What should be the ideal definite COVID-19 case definition?

Oguz Resat Sipahi*

*Ege University Medical Faculty, Department of Infectious Diseases and

Clinical Microbiology, Bornova, Izmir, Turkey

Corresponding author:

Ege University Medical Faculty,

Department of Infectious Diseases and

Clinical Microbiology

35100, Bornova, Izmir, Turkey

oguz.resat.sipahi@ege.edu.tr

oguz.resat.sipahi@gmail.com

Fax: 00902323904770

Tel: 00902323903305

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Coronavirus disease 2019-COVID-19 [novel coronavirus or new coronavirus or SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2)] has been influencing humanity very negatively since December 2019 [1-6]. This influence is probably more than any disease for living humans aged <100 who did not face the Spanish flu pandemic [3,7]. Globally, as of 11:54 AM (CEST), 5 July 2020, there have been 11.108.580 confirmed cases of COVID-19, including 527.835 deaths (4.75%), reported to World Health Organization [4]. Case-fatality rate estimates range from 0.6% to 7.2% by region and seem to be substantially higher than the 0.1% mortality rate of seasonal influenza [7].

Globally, the medical system, scientists as well as scientific publication systems worked enormously hard during this seven month period. Not only they cared millions of COVID-19 cases but also they already published 27.861 papers that are indexed in Pubmed database with the keyword “covid” by July, the 5th 2020. At least two treatment modalities ie. low dose steroid and remdesivir have been shown to improve the outcomes in randomized controlled studies [1,2,5,6].

COVID-19 diagnosis is currently based on routine RT-PCR (reverse transcriptase-polymerase chain reaction) +/- [clinical findings +/- chest radiology/computerized tomography-CT] [1,5,6,8-11]. RT-PCR is usually repeated on day 1 or 2 in cases, who has positive clinical findings? radiology positive but the first RT-PCR negative [1]. These cases create problems in the wards or ICUs. Even though we know that similar CT findings may also be seen in other viral respiratory diseases [11] and they are COVID-19 negative on the first swab/sample, should we treat these cases as they are COVID-19? On a series of 51 patients with chest CT and RT-PCR assay performed within 3 days of the initial presentation, the sensitivity of CT for COVID-19 infection was reported to be 98% compared to RT-PCR sensitivity of 71% ($p<.001$) on day 0 [12]. However, 15 of 51 cases with negative RT-PCR and

positive radiology at the initial presentation became RT-PCR positive between 1 and 7 days later. There is no detail about specificity of RT-PCR on that highly cited paper. Hence, moderate to severe cases are usually treated as they are COVID-19 in daily clinical practice.

Nearly all infectious agents contain DNA or RNA genomes, making sequencing an attractive approach for pathogen detection [16]. Metagenomic next-generation sequencing (NGS) is a promising approach for the diagnosis of infectious disease because a comprehensive spectrum of potential causes — viral, bacterial, fungal, and parasitic — can be identified by a single assay [13]. NGS is based on sequencing the whole microbial genetic material and finding with which microbe those sequences are compatible with [11,13-16]. Challenges of NGS include high cost, the length of time to results, and the need for technical and bioinformatics expertise. Furthermore, NGS data should be evaluated carefully especially in samples with normal flora. The cost of NGS has been reduced by several orders of magnitude since its advent in 2004, and it has emerged as an enabling technological platform for the detection and taxonomic characterization of microorganisms in clinical samples from patients [16]. NGS has been shown to improve the diagnosis in many important clinical problems such as meningitis, brain abscess, encephalitis, osteomyelitis, and pneumonia.

Could metagenomic analysis also help diagnosis in clinically and radiologically highly probable but COVID-19 RT-PCR negative cases? In the last issue of Clinical Infectious Diseases, Li et al [18] reported a very important COVID-19 case. She was a 57-year-old woman. who was a clinically and CT positive, but routine (as named by the authors) RT-PCR negative highly suspicious COVID-19 case. His husband was COVID-19 positive via routine RT-PCR but she had several consecutive negative RT-PCR swabs and other clinical sample results. The authors could not show the SARS-CoV-2 via viral culture. In addition, no data about the patient's contacts are given. However, metagenomic analysis of her

clinical samples via nanopore pathogen sequencing technology confirmed the presence of SARS-CoV-2. The virus of the patient's husband's virus is not compared to the patient's virus. Simultaneously, the ORF and NP (Nucleocapsid) gene variation of the infecting SARS-CoV-2 was shown. These genetic changes probably precluded the diagnosis via routine RT-PCR, which targeted the ORF1ab and NP gene. Furthermore, the micro-neutralization antibody (IgM and IgG) of SARS-CoV-2 from Guangdong CDC also was positive on hospital day 20. This important case report delineates that some of the CT positive but routine COVID-19 RT-PCR negative cases with highly suspected clinical findings may have mutated viruses. Furthermore, metagenomic analysis could improve the COVID-19 diagnosis in selected cases.

Critical data, which are not reported by Li et al [18], is the lack of the difference between the mutated nucleic acid sequence in the virus shown in the clinical sample of the presented case and the standard sequence used in routine (as specified in the paper) RT-PCR protocols. The difference is not mentioned probably due to the trade secrets. However, the traders with related secrets may check the difference between the defined mutations and the PCR sequences they use.

What should be the ideal definition of a COVID-19 case? We need a clearly defined -consensus-golden standard case definition and exclusion criteria to gain high quality data related to sensitivity and specificity of any diagnostic method for any infectious disease including COVID-19. However, there is no well-defined/consensus golden standard for the diagnosis as well as the exclusion of COVID-19 until now [1,8-10]. We need to ask and try to answer a few important questions for a consensus case definition. We may consider that one of the most important items, probably the most important one in such a definition, would be -as in every infectious disease- showing the presence of the infecting agent by viral culture. However, performing viral culture is limited to highly

equipped laboratories. Hence, confirming the presence of the viral nucleic acid in clinical samples via RT-PCR has been the most widely used method worldwide. Another question is regarding a case, who is symptom negative, radiology negative but RT-PCR positive. Could this be a false positive, if yes under which circumstances? Do we have to add serology to confirmed case definition for such cases? Should such a consensus definition require any symptom as a must or should we accept no symptom for the possible presence of totally asymptomatic cases [19]? Should we add this into consensus COVID-19 case definition; the viral culture-negative RT-PCR negative but metagenomic analysis -positive cases with or without symptoms or radiologic findings (such as in the paper of Li et al [18], which was confirmed also by serology)? Many experts should not oppose adding such cases into confirmed COVID-19 case definition. Unfortunately to my knowledge currently there is no study that uses both viral culture and metagenomic analysis and serology for evaluating diagnostic performance of routine RT-PCR.

An important question born by the presented case [18] is that we do not know how often the mutated viruses are present in the community. More frequent use of metagenomic approach in clinically/radiologically highly probable but routine RT-PCR negative cases -at least in centers with such technology- may help to find out the frequency of mutated viruses in clinical wards [11,18]. The possible additional diagnostic capacity of metagenomic analysis over routine RT-PCR may also be analysed in centers that are able to perform both techniques in a retrospective and/or prospective manner. If metagenomic analysis is not available, the question of whether mutated viruses are present in the community may also be checked-via implementing highly sensitive and specific serologic tests in CT positive routine RT-PCR negative cohorts. This may confirm the diagnosis retrospectively such as in the presented case by Li et al [18].

In conclusion, despite developments in medicine and infectious diseases, COVID-19 will continue to cause mortality. It is not easy or feasible to add metagenomic analysis into routine COVID-19 management flow chart [1,9]. Nevertheless, it may be recommended to add this powerful tool at least in national central laboratories. Although data related to the frequency of mutated variants described by Li et al [18] in the hospital settings are lacking, these defined mutations may increase the diagnostic capability of routine RT-PCR. Related societies should make a consensus related to definition of golden standard COVID-19 case criteria and definite exclusion criteria for evaluation of the diagnostic methods urgently. The International medical community may consider collaborating to create well-defined clinical sample collections that may also include metagenomic analysis, to facilitate well-defined diagnostic performance studies.

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