Consideration of the Cycle Threshold Values from Real-Time RT-PCR SARS-CoV-2 Interpretation for the Clinicians: Analysis of 339 Positive Cases from a Referral Laboratory in Jakarta, Indonesia

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ABSTRAK

Latar belakang: *real-time RT-PCR (rRT-PCR) merupakan metode yang direkomendasikan oleh WHO untuk diagnosis COVID 19. Nilai cycle threshold (Ct) diduga berkaitan dengan manifestasi klinis. Saat ini modalitas diagnosis lain untuk deteksi kuantitatif secara molekuler dan isolasi virus belum tersedia sebagai pemeriksaan rutin. Penelitian ini dilaksanakan untuk menganalisa hubungan antara nilai Ct dari rRT-PCR kualitatif dengan manifestasi klinis, serta mendeskripsikan faktor-faktor yang dapat mempengaruhi hasil pemeriksaan.* ***Metode:*** *Spesimen yang dikirimkan ke laboratorium rujukan pada bulan Maret sampai April 2020 dari berbagai institusi kesehatan menjadi sampel penelitian. Data karakteristik pasien dan manifestasi klinis diambil dari formulir penyidikan epidemiologi yang dikirim bersama spesimen. Spesimen diperiksa dengan metode rRT-PCR dan nilai Ct dikumpulkan. Data yang diperoleh diolah dengan uji statistik yang sesuai.* ***Hasil:*** *dari 339 hasil positif, diperoleh 176 (52%) kasus ringan-sedang dan 163 (48%) kasus berat. Wanita lebih banyak ditemukan pada kasus ringan-sedang (58%) sementara laki-laki lebih banyak pada kasus berat (60%). Median usia pada kasus ringan-sedang adalah 35 tahun dan kasus berat adalah 49 tahun. Terdapat hubungan antara manifestasi klinis dengan jenis kelamin ($p = 0.001$) dan usia ($p < 0.001$), tetapi tidak ditemukan hubungan antara nilai Ct dengan manifestasi klinis.* ***Kesimpulan:*** *situasi saat ini pada laboratorium rujukan dengan berbagai faktor pada pengambilan dan pemrosesan spesimen dapat mempengaruhi nilai Ct yang dihasilkan. Sebagai tambahan, respon imun pejamu merupakan faktor yang juga mempengaruhi tingkat keparahan penyakit, terlepas dari jumlah virus yang menginfeksi. Nilai Ct dari laboratorium rujukan antara pasien bergejala ringan-sedang dan berat tidak menunjukkan hubungan yang signifikan, dengan demikian interpretasi nilai Ct harus dilakukan dengan hati-hati.*

Kata kunci: *SARS-Cov-2, cycle threshold, rRT-PCR, interpretasi klinis.*

ABSTRACT

Background: *real-time RT-PCR was recommended by WHO for COVID-19 diagnosis. The cycle threshold (Ct) values were expected to have an association with clinical manifestation. However, the diagnostic modalities such as quantitative molecular detection and virus isolation were not yet available for the routine test. This study has been conducted to analyze the relationship between the Ct values of qualitative rRT-PCR and the clinical manifestation and to describe the factors determining the result.* ***Methods:*** *from March to April 2020, specimens were sent to our laboratory from different healthcare centers in Jakarta. The patient's characteristic and clinical manifestation were extracted from the specimen's epidemiology forms. The specimens extracted and tested using*

rRT-PCR, and the Ct value were collected. The data were analyzed using the appropriate statistic test. ***Results:*** *from 339 positive results, the mild to moderate case was 176 (52%) and the severe cases was 163 (48%). Female was dominant in the mild to moderate cases (58%), while the male was prevalent in the severe cases (60%). The median age for mild to moderate case was 35 years old and severe cases was 49 years old. Statistical analysis found relationship between both group with gender ($p = 0.001$) and age ($p < 0.001$), but not with the Ct value.* ***Conclusion:*** *many variables in specimen sampling and processing could affect the Ct value result. In addition, the disease's severity was depended with the host immune response, regardless the number of virus. There was suggested no significant difference between the Ct values of mild-moderate and severe COVID-19, and thus should not be loosely interpreted.*

Keywords: *SARS-Cov-2, Cycle Threshold, rRT-PCR, clinial interpretation.*

INTRODUCTION

Real-time reverse transcriptase (rRT) PCR has been recommended by WHO for SARS-CoV-2 detection in the recent guideline.[1] Several studies correlated cycle threshold (Ct) values with viral loads and disease severity.[2,3] Tom et al.[4] proposed Ct values to be considered in clinical decision making. However, publications from Canada and Singapore had different Ct values cutoff for infectivity.[5,6] The different result from the studies might come from the variety of approaches and methods of rRT-PCR and its Ct value interpretation.

The concept of Ct values inversely related to viral load is very tempting, especially for managing a patient's length of hospital stay during the pandemic. Han et al. addressed their concern for publications which used Ct values deliberately for viral quantification and correlated them with clinical manifestation.[7] Recently, many clinicians questioned the interpretation of Ct values for the patient management and disease control strategy. This study has been conducted to analyze the relationship between the Ct values of qualitative rRT-PCR and the clinical manifestation and to describe the factors determining the result.

METHODS

This cross-sectional study used the records in the epidemiology form of the specimens which sent to the Clinical Microbiology Laboratory of the Faculty of Medicine of Universitas Indonesia, from 13 March to 30 April 2020. Only specimens documented as the first sample with completed epidemiology form were included in this study for further analysis. Specimens documented as a follow up test were excluded. The clinical manifestation, gender, and age were collected. The clinical manifestation was grouped according to the criteria from WHO and the National Health Commission of People's Republic of China.[8] Therefore, the clinical manifestation was grouped as the mild to moderate case and the severe case.

The specimens were extracted with several methods of RNA extraction (Qiagen, Adbio, Da An, Viogen, Liveriver). All of the extraction kits were tested before use. The method used for SARS-CoV-2 detection was qualitative rRT-PCR. The reaction was shown following the commercial kit's protocol, with N gene and ORF gene as the target (Da An Gene, China). The Ct values under 40 for each gene were regarded as a positive result. Confirmatory rRT-PCR was conducted if the first reaction resulted in Ct values between 38 and 40. All data were analyzed using the appropriate test.

The variables collected were categorical and numerical data. The categorical variables were presented as number (percentage). The numerical variables were tested for normality and presented as mean (CI 95%, lower – upper) or median (interquartile range (IQR), number), depended on the normality result. The two categorical variables (clinical manifestation and gender) were analyzed with Chi-square test. The correlation between clinical manifestation and other numerical variables (age, Ct value), were assessed by one-way ANOVA or Kruskal-Wallis test, as indicated from normality test.

RESULTS

Patient characteristics were shown according to clinical manifestation in **Table 1**. Of 339

positive results, the mild to moderate cases contributed to 52% of all cases. The median age of mild to moderate cases was 35 years old (IQR, 25.25) and severe cases was 49 years old (IQR,22). The specimens were received from hospitals and other health care providers such as private clinics, primary health care, and laboratories. The median Ct values of all clinical manifestations were 34.7 (IQR, 5.33) for N gene and 35.4 (IQR, 5.23) for ORF gene.

There was an association between clinical manifestation and gender ($p = 0.001$). Female was more frequently found in the mild to moderate cases, while the male was prevalent in the severe cases (**Table 1**). There was a statistically significant difference of age in clinical manifestation as determined by Kruskal-wallis ($p < 0.001$). Older age was dominant in the severe cases, and younger age proportion were bigger in the mild to moderate cases (**Table 1**). Statistical analysis found no difference in the Ct value with the clinical manifestation ($p > 0.05$).

DISCUSSION

From Jakarta specimens, age and gender were related to clinical manifestation in concordance with other studies.[9,10] Advanced age was reported as the risk factor for hospital admission and male sex as the risk factor for severe disease.[11]

Table 1. Patients characteristics and the median Ct value according to clinical manifestation.

	Mild to moderate case	Severe case	p value
Age (years old), n (%)			
- < 10	2 (1.0)	1 (1.0)	0.000
- 10 – 19	15 (9.0)	4 (2.5.0)	
- 20 – 29	44 (25.0)	17 (10.0)	
- 30 – 39	43 (24.0)	23 (14.0)	
- 40 – 49	23 (13.0)	37 (23.0)	
- 50 – 59	30 (17.0)	41 (25.0)	
- ≥ 60	19 (11.0)	40 (24.5)	
Gender, n (%)			
- Male	74 (42.0)	98 (60.0)	0.001
- Female	102 (58.0)	65 (40.0)	
Ct value (Median, IQR)			
- N gene	35 (5.41)	34.49 (5.26)	0.874
- ORF gene	35.54 (5.72)	35.00 (4.92)	0.841
Total Cases, n (%)	176 (52.0)	163 (48.0)	

Regarding the Ct values and the clinical manifestation, no relationship was found between both subjects. It was similar to Tan et al's[12] report, in which Ct values were altered but could not be used to distinguish the disease severity. However, it was different from the previous studies by Yu et al.[2] and Zou et al.[3], suggesting the relationship between Ct values and disease severity. It was possible that the latter studies were conducted in a hospital laboratory where the specimen quality were highly supervised, or the swab materials, viral transport media, and the assay reagent came from similar manufacturer. In contrast, our specimens came from more than ten different hospitals and public-health centers in Jakarta. The specimens were collected at different times and stored for a while before delivery. Every health center had fluctuating swab supplies from different manufacturers and had health-care workers with different level of experience in swabbing the patients. From our observation, the variation of types and volume of virus transport medium (VTM) also became noticeable with the increasing number of specimens. These uncontrolled factors could affect the specimen and determine the test results.[13]

In this pandemic situation, the laboratory also had unstable supply of RNA extraction kits, and different methods were used in one month. This condition made a Ct values from the first test was unable to be compared with the follow up test because the specimen processing was not equal. In the combination with the sampling process and transportation, each extraction kit also generated a different number of extracted RNA, despite the correct methods. These factors could affect the PCR reaction and the Ct value result might confuse the clinicians when they got a lower Ct value from a follow up test, especially when the patient's general condition was stable or showed no sign of infections. Since some studies mentioned the correlation of infectivity with Ct values,[5,6] the current unstable Ct value results could create doubt when the clinicians were asked to release the recovered patients from the isolation state. In addition, the agreement on a quantification unit for every type of specimen was not yet established.

For respiratory samples using flocked swab, the viral load could be normalized by volume (RNA copies/ml of transport medium) or by cell number (RNA copies/median number of cells).[14] We also noticed since the study was conducted, several latest PCR kits also had different Ct values cutoff for positive results. This condition would affect the Ct values in the follow up test because of the different cutoff. Furthermore, a state of emergency made most of the referral laboratories employ many inexperienced technicians. These limitations would create slight alteration of Ct values between batches, in line with Han et al.'s notification about the batch effect on Ct values from rRT-PCR.[7]

Besides the technical problem, the unclear onset of diseases and the pathogenesis of COVID-19 also could affect the number of virus particle obtained from the patient's body. Although we already limited the specimen to the initial confirmed-specimen of the patients, currently we do not have any diagnostic tools or scoring instrument to assess whether the patients are in the early or the later stage of diseases. In addition, the diseases progressivity, the patient's immune response, and the viral clearance are different in every individual.[15] These factors could explain our result, which there was no difference of Ct value between the mild to moderate case and the severe case.

The limitation of this study was the short period of time to do the observation, when the situation was rapidly changing every month. Our findings represented the unstable nature of the pandemic situation, hence not applicable for different situations. Until this paper was made, there was no guideline in Indonesia for the quantification unit and Ct value standarization. There was also no current report on the correlation between Ct values and infectivity from the specimens in Indonesia. In addition, the variety of host immune response to SARS-CoV-2 infection also affected the clinical outcome. Therefore, clinicians should not consider Ct value results from rRT-PCR for patient management. Clinical decisions should be made through comprehensive assessment of patient's condition, other laboratory tests, and radiologic findings.

CONCLUSION

Ct values of rRT-PCR generated from a referral laboratory during a pandemic are not suitable as the additional data for disease control strategy, as long as the uncontrolled variables persisted. Future studies may include advances in virus quantification and development of regulation to reinforce the validation of the PCR kit performance before widely used for diagnostic test.

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CONFLICT OF INTEREST

Declarations of interest: none.

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