BRIEF REPORT

Lack of Prognostic Value of SARS-CoV2 RT-PCR Cycle Threshold in the Community

Miguel J. Martínez · Luca Basile · Antoni Sisó-Almirall ·

Victor Cristino · Genoveva Cuesta · Juan Carlos Hurtado ·

Mariana Fernandez-Pittol · María Mar Mosquera · Alex Soriano ·

Ana Martínez · Mª Angeles Marcos · Jordi Vila · Climent Casals-Pascual

ABSTRACT

The immense impact of the COVID-19 pandemic on health systems has motivated the scientific community to search for clinical prognostic factors for SARS-CoV-2 infection. Low cycle threshold values (Ct) of diagnostic real-time RT-PCR assays in hospitalized patients have been associated with a poor prognosis in several studies, whereas other studies did not find this association. We explored whether SARS-CoV-2 Ct values at diagnosis were associated with a poor outcome (admission to hospital and death) in 604 community patients diagnosed at primary health centers. Although lower Ct values were found in patients who died of COVID-19, the Ct value was not significantly associated with a worse outcome in a multivariate analysis, while age remained an independent prognostic factor. We did not find evidence to support the role of Ct values as a prognostic factor of COVID-19 in community cases.

M. J. Martínez · G. Cuesta · J. C. Hurtado ·
M. Fernandez-Pittol · M. M. Mosquera ·
M. A. Marcos · J. Vila · C. Casals-Pascual (✉)
Department of Microbiology, CDB, Hospital Clinic,
University of Barcelona, Barcelona, Spain
e-mail: ccasals@clinic.cat

M. J. Martínez · J. C. Hurtado · M. M. Mosquera ·
M. A. Marcos · J. Vila · C. Casals-Pascual
Barcelona Institute for Global Health (ISGlobal),
Barcelona, Spain

L. Basile · V. Cristino · A. Martínez
Public Health Agency of Catalonia, Generalitat of
Catalonia, Barcelona, Spain

A. Sisó-Almirall
Consorci d'Atenció Primària de Salut Barcelona
Esquerra (CAPSBE), Barcelona, Spain

A. Soriano
Department of Infectious Diseases, Hospital Clinic
of Barcelona-IDIBAPS, University of Barcelona,
Barcelona, Spain

Key Summary Points

Why carry out this study?

SARS-CoV-2 viral load correlated with worse clinical outcome in hospitalized patients in some studies, but its routine use to guide clinical decisions is controversial

We explored whether SARS-CoV-2 real-time Low cycle threshold values (Ct) values were associated with a poor prognosis in community patients diagnosed at primary health centers

What was learned from the study?

SARS-CoV-2 real-time Ct values were not associated with the severity of the disease in patients diagnosed at primary health centers

We did not find evidence to support the use of the Ct as a prognostic marker in patients diagnosed with COVID-19 in the community

INTRODUCTION

The utility of the real-time RT-PCR cycle threshold (Ct) value or viral load in nasopharyngeal samples (NPS) as a predictor of SARS-CoV-2 severe disease remains unclear. On the one hand, several studies have reported an independent association of (1) Ct values at hospital admission with the risk of intubation [1] and mortality [1, 2], (2) Ct values at diagnosis with disease severity, survival and sequelae [3] and (3) viral load (RNA copies/ml) at the time of diagnosis with mortality [4]. On the other hand, other studies found no significant association among (1) viral load at hospital admission and length of oxygen support, admission to intensive care unit or overall survival [5], (2) initial Ct values and in-hospital mortality [6] or (3) viral load at the emergency department and admission to ICU or mortality [7]. Most of the above-mentioned studies assessing the clinical prognostic value of SARS-CoV-2 Ct values were performed in hospitalized patients. A recent systematic review and meta-analysis found no association between the Ct value and risk of hospitalization. However, among hospitalized patients, lower Ct was associated with increased disease severity and mortality [8]. Here, we explored the potential association of the SARS-CoV-2 RT-PCR Ct value with the severity of the disease in the community by evaluating data of patients diagnosed at primary health care centers (PHCs).

METHODS

Study Design

All NPS for SARS-CoV-2 RT-PCR testing received from the PHCs in the catchment area of our hospital from June 2020 to January 2021 were included in the analysis. Duplicates were removed from the study, and only the first sample (or that closer to diagnosis) was evaluated. Epidemiological variables (age and sex), outcomes (hospital admission, intensive care admission and death) and the laboratory information of the patients were retrospectively retrieved from the medical and laboratory records of Hospital Clinic of Barcelona and data from the Public Health Agency of Catalonia, the public health authority responsible for the registration, notification and follow-up of SARS-CoV-2 infections in our region. To reduce Ct data variability associated with diagnostic platforms, we present data using a single method for all tested cases: a self-adapted robotic platform (Opentrons OT) for nucleic acid extraction and RT-PCR set-up [9] using the TaqPath™ COVID-19 CE-IVD RT-PCR kit (ThermoFisher Scientific, Waltham, MA). This kit detects three SARS-CoV-2 target genes (N, Orf1ab and S) and an internal control. The study was approved by the hospital Ethics and Research Committee (File HCB/2021/0122).

Databases and statistics

The main dependent variable explored was death. However, due to the low mortality of

community cases ($n = 4$), a composite outcome was defined (poor outcome) that included admission to hospital, admission to an intensive care unit (ICU) or death. The main independent variables studied were: age, sex and Ct values for the following genes: N, S and Orf1ab. The association of Ct with poor outcome or death was evaluated using a univariate (crude) and multivariate logistic regression adjusted for potential confounders. Statistical analysis was performed using Stata version 16.0 (StataCorp).

RESULTS AND DISCUSSION

All valid results obtained during the period of study (June 2020–January 2021) from samples submitted to our laboratory from five different PHCs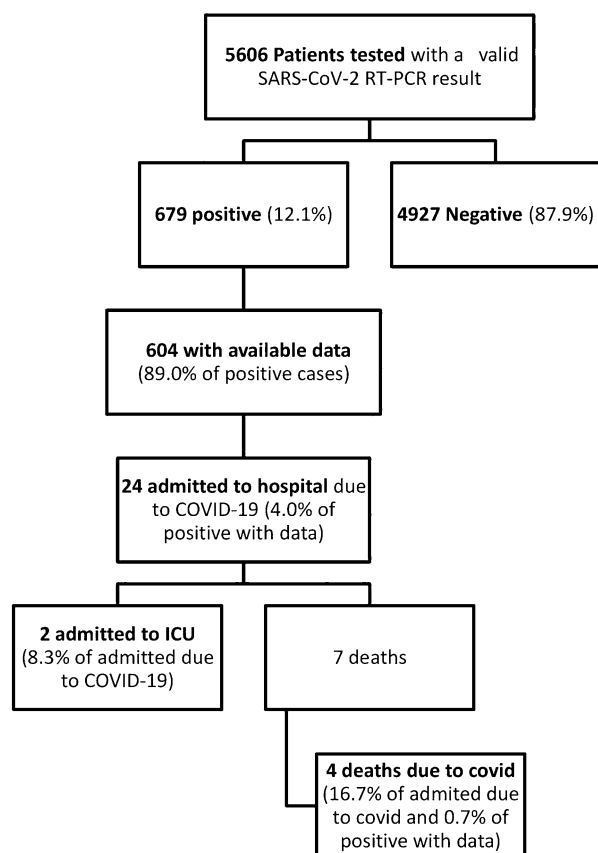 were analyzed. The study flowchart is shown in Fig. 1. More than 5500 patients were tested for SARS-CoV-2, and 679 resulted positive. Clinical and epidemiological data were available for 604 patients (89.0% of positive cases). Twenty-four patients were admitted to the hospital, two of them were admitted to an ICU, and four patients died due to COVID-19.

The median (IQR) age of the population studied was 37 (25–54) years. Of 604 cases, 318 (52.6%) were female. The median (IQR) Ct for
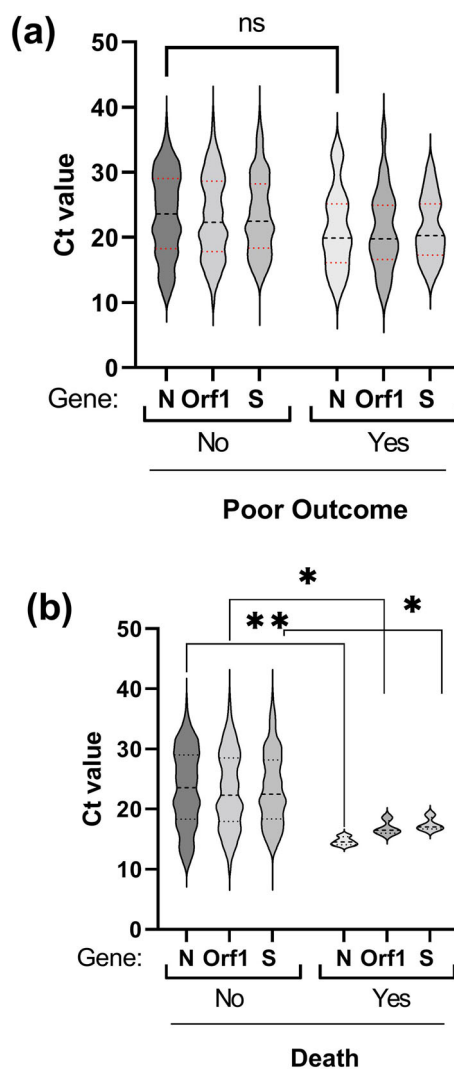

Fig. 1 SARS-CoV-2 RT-PCR results and clinical outcomes of patients screened at primary health centers

Fig. 2 SARS-CoV-2 cycle threshold (Ct) value for the genes N, Orf1ab and S. a Poor clinical outcome (admission, admission to critical care and death) and b only fatal outcome. *$P < 0.05$, **$P < 0.01$

the N gene was 23.5 (18.3–29.0) in the population studied and slightly lower [19.8 (16.1–25)] for those cases with a poor outcome (admission to hospital, ICU or death) although these differences were not statistically significant (p = 0.08) (Fig. 2a). When the analysis was repeated and Ct was compared between those who died (n = 4) and those who survived (N = 600), the median (IQR) differences were statistically significant (14.5 [14.1–15.2] vs. 23.5 [18.3–29], respectively; p = 0.007). This same pattern was observed for the S gene (p = 0.045) and for the ORF1 gene (p = 0.045) (Fig. 2b).

To further ascertain the prognostic role of the cycle threshold, we performed a logistic regression model. In the initial univariate model, the patient's age (OR: 1.06, 95% CI 1.04–1.08, p < 0.001) and male gender (2.8 [1.14–6.87, p = 0.02]) were associated with the composite poor outcome. In the multivariate model, age (OR: 1.07, 95% CI 1.04–1.09, p < 0.001) and male gender (OR: 5.48, 95% CI 1.99–15.1, p = 0.001) remained associated with a poor outcome. The following variables were associated with death in the univariate analysis: the cycle threshold for the N gene (OR: 0.73, 95% CI 0.55–0.96, p = 0.02) and the patient's age (OR: 1.13, 95% CI 1.03–1.25, p = 0.008). Only age remained as an independent prognostic factor for death (OR: 1.13, 95% CI 1.02–1.26, p = 0.018) whereas the Ct value was no longer significantly associated with a fatal outcome (OR: 0.67, 95% CI 0.42–1.06, p = 0.08) (Table 1).

In agreement with other studies, we observed a significant association of age and sex with poor COVID-19 outcome [3, 10]. Our results do not support the concept that the Ct value per se is a significant predictor of SARS-CoV-2 infection outcome among outpatients. SARS-CoV-2 viral load is higher in the first days of symptoms [11] and seems not to differ by age in people > 20 years [12, 13]. Interestingly, SARS-CoV-2 viral loads in asymptomatic infections have been reported to be as high as in symptomatic patients [14, 15]. A wide range of real-time RT-PCR diagnostic assays that provide Ct values is available for SARS-CoV-2. However, significant variations in the Ct can be observed depending on the viral target gene amplified, amplification platform, RNA extraction method or quality of the clinical sample [16, 17]. Several authors have highlighted these limitations suggesting that SARS-CoV-2 Ct values may not be recommended to guide clinical decisions as a general approach and rather should be interpreted with caution [18–20]. As NPS are not homogeneous in their cellular content and

Table 1 Uni- and multivariate analysis of association of cycle threshold, age and sex in relation to poor outcome and death

Variables	Crude		Adjusted	
	OR (95% CI)	p value	OR (95% CI)	p value
Poor outcome (admission/ICU/death)				
N gene cycle threshold	0.94 (0.88–1.00)	0.09	0.97 (0.94–1.04)	0.4
Age (years)	1.06 (1.04–1.08)	< 0.001	**1.07 (1.04–1.09)**	< 0.001
Sex (male)	2.8 (1.14–6.87)	0.024	**5.48 (1.99–15.1)**	0.001
Death				
N gene cycle threshold	0.73 (0.55–0.96)	0.027	0.67 (0.42–1.06)	0.088
Age (years)	1.13 (1.03–1.25)	0.008	**1.13 (1.02–1.26)**	0.018
Sex (male)	0.36 (0.038–3.56)	0.388	na	na

Statistically significant values in bold

Na not applicable, as the variable was not selected in the univariate analysis

depend on the collection of the sample, normalization of the viral gene Ct value with a cellular target has been proposed as a method to control evaluation errors that may occur using raw Ct data [21, 22]. Heterogeneity in the patients' clinical features, laboratory methods used and the clinical and laboratory variables analyzed among the different studies may explain the fact that the association of SARS-CoV-2 viral load and disease outcome is not consistent in the literature. Standardization of the laboratory methodology (including normalization by cellular control genes) together with the inclusion of the main variables currently known to influence the course of the disease may result in a better understanding of the impact of viral load on COVID-19 infected patients.

This study has several limitations. First, the number of patients with a fatal outcome was low (N = 4), affecting the robustness of the initial multivariate model using death as dependent variable, and therefore studies including a larger number of deaths from patients diagnosed at the community would be desirable. However, the study was adequately powered to find differences in the rates of poor outcome as defined by admission to hospital or to a critical care unit or death in relation to the Ct. Second, the number of variables available for these patients was limited, and some important risk factors and comorbidities could have affected the study of these associations. Finally, the use of a single diagnostic platform, while it provides consistent and comparable values across all patients studied, may not translate well to other platforms, and further studies using different platforms should confirm these findings. In conclusion, as previously shown, age is a strong predictor of mortality and poor outcome. We could not find evidence to support the use of the Ct as a prognostic marker in patients diagnosed with COVID-19 in the community. Nonetheless, the Ct value seems relevant in terms of infection control for the identification of cases with high SARS-CoV-2 viral loads. The Ct/viral load values have been shown to correlate with the probability of viral isolation in cell culture and disease transmissibility. In fact, several studies have reported that a small proportion of cases with very high viral loads might be responsible for a significant number of secondary cases [12, 23, 24].

study are available from the corresponding author on reasonable request.

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