasopharyngeal swab PCR
Bronchoalveolar lavage/sputum PCR
IgM antibody
IgG antibody
Virus isolation from respiratory tract
Stool PCR
Re-Infection

- Reinfection: SARS-CoV-2 subsequent infection after recovery from previous episode of the infection (2 or 3 month later).

- Overall, (i) if the time lag between discharge and RP of SRAS-CoV-2 is at most 28 days, these might be reactivation or relapse of previous infection; (ii) if the time lag is 2 months, it is more likely to be reinfection; and (iii) if the time lag is 3 months or above, it is very likely to be true reinfection.

- However, the most reliable way is to perform sequencing twice and get two different strains of the virus.

- In following study “Assessment of SARS-CoV-2 reinfection 1 year after primary infection in a population in Lombardy, Italy. Vitale et al. JAMA Internal Medicine (May 28, 2021)” just 45 person verified as reinfection case among 15000
guidelines to ensure the identification of re-infection cases. Specifically, CDC states that ‘a gold-standard confirmation of SARS-CoV-2 re-infection will require confirmation of initial infection and virus detection across two distinct time periods with genetic sequencing data.

One of the most reported mutation to the SARS-CoV-2 Spike (S) protein is D614G. This mutation is thought to facilitate the infectivity of the virus, possibly by increasing the binding affinity between the spike protein and the host cell receptor, ACE2. now they account for over 90% of infections.

Re-infection is well-documented for the known ‘common-cold’- causing coronaviruses, e.g. hCoV-OC43 and hCoV-HKU1, with an estimated period of 45 weeks of protective immunity.

Variables such as the virus load, changes in the person’s overall health, occurrence of antibody-dependent enhancement (ADE), and differences between variants of SARS-CoV-2 could all affect the severity of a re-infection
Can immunity end the SARS-CoV-2 pandemic?

Early in the pandemic, an amino acid mutation (D614G) in the original Wuhan strain was identified in the highly antigenic SARS-CoV-2 spike (S) glycoprotein. Despite being rare at the time of identification (in March 2020), it quickly became dominant, with variants encoding the mutation now accounting for over 90% of cases worldwide. The D614G signature separates SARS-CoV-2 into two major clades: 19D, which includes the original Wuhan isolate from 2019, and 20G.

Upon exposure to SARS-CoV-2, the body mounts an anti-viral response, depending on several factors, which include the viral load, variant, and the patient's health status. This response may vary. Typically, clearance of the infection requires a coordinated innate and adaptive immune response that produces virus-specific antibodies, including long-lasting IgGs. A memory B cell response is also generated for future protection. However, if the initial SARS-CoV-2 infection is cleared without the production of IgG antibodies or the formation of a memory B cell reservoir, the patient may be susceptible to re-infection. As the pandemic continues, there is a number of reported cases of confirmed re-infection by genetically different SARS-CoV-2 variants.

With over 30 million cases of COVID-19, re-infection cases are still considered rare events. Close monitoring and a better understanding of re-infection cases are essential for the development of an effective and long-lasting vaccine.
Re-activation

- **Reactivation** is the process by which a latent virus switches to a lytic phase of replication.

- A latent viral infection is a type of persistent viral infection which is distinguished from a chronic viral infection. Latency is the phase in certain viruses' life cycles in which, after initial infection, proliferation of virus particles ceases.

- However, the viral genome is not eradicated. The virus can reactivate and begin producing large amounts of viral progeny (the lytic part of the viral life cycle) without the host becoming re-infected by new outside virus, and stays within the host indefinitely.
Episomal latency: refers to the use of genetic episomes during latency. In this latency type, viral genes are stabilized, floating in the cytoplasm or nucleus as distinct objects, either as linear or lariat structures. One example is herpes virus family, Herpesviridae.

Proviral latency: A provirus is a virus genome that is integrated into the DNA of a host cell. One of the best-studied viruses that does this is HIV.

Latency is generally maintained by viral genes expressed primarily during latency.
Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of human cells

- Reactivation in SARS-CoV-2: also known as relapse, a re-detectable positive SARS-CoV-2 viral RNA in recovered patient which occurs within the first 4 weeks of previous infection.
- Some studies reported that some parts of SARS-CoV-2 genome integrated into the human genome.
- We found target site duplications flanking the viral sequences and consensus LINE1 (long interspersed nuclear element 1).
- We also found this integration in some patient-derived tissues. we have detected only sub-genomic sequences derived mainly from the 3’ end of the viral genome integrated into the DNA of the host cell.
- nonretroviral RNA virus sequences have been detected in the genomes of many vertebrate species such as vesicular stomatitis virus or lymphocytic choriomeningitis virus (LCMV)
expression of endogenous LINE1 and other retrotransposons in host cells is commonly up-regulated upon viral infection, including SARS-CoV-2 infection.

A majority of chimeric junctions mapped to SARS-CoV-2 nucleocapsid (N) sequence. This is consistent with the finding that nucleocapsid (N) RNA is the most abundant SARS-CoV-2 sub-genomic RNA, and thus is most likely to be a target for reverse transcription and integration.

it has been estimated that only between 1 in 1,000 and 1 in 100,000 mouse cells infected with LCMV show integration.

One recent study reported that such events are, at most, extremely rare in vivo, and therefore are unlikely to drive oncogenesis or explain post-recovery detection of the virus.
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