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Prevalence of COVID-19 at the Wahgnion-Gold mining site in Burkina Faso and use of RT-PCR initial cycle threshold to monitor the dynamics of SARS-CoV-2 load

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Abstract:

Background: To control the spread of coronavirus disease-19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), it is necessary to adequately identify and isolate infectious patients particularly at the work place. Real time polymerase chain reaction (RT-PCR) assay is the recommended confirmatory method for the diagnosis of SARS-CoV-2 infection. The aim of this study was to determine the prevalence of SARS-CoV-2 infection in Burkina Faso and to use the initial cycle threshold (Ct) values of RT-PCR as a tool to monitor the dynamics of the viral load.

Methodology: Between September 2021 and February 2022, oropharyngeal and/or nasopharyngeal swab samples of consecutively selected COVID-19 symptomatic and apparently healthy workers from the Wahgnion mining site in the South-western Burkina Faso who consented to the study were collected according to the two weeks shift program and tested for SARS-CoV-2 using RT-PCR assay. Patients positive for the virus were followed-up weekly until tests were negative. Association of the initial RT-PCR Ct values with disease duration was assessed by adjusted linear regression approach. Two-sided p value < 0.05 was considered statistically significant.

Results: A total of 1506 (92.9% males) participants were recruited into the study, with mean age and age range of 37.1 ± 8.7 and 18-68 years respectively. The overall prevalence of SARS-CoV-2 infection was 14.3% (216/1506). Of the 82 patients included in the follow-up study, the longest duration of positive RT-PCR test, from the first positive to the first of the two negative RT-PCR tests, was 33 days (mean 11.6 days, median 10 days, interquartile range 8-14 days). The initial Ct values significantly correlated with the duration of RT-PCR positivity (with β=-0.54, standard error=0.09 for N gene, and β=-0.44, standard error=0.09 for ORF1ab gene, $p<0.001$). Participants with higher Ct values corresponding to lower viral loads had shorter viral clearance time than those of lower Ct values or higher viral loads.

Conclusion: Approximately 1 out of 7 tested miners had SARS-CoV-2 infection and the duration of their RT-PCR tests positivity independently correlated with the initial viral load measured by initial Ct values. As participants with lower initial Ct values tended to have longer disease duration, initial RT-PCR Ct values could be used to guide COVID-19 patient quarantine duration particularly at the work place.

Keywords: SARS-CoV-2, COVID-19, RT-PCR, cycle threshold, prevalence, Burkina Faso

Prévalence du COVID-19 sur le site minier de Wahgnion-Gold au Burkina Faso et utilisation du seuil de cycle initial de RT-PCR pour surveiller la dynamique de charge du SARS-CoV-2

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Résumé:

Contexte: Pour contrôler la propagation de la maladie à coronavirus 19 (COVID-19) causée par le syndrome respiratoire aigu sévère coronavirus-2 (SRAS-CoV-2), il est nécessaire d'identifier et d'isoler de manière adéquate les patients infectieux, en particulier sur le lieu de travail. Le test de réaction en chaîne par polymérase en temps réel (RT-PCR) est la méthode de confirmation recommandée pour le diagnostic de l'infection par le SRAS-CoV-2. Le but de cette étude était de déterminer la prévalence de l'infection par le SRAS-CoV-2 au Burkina Faso et d'utiliser les valeurs du seuil initial du cycle (Ct) de la RT-PCR comme outil de suivi de la dynamique de la charge virale.
Méthodologie: Entre septembre 2021 et février 2022, des écouvillonnages oropharyngés et/ou nasopharyngés de travailleurs symptomatiques COVID-19 et apparemment en bonne santé sélectionnés consécutivement du site minier de Wahgnion dans le sud-ouest du Burkina Faso qui ont consenti à l'étude ont été prélevés selon les deux programme de quart de semaines et testé pour le SRAS-CoV-2 à l'aide d'un test RT-PCR. Les patients positifs pour le virus ont été suivis chaque semaine jusqu'à ce que les tests soient négatifs. L'association des valeurs Ct initiales de la RT-PCR avec la durée de la maladie a été évaluée par une approche de régression linéaire ajustée. Une valeur p bilatérale < 0,05 a été considérée comme statistiquement significative.
Résultats: Un total de 1506 participants (92,9% d'hommes) ont été recrutés dans l'étude, avec un âge moyen et une tranche d'âge de 37,1 à 8,7 ans et de 18 à 68 ans, respectivement. La prévalence globale de l'infection par le SRAS-CoV-2 était de 14,3% (216/1506). Sur les 82 patients inclus dans l'étude de suivi, la plus longue durée de test RT-PCR positif, du premier test positif au premier des deux tests RT-PCR négatifs, était de 33 jours (moyenne 11,6 jours, médiane 10 jours, intervalle interquartile 8-14 jours). Les valeurs Ct initiales étaient significativement corrélées à la durée de positivité de la RT-PCR (avec $\beta=-0{,}54$, erreur standard=0,09 pour le gène N et $\beta=-0{,}44$, erreur standard=0,09 pour le gène ORF1ab, $p<0{,}001$). Les participants avec des valeurs de Ct plus élevées correspondant à des charges virales plus faibles avaient un temps de clairance virale plus court que ceux avec des valeurs de Ct plus basses ou des charges virales plus élevées.
Conclusion: Environ 1 mineur testé sur 7 était infecté par le SRAS-CoV-2 et la durée de la positivité de ses tests RT-PCR était indépendamment corrélée à la charge virale initiale mesurée par les valeurs Ct initiales. Comme les participants avec des valeurs Ct initiales inférieures avaient tendance à avoir une durée de maladie plus longue, les valeurs Ct initiales de la RT-PCR pourraient être utilisées pour guider la durée de la quarantaine des patients COVID-19, en particulier sur le lieu de travail.

Mots clés: SRAS-CoV-2, COVID-19, RT-PCR, seuil de cycle, prévalence, Burkina Faso

Introduction:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing the 2019 coronavirus disease pandemic (COVID-19) was first identified in December 2019 in Wuhan, China (1,2). The outbreak subsequently spread worldwide, affecting over 522 million persons globally with approximately 6.25 million deaths as of May 22, 2022 (3,4). The first case in Burkina Faso was identified on March 9, 2020, and as of 20 June 2022, 21,044 cases and 387 deaths were reported according to the country's health authority records (5).

To counter the spread of the disease and limit its public health implications, the World Health Organization (WHO) cautioned the public to take responsive care and the public care strategies have included handwashing, wearing of face masks, physical distancing, avoiding mass gathering and assemblies, and total lockdowns, which have flattened the transmission curve allowing the reopening of public services (3). Testing has been crucial to track the spread of the disease during the pandemic, and to swiftly implement the needed public health interventions such as isolation, quarantine, and appropriate clinical management of cases.

Real-time reverse-transcription polymerase chain reaction (RT-PCR) test, which is a nucleic acid amplification test (NAAT) to detect viral nucleic acid, has routinely been used to detect SARS-CoV-2 in oropharyngeal or nasopharyngeal swabs of individuals suspected of COVID-19, and currently represents the 'gold standard' diagnostic methods for COVID-19 (6). Different viral target gene structures, including the nucleocapsid (N) and the open reading frame (ORF) 1 (O) gene, have been used for the diagnosis of SARS-CoV-2 infection (7). During

the amplification process in RT-PCR assay, cycle threshold (Ct) values are used to assess the viral nucleic acid amplification for the target gene structure, and values that cross the threshold are used to discriminate positive from negative samples (8).

Although case diagnosis and optimal quarantine strategies have been an important component for the public health strategies of SARS-CoV-2 transmission prevention (9), the procedure consists of several steps, and needs laboratory equipment that makes the process tedious and difficult to be conducted outside the laboratory setting particularly in resource cons-traint settings (10). Thus, finding ways to red-uce the burden of samples to be tested would substantially reduce the work load, and thus allow a more efficient use of resources in sub-Saharan African countries.

Exploring the relationship between the initial PCR Ct values could tentatively indicate the duration of the PCR test positivity, and thus help eliminate unnecessary testing. However, research studies assessing the association of the initial PCR Ct values and the duration of the PCR test positivity are uncommon and included very limited sample size which make the estimates less precise (11). The aim of this study is to determine the prevalence of SARS-CoV-2 infec-tion at a mining site in Burkina Faso, and assess the initial PCR Ct values as an indirect measure of the dynamics of the viral load in COVID-19 patients.

Materials and method:

Study area and design
This was a prospective surveillance stu-dy integrated to the work shift of mine workers at the Wahgnion-Gold mining site in south-western Burkina Faso. Miners were required a negative PCR test before returning to their work place. All apparently healthy or COVID-19 sym-ptomatic miners who consented to participate and adhere to the study procedures were syste-matically included in a consecutive and sequen-tial manner as they visited the COVID-19 testing center located on the mining site over the study period between August, 2021 and January, 2022.

Ethics approval and consent to participate
This was a pandemic response/surveil-lance data and the study protocol ethical clear-ance was obtained from the national ethics com-mittee of health research of Burkina Faso (clear-ance certificate number CERS-2020-7-126). In addition, data were fully anonymized to protect participants' identities and personal data. Usage

was done in accordance ethical regulations.

Sample and data collection
For each participant, oropharyngeal (OP) or nasopharyngeal (NP) swabs were collected, and participants socio-demographic and medical characteristics were collected using interviewer-administered data collection form. All patients tested positive for SARS-CoV-2 were included in the follow-up study if they agreed to participate and had their follow-up swabs assessed by RT-PCR. Follow up swabs were collected each 3-7 days interval according to the patient availability until two consecutive negative RT-PCR test res-ults were obtained.

Sample management and RNA extraction
Samples were stored in universal trans-port medium (Copan Diagnostics) and transfer-red to the National Influenza Reference Labora-tory (NIRL) for analysis. Viral RNA was manually extracted from 200µL of virus transport medium containing NP and OP swabs by using QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany), and eluted into 60 µL elution buffer according to the manufacturer's instruction. The eluted RNAs (templates for RT-PCR) were stored immedia-tely at minus 80°C until use.

SARS-CoV-2 RT-PCR assays
Two different real-time reverse transcri-ption polymerase chain reaction (rRT-PCR) as-says; the Viasure SARS-CoV-2 RT-PCR detection kit (Certest Biotec SL., Spain) and the detection kit for 2019 novel coronavirus (2019-nCoV) RNA (PCR-fluorescence probing) manufactured by Da An Gene Co., Ltd. of Sun Yat-sen University, were used to detect N and ORF 1ab genes using the Applied Biosystems 7500 RT-PCR instrument (Thermo Fisher Scientific). The Da An Gene co kit was used for initial detection of COVID-19 positive subjects. Positive samples were further retested with the Viasure kit to establish the baseline cycle threshold (Ct) values and patients were followed up until the time of the first nega-tive test.

The detection kit for 2019 novel corona-virus (2019-nCoV) RNA of Da An Gene Co., Ltd has the 2019-nCoV primer and probe sets desi-gned to detect N and ORF1ab gene sequences. Further, human housekeeping gene RNP (Ribo-nuclease P) was developed as the target gene for the internal control for monitoring the speci-men collection, nucleic acid extraction and PCR amplification processes, to reduce occurrence of false negative results. Each 25µL of reaction mix contained 17µL of NC (ORF1ab/N) PCR reaction solution A, 3µL of NC (ORF1ab/N) PCR reaction solution B, and 5µL of RNA. Thermal cycling was performed at 50°C for 15min and 95°C for 15

min followed by 45 cycles of 94°C for 15 seconds and 55°C for 45 seconds. Each run included NC (ORF1ab/N) negative control and NC (ORF1ab/N) positive control. Specimens with a cycle threshold (Ct) value ≤ 40 for SARS-CoV-2 ORF1ab and N gene targets were considered positive.

The Viasure SARS-CoV-2 RT-PCR detection kit contains in each well of the PCR plate all the components necessary for RT-PCR assay (specific primers/probes, dNTPs, buffer, polymerase and retro-transcriptase) in a stabilized format, as well as an internal control to monitor PCR inhibition. Briefly, each 20µL of reaction mix contained 15µL mix of enzymes, primers, probes, buffer, dNTPs, stabilizers and internal control in stabilized format which is reconstituted of the rehydration Buffer and 5µL of RNA. Thermal cycling was performed at 45°C for 15min and 95°C for 02 m, followed by 45 cycles of 95°C for 10 seconds and 60°C for 50 seconds. Each run included reconstituted SARS-CoV-2 positive control and negative control.

A sample is considered positive if the Ct value obtained is less than 38 for SARS-CoV-2 ORF1ab and N genes or ORF1ab SARS-CoV-2 gene only and the internal control shows or not an amplification signal. If N target only was positive, the interpretation was SARS-CoV-2 presumptive positive and additional confirmatory testing was conducted by the reference laboratory.

Statistical analysis

Data were entered onto an Excel database and transferred onto R statistical software (R Development Core Team, 2021) for statistical analysis. We carried out a patient-level analysis by first describing the distribution of their sociodemographic and medical characteristics. To assess the association of patients COVID-19 initial RT-PCR Ct values with duration of the illness, we calculated the duration as the difference between the initial diagnosis date and the date of the first of two negative test results. Next, we modeled the association using adjusted general linear regression models to calculate effect estimates and 95% confidence interval (CI) per Ct value. Means were compared by the *t*-test and medians were compared by Wilcoxon exact test. Statistical significance was determined at two-sided *p* value < 0.05.

Results:

Characteristics of study participants

A total of 1506 participants were tested, with a male predominance (ratio of 13:1), mean age of 37.1 ± 8.7 years and age range of 18-68 years. The city of Bobo-Dioulasso was the most represented residency area (58.2%) with the others, representing 19.5%, 10.6%, 7.5%, and 2.5% for Niankorodougou, Sindou, Banfora, and Ouagadougou respectively. Majority of the study participants (82.7%) were nationals from Burkina Faso with others (17.3%) being expatriate workers.

The oropharyngeal and nasopharyngeal swabs represented 60.2% and 39.6% respectively of the total samples and two participants had mix (nasopharyngeal and oropharyngeal) swabs (Table 1).

Prevalence of SARS-CoV-2 infections

The overall prevalence of SARS-CoV-2 infection was 14.3% (216/1506). Participants from the cities of Sindou, Bobo-Dioulasso, and Niankorodougou, were the most infected with 22.6%, 15.3% and 13.3% of their respective total participants. The prevalence of SARS-CoV-2 infections was not significantly different according to participants gender (13.1% for female and 14.4% for male, *p*=0.9) or the type of swab samples collected (18.4% vs 11.7%, *p*=0.32).

Cycle threshold values and duration of disease

Of the 82 participants positive for SARS-CoV-2 and who had RT-PCR follow-up data available, the median duration from initial positive PCR test results to the first results of the two consecutive negative PCR results was 10 days, with range of 3-33 days. Half of the participants had their duration between 8 and 14 days (first and third quartiles). The association of initial Ct values with the duration of the illness is shown in Fig 1 for the N-gene and Fig 2 for the ORF 1ab gene.

The duration of positive RT-PCR test was negatively correlated with the initial Ct value. The longest duration of positive RT-PCR was 33 days from the first positive RT-PCR test results. The mean duration was 11.6 days from initial positive RT-PCR test to the first of the two consecutive negative RT-PCR tests, and for each additional Ct value, the average illness duration significantly decreased by 0.54 day for the N-gene and 0.44 day for the ORF1ab gene (*p*< 0.001) when adjusted with the patient age, gender, and type of samples (nasopharyngeal or oropharyngeal).

Table 1: Characteristics of study participants at the Wahgnion-Gold mining site in Burkina Faso

Characteristics	Measured statistic
Age (years)	
Mean age (mean ± SD)	37.1± 8.7
Age range	18-68
Gender	
Male, n (%)	1399 (92.9)
Female, n (%)	107 (7.1)
Origin country	
National n, (%)	1245 (82.7)
Expatriate n, (%)	261 (17.3)
Residency	
Bobo Dioulasso, n (%)	877 (58.2)
Sindou, n (%)	159 (10.6)
Banfora, n (%)	113 (7.5)
Niankorodougou, n (%)	293 (19.5)
Ouagadougou, n (%)	37 (2.5)
Others, n (%)	27 (1.8)
Type of swabs	
Oropharyngeal, n (%)	907 (60.2)
Nasopharyngeal, n (%)	597 (39.6)
Mix (oropharyngeal and nasopharyngeal), n (%)	2 (0.1)
Cycle threshold at first PCR test	
N-gene, (median, range)	26.57 (11.68 -38.24)
ORF1ab gene, (median, range)	25.43 (13.79-40)

SD = standard deviation; PCR = polymerase chain reaction; Ct = cycle threshold; n= total count, % = percentage
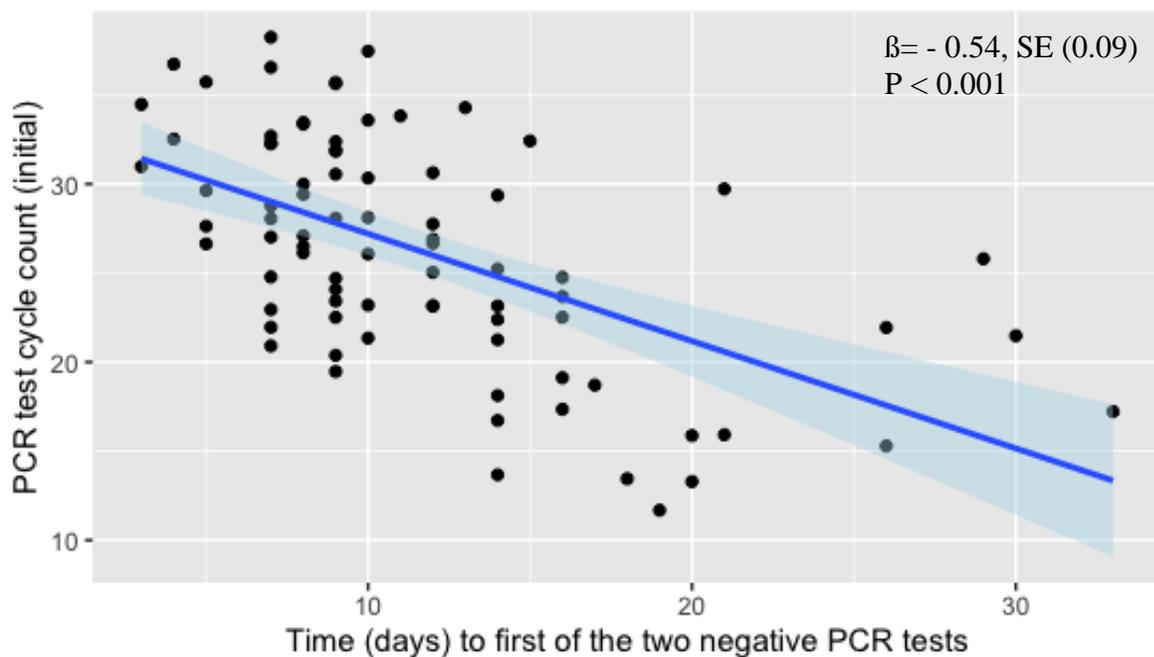

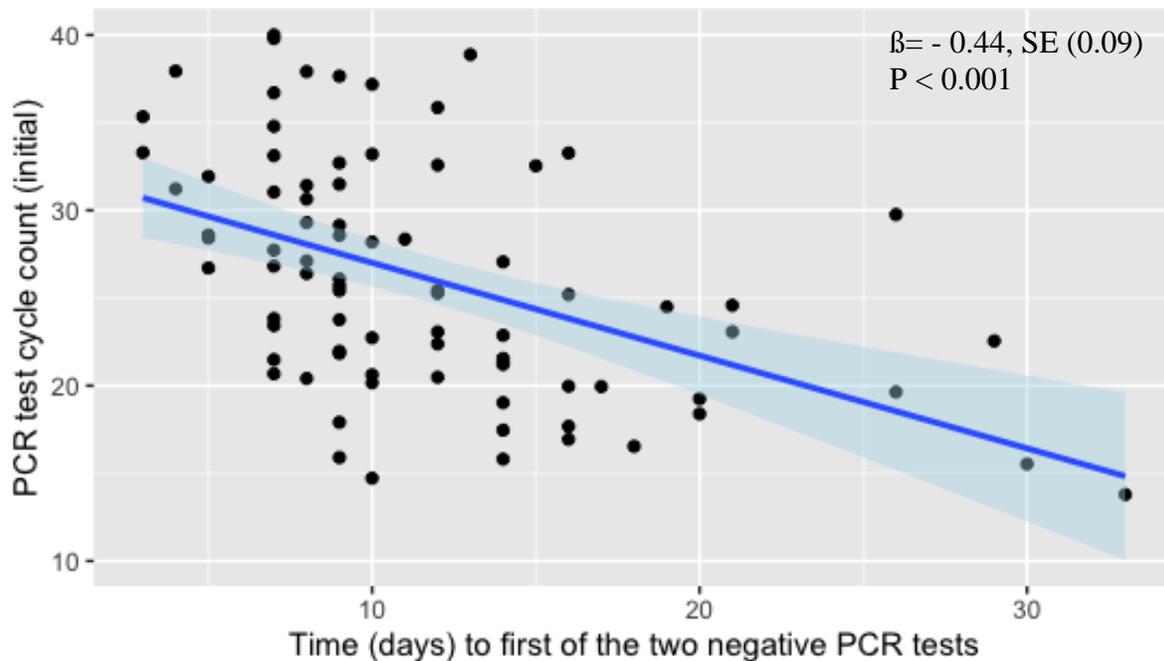Fig 1: Duration of positive SARS-CoV-2 RT-PCR test according to initial cycle threshold (Ct) value for N-gene

Fig 2: Duration of positive SARS-CoV-2 RT-PCR test according to initial cycle threshold (Ct) value for ORF1ab gene

Discussion:

Nearly one out of seven participants had a RT-PCR detected SARS-CoV-2 infection at the testing site of the Wahgnion mine in Burkina Faso. This suggest that miners could represent an important reservoir of SARS-CoV-2 transmission into the general population. Indeed, miners regularly travel to their original residency when they are not on duty and adequately identifying and isolating positive patients would be crucial to break the chain of transmission either in the general population or in the mine, which could help lower the impact of the disease on the miners productivity (12).

The prevalence of SARS-CoV-2 infection in this study was higher than that reported in the overall population (with prevalence of < 1%) and could accentuate the gradient of SARS-CoV-2 transmission from the mine to the general population (13). Much higher figures were reported in the country among high risk population including person with recent travel history from high risk countries, contact cases of COVID-19, clinically suspected cases and healthcare workers (5). Therefore, continuous surveillance is needed to timely identify transmission reservoirs, areas and persons most at risk for targeted preventive interventions. In this study, most of positive cases were from the nearby cities of the mining site, particularly among the local populations and suggest that population close to

mining sites are more at risk, and need continuous surveillance and adequate preventive interventions.

Higher prevalence of SARS-COV-2 infection (although not statistically significant) was detected using the nasopharyngeal swabs compared to oropharyngeal swabs in our study. Many studies have reported that nasopharyngeal swabs may be more suitable than oropharyngeal swabs for the detection of SARS-CoV-2 infection (14–18). This suggest that routine surveillance of SARS-CoV-2 infection could primarily use nasopharyngeal swabs to increase the odds of detection. The RT-PCR assay is considered the 'gold standard' for the qualitative and quantitative molecular detection of SARS-CoV-2 nucleic acids (6). The technique has the advantage of test sensitivity of 95% (19), with a detection limit below 10 copies/reaction which allows early detection of low viral titers (20).

Initial cycle threshold (Ct) values are used to discriminate positive from negative samples, and we hypothesized that the initial Ct values could well correlate with the duration of the disease and thus, guide the frequency of the control testing and the return to daily activities (8). We also anticipate that adequately interpreting the initial Ct values could potentially help to predict the duration of the viral RNA shedding, and thus the possible duration of infectivity of COVID-19 patients. In this study, we found a significant correlation between initial viral load,

estimated by the initial RT-PCR test Ct values, and the duration of positive RT-PCR test results for both SARS-CoV-2 N and ORF1ab genes. Indeed, initial Ct values negatively correlated with the duration of the illness, indicating that individuals with an increased initial Ct value or lower viral load rapidly cleared their SARS-CoV-2 infection as compared to those with lower Ct values or higher viral load. For example, 42 (89.4%) of the 47 participants with Ct value over 25 could clear the infection within 14 days, while only 14 (40%) of the 35 participants with Ct value below 25 could clear the viral RNA within the same period. In the meantime, a total of 22% participants could not clear the viral RNA within the same period irrespective of the initial Ct values. Several studies have reported that RT-PCR Ct values strongly correlated with cultivable virus and the probability of culturing virus declines to 8% in samples with Ct >35 and 6% 10 days after onset of the disease irrespective of the presence of symptoms (21,22). Therefore, Ct values, could be used as criteria to approximate the duration of quarantine, given virus shedding is not associated with the presence of clinical symptoms (22).

Indeed, using the presence of clinical symptoms as a surrogate for decision making for daily activity return could lead to a higher risk of contamination at the work place, as their presence do not correlate with the infectivity period (22), and thus Ct values would play an important role for decision making. A study already reported a significant correlation between initial RT-PCR Ct values and the duration of the disease, however, their estimates could not be more precise due to their small sample size (11). Indeed, in this cited study, the average reduction time was 1.3 days per additional cycle ($\beta = -1.29 \pm 0.26$) using a sample of 25 patients, with larger standard errors. Our current study provided more accurate estimates, as higher sample size was used for the modeling, and this finding supports the use of Ct value to predict the duration of the illness, and thus allows a more efficient use of resource in low-income settings particularly the sub-Saharan Africa where infrastructure and qualified personnel are limited.

The knowledge of the relationship between Ct values and the duration of the disease can be used for targeted measure to reduce risk of transmission. In a recent study, longitudinal assessment of RT- PCR test results in individuals requiring 15–30 days to clear SARS-CoV-2 RNA showed that groups with initial high viral load (Ct values ≤25) and intermediate viral load (Ct values 26–30) exhibited a significant reduction of viral load between 8 and 14 days, and thus concluded that in patient with longer duration of disease, they may be of reduced infectivity after 14 days of isolation, however, the study could not confirm the absence of transmission beyond that period. Although we hypothesize from our study that the infectivity would be lower or even absent in high Ct values patients, we recommend that minimum prevention measures should be adopted by patients even after their return to daily activities.

Conclusion:

Our finding indicates that SARS-CoV-2 load estimates by Ct values on RT-PCR assay can be used to predict the duration of COVID-19 and infer the period of virus transmission. As participants with lower initial Ct values tended to have longer disease duration, initial RT-PCR Ct value could be used as a criterion to guide the return to work and daily activities.

Contributions of authors:

The study was conceived by ZT. Data were collected by ZT, and AC. Data analysis was done by ML. The manuscript was drafted by ML. AC and ZT made important contributions to the final manuscript. All authors read and approved the final manuscript.

Conflicts of interest:

Authors declare no conflict of interest.

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