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Research article

RT-PCR Ct values combined with age predicts invasive mechanical ventilation and mortality in hospitalized COVID-19 patients in a MERS-CoV-endemic country

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ABSTRACT

Background: Several risk factors have been used to predict severity of coronavirus disease 2019 (COVID-19), real-time reverse transcriptase polymerase chain reaction (RT-PCR) cycle threshold (Ct) values have not been included.

Methods: A retrospective analysis of laboratory-confirmed COVID-19 patients who were hospitalized between March 2 and September 1, 2020, in an academic hospital in Riyadh that serves as a Middle East respiratory syndrome coronavirus (MERS-CoV) referral center was conducted. Nasopharyngeal (NP) and endotracheal (ET) samples were tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by RT-PCR, and viral load (VL) was determined based on the Ct values of E genes. The Ct values were distributed into four groups, with group Ct1 ($\leq$19) indicating the highest VL and Ct4 ($\geq$31) indicating the lowest VL. Univariate logistic regression was used to analyze age, gender, and comorbidities in relation to Ct groups for a primary endpoint of either invasive mechanical ventilation (IMV) or mortality. Significant variables were further analyzed by multivariate logistic regression.

Results: The analysis included 728 patients hospitalized with COVID-19 (38% female; median age = 53 years; 41.3% diabetic; 39.4% hypertensive). Overall, 13.6% of these patients required IMV, and the in-hospital mortality rate was 15.5%. The IMV rate was higher in the Ct1 and Ct2 groups (15.2% and 15.5%, respectively) than in the Ct4 group (6.4%; $p = 0.01$). The mortality rate was also higher in the Ct1 and Ct2 groups (19.4% and 18.9%, respectively) than in the Ct4 group (8.9%; $p = 0.02$). The univariate analysis showed that lower Ct values and increasing age were associated with an increased risk of IMV (OR: 1.03; 95% CI: 1.01, 1.04; $P < 0.0001$) and mortality (OR: 1.04; 95% CI: 1.03, 1.06; $P < 0.0001$). The multivariate analysis showed that Ct1 was associated with the highest risk of mortality (OR: 2.29; 95% CI: 1.16, 5.52; $P = 0.016$), while Ct2 was associated with the highest risk of IMV (OR: 3.1; 95% CI: 1.47, 6.53; $P = 0.003$).

Conclusion: The SARS-CoV-2 RT-PCR Ct values of hospitalized COVID-19 patients can be used as predictors of IMV and mortality, and this effect increases when combined with age. Clinicians could use these predictors to triage older patients for risk stratification and allocate IMV.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 (COVID-19) pandemic, which has infected nearly 110 million people worldwide and caused more than 2 million deaths as of February 2021 [1]. Among hospitalized patients, 12%–33% require invasive mechanical ventilation (IMV), and 10%–21% die [2, 3].

The diagnosis of COVID-19 primarily depends on the use of real-time reverse transcriptase polymerase chain reactions (RT-PCRs) on nasopharyngeal (NP) or endotracheal aspirate (ET) samples. A cycle threshold (Ct) value is the cycle number of the point at which an RT-PCR amplification curve crosses the threshold of detection. The Ct value of a tested

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sample can be established by setting a threshold line and calculating its intersection with each curve. Previous studies have demonstrated that Ct values correlate with viral RNA load [4], disease infectiousness [5, 6], and mortality [7]. In particular, Ct values are inversely proportional to viral load (VL), such that a Ct value increase of approximately 3.3 correlates with a nearly ten-fold reduction in the initial starting RNA amount [4]. Higher Ct values indicate later amplification, which is usually due to insufficient starting template or high levels of sample degradation.

The Kingdom of Saudi Arabia (KSA) is among the top forty countries in caseloads of COVID-19, with 374,029 reported cases and 6,450 reported COVID-19-related deaths [1] as of February 2021. It is also the only country in the world with another coronavirus that is endemic. The Middle East respiratory syndrome coronavirus (MERS-CoV) was first reported in 2012 [8] and is still causing nosocomial outbreaks [9] and sporadic community cases [10].

Several risk factors for COVID-19 disease severity have been identified, including lymphopenia, high levels of lactate [11], and D-dimer [12]. Furthermore, the Quick COVID-19 Severity Index has been validated for the prediction of respiratory failure based on respiratory rate, oxygen saturation, and oxygen flow rate [13].

The correlation between Ct values and age as predictors of IMV and mortality has not been studied extensively. Therefore, we aimed to determine how RT-PCR Ct values correlate with mortality and need for IMV among hospitalized COVID-19 patients.

2. Materials and methods

2.1. Study population

A retrospective analysis was performed on all RT-PCR-confirmed SARS-CoV-2 cases who required hospitalization due to COVID-19 to King Khalid University Hospital (KKUH) from March 2 to September 1, 2020. Patients younger than 14 years of age and patients with no reported Ct values within the first 24 h of admission were excluded from the study. A MERS-CoV PCR was conducted on each patient who fit the Ministry of Health's (MOH's) MERS-CoV case definition [14] or per their clinician's discretion. Data concerning the patients' demographics and comorbidities were collected from their electronic medical records. Nylon flocked swabs (FLOQSwab™, Copan, Italia S.p.A.) were used to collect NP specimens. All samples were transported via Universal Transport Medium™ (UTM®, Copan, Italia S.p.A.). Real-time RT-PCRs were performed at our institution's molecular laboratory. A positive result was considered if both SARS-CoV-2 E and S genes were detected using a RealStar® SARS-CoV-2 RT-PCR kit (Altona®-Diagnostics, Hamburg, Germany) and a Rotor-Gene Q system (Qiagen®, Santa Clarita, CA, USA). Each report was either positive or negative, and positive samples were reported with Ct values. Specifically, we used the Ct values of E genes; these are excellent targets because they are not present in MERS-CoV or seasonal coronaviruses. For simplification purposes, each value was rounded to the nearest integer. When indicated, we used the same samples to test for MERS-CoV RNA. After extraction, the RNA was reverse-transcribed to cDNA, which was then amplified and screened for MERS-CoV upE and orf1a genes using the specific primers and probes of the RealStar® MERS-CoV RT-PCR kit (Altona®-Diagnostics) on a Rotor-gene Q instrument (Qiagen®). This study was approved by the Institutional Review Board of the College of Medicine and King Saud University Medical City (IRB-E20-4979), all methods were carried out in accordance with relevant guidelines and regulations, an informed consent was obtained from all participants.

2.2. Statistical analysis

Descriptive statistics—including means, standard deviations, and estimations of proportions—were calculated where appropriate. Significance was determined at the 0.05 level. For the purpose of this study the Ct values were divided into four groups as quartiles (Ct1: ≤19, Ct2: 20–25, Ct3: 26–30, Ct4: ≥31) for grouping samples and help study the significance of viral load. Similarly, age was grouped into four categories (<40, 41–53, 54–63, and >63 years).

An analysis of variance (ANOVA) and a chi square (Fisher's exact) test were used to determine if significant differences occurred between the variables within each Ct value group. Correlations between variables were checked with a Spearman analysis. A univariate logistic regression was carried out for the dichotomous dependent variables (mortality and need for IMV). The independent variables included gender, age group, Ct value group, cardiac disease, respiratory disease, diabetes, hypertension, smoking, and chronic kidney disease (CKD). Any variables that were significant in any of the univariate logistic analyses were carried on to the multivariate logistic regression. The area under the receiver operating characteristic (ROC) curve was estimated for each outcome variable to compare Ct value as a single predictor with the positive predictors of the multivariate logistic regression. Stata 15 software was used to carry out these analyses.

3. Results

During the study period, 743 participants over 14 years old were admitted to hospital with RT-PCR-confirmed COVID-19, 284 (38.22%) of which were female and 459 (61.78%) of which were male. Fifteen patients had Ct values beyond 24 h after admission and were thus excluded from the analysis, resulting in a total of 728 participants. The median duration of illness before admission was five days, and the duration of hospitalization was 12 days.

The mean Ct value (SD) was 24.38 (7.5), with a median of 25 and a range of 4–38. The mean (SD) age was 52.26 (16.87) years, with a median of 53 years and a range of 14–101 years. The mean (SD) body mass index (BMI) was 29.79 (6.6), with a median of 29 and a range of 17–55.16. Of all the participants, 99 (13.60%) required IMV and 113 (15.52%) died. A total of 266 patients (36.54%) underwent MERS-CoV PCRs, and all tested negative. None of the participants had laboratory-confirmed MERS-CoV in the past. The ANOVA showed no significant differences in age or BMI between the four Ct groups. The chi square test suggested that the number of smokers and the probability of needing IMV or dying differed significantly between the four Ct groups (Table 1).

A univariate logistic regression was performed for each of the dependent variables (mortality and IMV) with each of the independent variables (Table 2). Preexisting respiratory disease and BMI were not significant in the univariate regressions and thus were not carried into multivariate logistic regression. Smoking was very infrequent and thus excluded as well. The remaining variables were included in both multivariate regressions, and significance emerged only for the different age and Ct groups. Specifically, the odds of mortality and IMV increased with increasing age. Conversely, the odds of mortality and IMV increased with decreasing Ct values, most notably in the first and second Ct groups (Table 3).

With Ct value as the sole predictor, mortality had an area under the curve (AUC) of 0.59, which increased to 0.71 when age was added as a predictor (Figure 1).

A similar finding was obtained for the estimation of need for IMV, which had an AUC of 0.6 using Ct value alone and an AUC of 0.68 when age was added (Figure 2). These findings indicate that age and Ct value when combined contribute to both outcomes more than Ct value alone.

4. Discussion

Hospitalized COVID-19 patients exhibit variable disease severity. Therefore, studying different risk factors for severe disease is vital to predicting unfavorable outcomes that may help in triage, early intervention, and management of such patients [15]. In this observational study of a country endemic to MERS-CoV, we found that Ct values were

Table 1. Demographics and clinical outcomes related to Ct value for 728 participants.

Ct Group n		Ct1 = 191	Ct2 = 206	Ct3 = 174	Ct4 = 157	P value
Ct value ranges		≤19	20–25	26–30	≥31	
Ct value m (sd)		14.85 (3.5)	22.5 (1.7)	27.9 (1.4)	34.5 (2.9)	p < 0.0001
Age m (sd)		53.9 (17.3)	53.2 (16.6)	51.7 (15.5)	49.5 (17.9)	p = 0.073
BMI m (sd)		30.1 (7.5)	29.6 (6.4)	30 (6.5)	29.4 (5.9)	p = 0.79
Gender n (%)	Female	64 (33.5%)	79 (38.4%)	65 (37.4%)	69 (44%)	p = 0.26
	Male	127 (66.5%)	127 (61.7%)	109 (62.6%)	88 (56%)	
Diabetes n (%)	Yes	74 (38.7%)	89 (43.2%)	80 (46%)	58 (37%)	p = 0.3
	No	117 (61.3%)	117 (56.8%)	94 (54%)	99 (63%)	
Hypertension n (%)	Yes	82 (42.9%)	81 (39.3%)	68 (39.1%)	56 (35.7%)	p = 0.6
	No	109 (57.1%)	125 (60.7%)	106 (60.9%)	101 (64.3%)	
Smoker n (%)	Yes	18 (9.8%)	9 (4.6%)	4 (2.5%)	2 (1.3%)	p = 0.002
	No	165 (90.2%)	185 (95.4%)	159 (97.5%)	151 (98.7%)	
Cardiac disease n (%)	Yes	30 (15.7%)	29 (14.1%)	18 (10.3%)	25 (15.9%)	p = 0.4
	No	161 (84.3%)	177 (85.9%)	156 (89.7%)	132 (84.1%)	
Lung disease n (%)	Yes	18 (9.4%)	18 (8.7%)	14 (8.1%)	11 (7%)	p = 0.9
	No	173 (90.6%)	188 (91.3%)	160 (91.9%)	146 (93%)	
CKD n (%)	Yes	15 (7.9%)	9 (4.4%)	9 (5.2%)	10 (6.4%)	p = 0.5
	No	176 (92.1%)	197 (95.6%)	165 (94.8%)	147 (93.6%)	
Invasive mechanical ventilation n (%)	Yes	29 (15.2%)	38 (15.5%)	22 (12.6%)	10 (6.4%)	p = 0.01
	No	162 (84.8%)	168 (84.5%)	152 (87.4%)	147 (93.6%)	
Death n (%)	Yes	37 (19.4%)	39 (18.9%)	23 (13.2%)	14 (8.9%)	p = 0.02
	No	148 (80.6%)	146 (81.1%)	156 (86.6%)	165 (91.1%)	

Ct: cycle threshold, BMI: body mass index, CKD: chronic kidney disease. Ct, age, BMI are reported in mean and standard deviation [mean (sd)], remaining variables are reported in proportions.

Table 2. Univariate analysis of variables related to invasive mechanical ventilation or mortality.

		Death				Invasive mechanical ventilation			
		OR	SE	CI	P-Value	OR	SE	CI	P-Value
Gender	**Male**	1.17	0.25	0.78, 1.78	0.44	1.24	0.01	1.03, 1.06	<0.0001
	Female	Ref							
Age (years)	**<40**	Ref				Ref			
	41–53	2.56	1.6	1.48, 8.6	0.005	3.6	1.52	1.57, 8.26	0.002
	54–63	7.97	3.37	3.48, 18.27	<0.0001	6.52	2.62	2.96, 14.35	<0.0001
	>63	9.59	4.03	4.2, 21.87	<0.0001	4.92	2.02	2.19, 11.01	<0.001
Age (continuous)		1.04	0.01	1.03, 1.06	<0.0001	1.03	0.01	1.01, 1.04	<0.0001
BMI (continuous)		1.01	0.02	0.98, 1.04	0.55	1.01	0.02	0.98, 1.05	0.38
Diabetes		2.23	0.46	1.49, 3.33	<0.0001	1.83	0.39	1.21, 2.79	0.005
Hypertension		2.65	0.55	1.77, 3.98	<0.0001	1.82	0.39	1.2, 2.77	0.005
Cardiac disease		2.38	0.59	1.47, 3.87	<0.0001	1.46	0.41	0.84, 2.52	0.18
Pulmonary disease		1.77	0.56	0.96, 3.28	0.07	1.52	0.52	0.78, 2.97	0.22
CKD		2.12	0.75	1.06, 4.25	0.03	1.66	0.65	0.77, 3.55	0.2
Ct value Group:									
Ct1	**≤19**	2.45	0.8	1.27, 4.73	0.007	2.63	1	1.24, 5.59	0.012
Ct2	**20–25**	2.39	0.79	1.25, 4.57	0.009	3.33	1.23	1.6, 6.9	0.001
Ct3	**26–30**	1.56	0.56	0.77, 3.14	0.22	2.13	0.84	0.97, 4.65	0.058
Ct4	**≥31**	Ref				Ref			

Ct: cycle threshold, BMI: body mass index, CKD: chronic kidney disease, OR: Odds ratio, SE standard error, CI: Confidence interval.

clearly correlated with both IMV and mortality. High VL, as indicated by low Ct values, may be used as a disease severity surrogate; this can be enhanced by considering age, as old age and low Ct values are both associated with IMV and death.

Serial SARS-CoV-2 RT-PCRs of throat and sputum samples have shown that VL peaks at 5.5 days from symptom onset [16]. Given that our cohort had a median symptom duration of five days prior to hospitalization, the participants' VLs may have peaked upon hospital admission. Furthermore, patients with progressive disease have been shown to have higher VLs than recovering patients [17]. The median Ct value of our cohort was 25, with a median illness duration of five days. This finding is consistent with an epidemiological study that showed a median Ct value of 26 within the first seven days from symptom onset and a median Ct value of 35 at 21 days or more from symptom onset [18].

Mortality has been found to correlate with old age. In a large case series of nearly 72,000 patients from China, the case fatality rate (CFR)

Table 3. Multivariate analysis of variables related to invasive mechanical ventilation or mortality.

		Death				Invasive mechanical ventilation			
		OR	SE	CI	P-Value	OR	SE	CI	P-Value
Gender	Male	1.1	0.25	0.7, 1.7	0.68	1.11	0.27	0.69, 1.78	0.67
	Female								
Age	<40	Ref				Ref			
	41–53	3.1	1.4	1.26, 7.65	0.014	3.26	1.41	1.39, 7.6	0.007
	54–63	6.01	2.7	2.49, 14.52	<0.0001	5.34	2.3	2.29, 12.43	<0.0001
	>63	5.97	2.82	2.4, 15.07	<0.0001	3.48	1.63	1.39, 8.72	0.008
Diabetes		1.21	0.3	0.75, 1.97	0.43	1.27	0.33	0.77, 2.1	0.36
Hypertension		1.12	0.29	0.67, 1.87	0.67	0.97	0.26	0.57, 1.65	0.9
Cardiac disease		1.43	0.4	0.82, 2.47	0.2	1.08	0.34	0.58, 2	0.81
CKD		1.33	0.51	0.62, 2.83	0.461	1.4	0.59	0.61, 3.21	0.42
Ct value Group:									
Ct1	≤19	2.29	0.79	1.16, 5.52	0.016	2.48	0.97	1.15, 5.3	0.02
Ct2	20–25	2.27	0.78	1.16, 4.56	0.017	3.1	1.18	1.47, 6.53	0.003
Ct3	26–30	1.47	0.54	0.71, 3.03	0.3	1.9	0.77	0.86, 4.29	0.11
Ct4	≥31	Ref				Ref			

Ct: cycle threshold, CKD: chronic kidney disease, OR: Odds ratio, SE standard error, CI: Confidence interval.

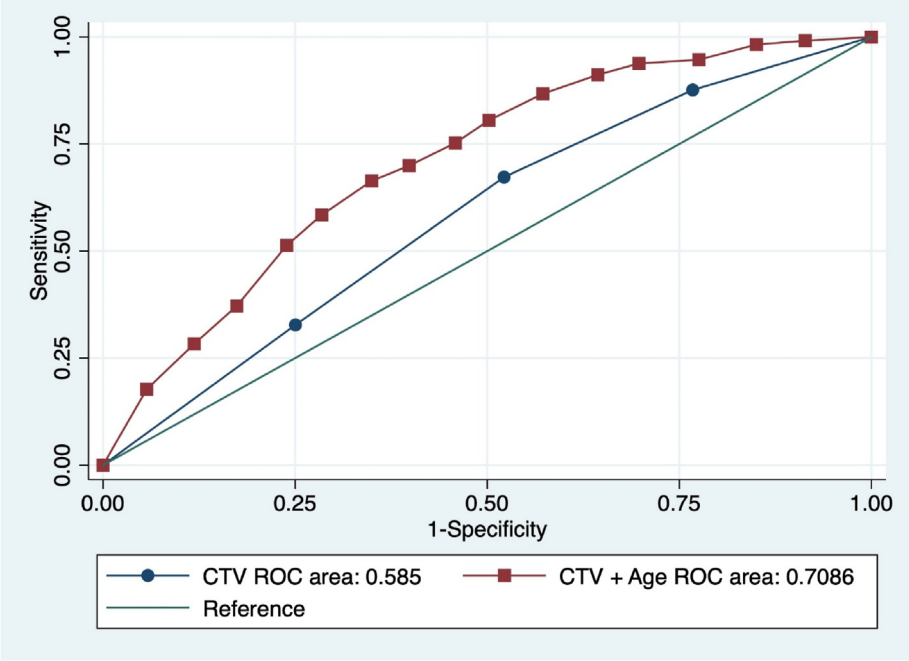

Figure 1. Receiver operating characteristic (ROC) curve for mortality. ROC: receiver operating characteristic, CTV: cycle threshold value.

was 8% in patients aged between 70 and 80 years and 15% in older patients [19]; however, RT-PCR Ct values were not reported in this case series. Although the present study did find a higher mortality rate among participants aged 54 years and older, this study did not specifically examine the age group of those over 70 years old. Another study found relatively low Ct values among patients under 18 years old [18]; however, they reported that there were only three such participants, and those participants exhibited no or minimal symptoms.

A large multicenter retrospective study of nearly 240,000 COVID-19 patients in Saudi Arabia showed that mortality increased with increasing age, which remained significant in a multivariable model containing all variables; however, Ct values were not examined in this study [20]. Although the present sample size was smaller and sourced from a single center, our analysis does suggest that associating Ct values with age may be useful for the stratification of patients within the same age group. In a large prospective study of 1,145 patients from New York City, VL upon diagnosis was found to be independently associated with mortality; as in the present study, this association was maintained after adjusting for comorbidities [21].

In a recent retrospective analysis of nearly 1,000 patients, Ct values were positively correlated with patient survival; this was demonstrated when the Ct values were divided into quartiles, as the highest mortality rates occurred in the groups with the lowest Ct values [7]. The present findings validate these results and similarly suggest that grouping Ct values according to VL may produce a useful predictive clinical tool.

Two previous studies reported no significant differences in VLs between severely symptomatic and mildly symptomatic or asymptomatic COVID-19-infected patients [22, 23]; however, both of these studies used

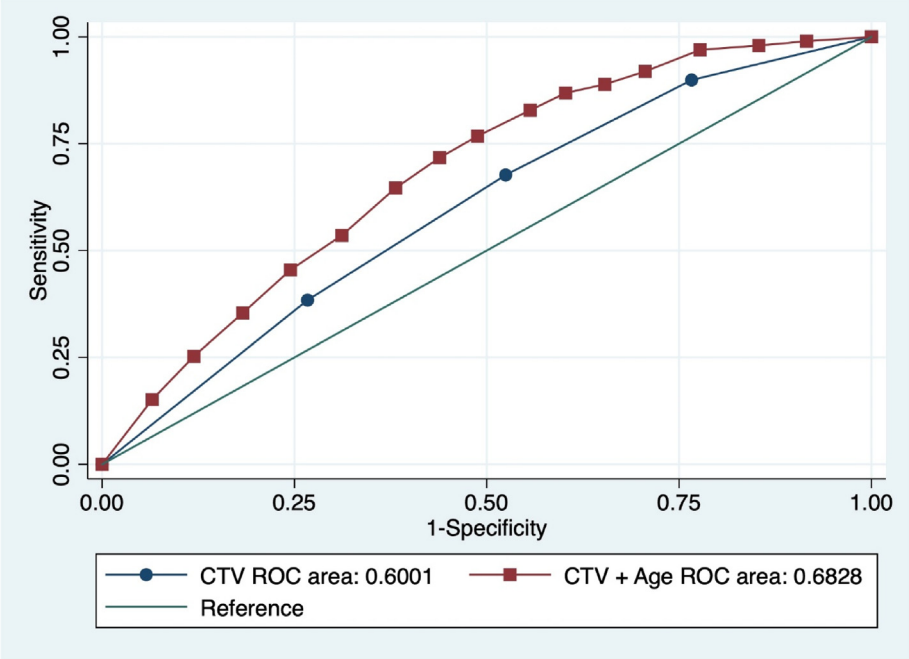

Figure 2. Receiver operating characteristic (ROC) curve for invasive mechanical ventilation. ROC: receiver operating characteristic, CTV: cycle threshold value.

relatively small patient samples. In a study of 62 patients with mild to moderate disease upon admission, low Ct values were predictive of disease progression [24].

Another study examining the respiratory, serum, and stool samples of 96 patients showed significantly higher VLs in the respiratory samples of patients with severe disease than in the other samples [25]; however, this study did not analyze mechanically ventilated patients, and none of the patients in the study cohort died.

Another previous study of 678 hospitalized patients demonstrated that low Ct values were associated with mortality and need for mechanical ventilation [26]; the researchers also found that high VL was associated with increased age, comorbidities, and smoking. The present study did not reveal any independent associations between VL and any comorbidity or high BMI; however, we did find a linear independent association between VL and increasing age. A meta-analysis including nearly 3,500 obese patients showed a significant association between VL and poor outcomes [27]. Similarly, in a meta-analysis of 731 patients, those with histories of smoking showed significantly high levels of progression to severe disease [28], neither of these studies included Ct values in their analyses; however, the present study did not analyze smoking due to the low number of smokers among the study participants.

In a systematic review of 18 studies on Ct values and VL [29], only one study including 308 patients showed an association between viral load and mortality [30]; this finding was consistent with the present findings. While one study showed lower Ct values in patients who progressed to severe disease [24], there is little data on the correlation of Ct values with need for IMV. Lie et al. found that Ct values are linked to the PaO_2/FiO_2 ratio but not to Acute Physiology and Chronic Health Evaluation II (APACHE II) scores [31]. In the present study, we found an independent association between lower Ct values and need for IMV. This data could be useful for predicting which patients may require such intervention, especially during surges of the pandemic, when ventilator allocation may be scarce.

COVID-19 Severity Index utilizes several parameters to help determine which hospitalized patients need ICU care [32], however it does not distinguish which ICU patients need IMV and does not predict mortality. The 4C Mortality Score that use eight variables has been shown to have high discrimination for mortality [33], although both use age within their scoring systems, none utilize Ct values, the current study supports developing similar scoring tools with Ct values as part of their parameters.

Another interesting finding of the present study was the lack of any MERS-CoV coinfection in tested patients. The World Health Organization's (WHO's) June to December 2020 Situation Update reported very few cases of MERS-CoV throughout Saudi Arabia, most of which were related to camel exposure [34]. However, a recent report confirmed MERS-CoV co-infection in eight critically ill patients with COVID-19 [35], although the authors were unable to pinpoint the exact type of exposure that caused the co-infections, the data in our study may be limited as MERS-CoV was selectively tested as per clinicians' discretion for patients exposed to camels or camel products.

The present study had several limitations: it was conducted in a single center, Ct values within the first 24 h of hospitalization were utilized with no serial measurements, the effects of Ct values on inflammatory markers and radiological features were not analyzed, and the age group over 80 years old was not specifically analyzed. In addition, Ct value ranges are not standardized between platforms; therefore, it may not be feasible to implement them clinically on a wider scale. Larger prospective studies are urgently warranted. However, the present study adds to the growing body of literature on the association between COVID-19 severity and SARS-CoV-2 RT-PCR Ct values, and in our center a cut-off below or equal to 19 is more predictable for IMV and/or mortality.

In conclusion when correlated with age within 24 h of hospitalization, SARS-CoV-2 RT-RCR Ct values can be used as clinical tools for the prediction of IMV and in-hospital mortality.

Declaration

Author contribution statement

Mazin Barry, Taim Muayqil: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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