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SARS CoV2 RT-PCR cycle threshold (Ct) values and the risk of developing acute respiratory distress syndrome in patients with Covid19

Valeurs des cycles seuils (Ct) de la RT-PCR du SARS CoV2 et le risque de développer un syndrome de détresse respiratoire aiguë chez les patients atteints de Covid19

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ABSTRACT

Background. SARS-CoV-2 viral loads may aid in the risk stratification of patients with COVID-19. **Methods.** 486 patients tested positive for SARS Cov2 by real time RT-PCR were included in this study. All the tests were performed on nasopharyngeal swabs during the first week after symptom onset using Sansure Biotech™ SARS Cov2 real time RT-PCR kits. Patient's condition was monitored over a period of one month after the onset of symptoms. **Results.** The mean Ct value in the group of patients who developed acute respiratory distress syndrome (ARDS +) was 18.27 (95% CI: 17.43-19.10) while for the ARDS- group it was 33.06 (95% CI: 32.77-33.34). **Discussion.** The Ct values in the group of patients who developed ARDS (ARDS +) were significantly lower than those observed in the ARDS- group. By setting a cut-off value, the determination Ct values (on a qualitative technique) from nasopharyngeal swabs performed during the first week after symptom onset will assist clinicians in risk-stratifying patients. **Conclusion.** Our data show that the determination of SARS CoV2 RT-PCR cycle threshold values from nasopharyngeal swabs performed during the first week after symptom onset may aid in the risk stratification of patients with COVID-19

Keywords: SARS-CoV2, real time RT-PCR, ARDS, COVID 19, Cycle Threshold value.

RÉSUMÉ

Contexte. Les charges virales du SRAS-CoV-2 peuvent aider à la stratification du risque chez les patients atteints de COVID-19. **Méthodes.** 486 patients testés positifs pour le SRAS-CoV2 par RT-PCR en temps réel ont été inclus dans cette étude. Tous les tests ont été réalisés sur des écouvillons nasopharyngés au cours de la première semaine après l'apparition des symptômes à l'aide des kits de RT-PCR en temps réel Sansure Biotech™ SARS Cov2. L'état des patients a été suivi sur une période d'un mois après l'apparition des symptômes. **Résultats.** La valeur moyenne de Ct dans le groupe de patients ayant développé un syndrome de détresse respiratoire aiguë (SDRA +) était de 18,27 (IC 95 % : 17,43-19,10) tandis que pour le groupe (SDRA -) elle était de 33,06 (IC 95 % : 32,77-33,34). **Discussion.** Les valeurs de Ct dans le groupe de patients ayant développé un SDRA (SDRA +) étaient significativement plus basses que celles observées dans le groupe (SDRA-). En fixant une valeur seuil, la détermination des valeurs de Ct (selon une technique qualitative) à partir d'écouvillons nasopharyngés réalisés au cours de la première semaine après le début des symptômes aidera les cliniciens à stratifier les patients en fonction du risque. **Conclusion.** Nos données montrent que la détermination des valeurs des cycles seuils de la RT-PCR du SARS Cov2 à partir d'écouvillons nasopharyngés réalisés au cours de la première semaine après l'apparition des symptômes peut aider à la stratification du risque chez les patients atteints de COVID-19.

Mots-clés : SARS-CoV2, RT-PCR en temps réel, SDRA, COVID 19, valeur de cycle seuil.

INTRODUCTION

Early risk stratification in COVID-19 remains a challenge, in prior analyses of the SARS-CoV-1 outbreak, viral load within the nasopharynx was associated with poor disease outcome (1,2). Some SARS CoV-2 studies have demonstrated this same association (3,4,5). Quantitative PCR techniques are less available and more expensive, so the aim of this study is to find out whether Ct values can be correlated with severe forms of COVID -19.

MATERIALS AND METHODS

All the tests were performed on nasopharyngeal swabs during the first week after symptom onset using Sansure Biotech™ SARS Cov2 real time RT-PCR kits on Rotor-Gene™ thermocycler. For each sample, the average Ct value for both ORF-1ab region and the N gene was calculated. if the Ct value was greater than 40 or there was no amplification for one of the 2 target genes, the Ct value was considered to be equal to 40 for this gene. Patient's condition was monitored over a period of 1 month after the onset of symptoms. Acute respiratory destress syndrome (ARDS) case determination was based on the 2012 Berlin definition (6): PaO2/FiO2 <300mm Hg with a minimum of 5 cmH20 PEEP (Positive End Expiratory Pressure). Statistical analyses were carried out using MedCalc™ software.

RESULTS

Of the 486 patients, 46 (9,47%) had an acute respiratory destress syndrome, the average age of this group of patients was 55 years (ranging from 31 to 92 years), 73,9% (34) of them have cardiovascular disease, diabetes and / or hypertension as a comorbidity.

The mortality rate was 5,14% (25/486).

Four hundred forty patients (90,53%) had less severe forms ranging from asymptomatic to moderate forms. the average age of this group of patients was 51 years (ranging from 28 to 91 years), with 46,8% (206) having cardiovascular disease, diabetes and / or hypertension as a comorbidity. The SARS CoV2 RT-PCR Ct values obtained in the two groups of patients are shown in Fig.1.

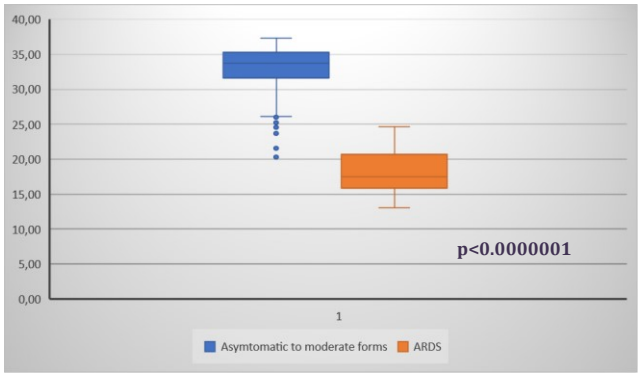

Figure.1. SARS Cov2 RT-PCR Ct values depending on the severity of the disease.

The average of Ct value in the ARDS group was 18,27 ranging from 13,01 to 24,66 (95% CI: 17.43-19.10), on the other hand the average of Ct value in the asymptomatic to moderate forms group was 33,06 ranging from 20,29 to 37,29 (95% CI: 32.77-33.34) (independent samples t-test: p<0.0000001).

The Receiver Operating Characteristics curve for the different Ct criterions is shown in Fig.2, the contingency table of Acute Respiratory Distress Syndrome (ARDS) and Ct values (>24.66 and ≤24.66) is shown in fig.3.

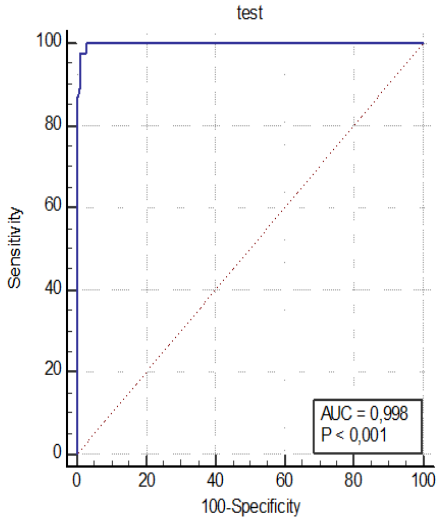

Figure 2. Receiver Operating Characteristics curve for the different Ct criterions.

Table 1. Relationship between SARS CoV2 RT-PCR cycle threshold values and risk of developing acute respiratory distress syndrome (ARDS). (P<0.0000001).

	ARDS +	ARDS -
Ct < or =24.66	46	13
Ct >24.66	0	427

DISCUSSION

Across the spectrum of viral infections, the extent of viral load has been a predictor of disease severity and progression, including for HIV (7, 8), Ebola (9), influenza, SARS CoV1 and other non-COVID-19 respiratory viral infections (1,2,10,11,12). The association between SARS CoV2 viral load and the poor disease outcome was proven by some studies (3,4,5), but the quantitative SARS CoV2 RT-PCR techniques are less available and more expensive, on the other hand, in some studies Ct values were used as an approximation of viral loads (13,14,15), the aim of the current study is to find out if such approximation can be used in risk stratification of patients with COVID-19, so we conducted an analysis of Ct values and disease progression in 486 patients.

The Ct values in the group of patients who developed ARDS (ARDS +) were significantly lower than those observed in the group of patients with less severe symptoms (ARDS -), (p<0.000 student test) for the ARDS + group the mean Ct was 18.27 (95% CI: 17.43-19.10) while for the ARDS- group the mean Ct value was 33.06 (95% CI: 32.77-33.34).

By creating a receiver operating characteristic curve (ROC curve) (fig. 2), it is clear that the Ct values are a good classifier for predicting the risk of ARDS following infection with SARS CoV2: The Area Under the ROC Curve AUC= 0.998 with a significance level P<0.0001.

The optimal Ct cut-off value was $Ct_{cut\text{-}off}$= 24.66 (Youden Index J= 0,9705), with a sensitivity and specificity of 100% and 97.05% respectively, the negative predictive value of this cut-off value was 100% while the positive predictive value was 78 % (95% CI: 67,48-85,83).

It is true that the determination of a cut-off value of Ct remains an approximate approach, since such a cut-off value depends on the PCR kit used and it's clear that the cycle threshold (Ct) values from RT-PCR can be affected by batch effect, and cannot be directly interpreted as viral load (16) but, despite that, the current study proves that there is a good correlation between Ct values and the risk of developing an ARDS (P<0.0000001-Fisher exact test-,fig.3), these results agree with those of other studies using viral loads and having demonstrated an association between disease progression, increased risk of death and SARS-CoV-2 viral loads (3,4,17).

CONCLUSION

Our data show that the determination of SARS CoV2 RT-PCR cycle threshold values from nasopharyngeal swabs performed during the first week after symptom onset may aid in the risk stratification of patients with COVID-19, using Sansure Biotech™ SARS Cov2 RT-PCR kits on Rotor-Gene™ thermocycler we have been able to set Ct=24.66 as a cut-off.

Conflict of interest

The authors declare that they have no conflict of interests.

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