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BRIEF REPORT

Cycle threshold predicted mortality in a cohort of patients with hematologic malignancies infected with SARS-CoV-2

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Abstract When a SARS-CoV-2 RT-qPCR test is performed, it may determine an indirect measure of viral load called cycle threshold (Ct). Respiratory samples with Ct <25.0 cycles are considered to contain a high viral load. We aimed to determine whether SARS-CoV-2 Ct at diagnosis could predict mortality in patients with hematologic malignancies (lymphomas, leukemias, multiple myeloma) who contracted COVID-19. We included 35 adults with COVID-19 confirmed by RT-qPCR performed at diagnosis. We evaluated mortality due to COVID-19 rather than mortality due to the hematologic neoplasm or all-cause mortality. Twenty-seven (27) patients survived and 8 died. The global mean Ct was 22.8 cycles with a median of 21.7. Among the survivors, the mean Ct was 24.2, and the median Ct value was 22.9 cycles. In the deceased patients, the mean Ct was 18.0 and the median Ct value was 17.0 cycles. Using the Wilcoxon Rank Sum test, we found a significant difference ($p=0.035$). SARS-CoV-2 Ct measured in nasal swabs obtained at diagnosis from patients with hematologic malignancies may be used to predict mortality.

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PALABRAS CLAVE

Umbral de ciclado;
Reacción en cadena
de la polimerasa;
Neoplasias
hematológicas;
SARS-CoV-2;
COVID-19

El umbral de ciclado predijo la mortalidad en una cohorte de pacientes con neoplasias hematológicas infectados con SARS-CoV-2

Resumen Cuando se realiza una RT-qPCR para SARS-CoV-2, es posible determinar una medida indirecta de la carga viral llamada umbral de ciclado (Ct). Las muestras respiratorias con Ct <25,0 ciclos se consideran de alta carga viral. Nos propusimos determinar si el Ct para SARS-CoV-2 al diagnóstico predice la mortalidad en pacientes con neoplasias hematológicas (linfomas, leucemias, mielomas) que contrajeron COVID-19. Incluimos 35 adultos con COVID-19 confirmado por RT-qPCR al diagnóstico. Evaluamos la mortalidad por COVID-19, no la mortalidad por la neoplasia hematológica o la mortalidad por cualquier causa. De los 35 pacientes, 27 sobrevivieron y 8 fallecieron. El Ct global medio fue 22,8 ciclos con una mediana de 21,7 ciclos. Entre los sobrevivientes, el Ct medio fue 24,2 ciclos con una mediana de 22,9 ciclos. Entre los fallecidos, el Ct medio fue 18,0 y el Ct mediano fue 17,0 ciclos. Empleando la prueba de suma de rangos de Wilcoxon, encontramos una diferencia significativa ($p = 0,035$). En pacientes con neoplasias hematológicas infectados con coronavirus, el Ct de SARS-CoV-2 medido en hisopados nasales al momento del diagnóstico podría ser utilizado para predecir la mortalidad.

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Since the beginning of the SARS-CoV-2 pandemic, there has been a remarkable effort to find a reliable laboratory determination that could predict the severity and mortality of COVID-19. Much of this attention was placed on past evidence from SARS-Coronavirus, since higher viral load was associated with worse outcomes^{2,8}. For this purpose, a proxy for viral load called cycle threshold has been investigated in the general population.

After over 2 years of pandemic, it is widely known that the standard molecular method for COVID-19 diagnosis is via RT-PCR¹³. The information derived from RT-qPCR goes beyond a binary “positive-negative” result. RT-qPCR measures the viral RNA in terms of Ct, which is the number of cycles that the fluorescent signal requires to become detectable, and is inversely proportional to the viral load. Values ≥ 40.0 cycles are considered negative. It has been determined that respiratory samples with Ct <25.0 are considered to carry a high viral load, which was independently associated with an increased risk of death in the general population^{4,9}.

The overall data regarding the correlation between the viral load, or the Ct, and COVID-19 severity and risk of death is controversial, whether derived from case series or systematic reviews^{3,11,12}. Several factors may affect Ct values. These have been grouped into pre-analytic (collection technique, type of specimen, sampling time), analytic (internal control, type of RT-PCR used, purity of reagents, pipetting defects), and post-analytic categories (interpretation of the reports)⁹.

It has been demonstrated that, among oncology patients, those with hematologic malignancies have a 1.57-fold risk of experiencing severe denouements compared with patients with solid organ tumors⁷. Westblade et al. conducted a multicenter, observational, cohort study of patients with and without cancer. Not only did they find that those with hematologic malignancies had higher viral loads than non-cancer patients (Ct = 25.0 and 29.2, respectively),

but also that the presence of a hematologic malignancy was independently associated with having a higher viral load compared with non-cancer patients (aOR = 2.52; 95% CI = 1.3–4.88; $p = 0.006$). Moreover, death was statistically higher in patients with a high viral load, as defined above¹⁵.

We conducted a multicenter, retrospective study with the aim of assessing whether SARS-CoV-2 Ct at diagnosis could predict mortality in patients with hematologic malignancies who contracted COVID-19. Two (2) tertiary centers in Buenos Aires participated.

Inclusion criteria: (1) Adult patients with hematologic malignancies (lymphomas, leukemias, multiple myeloma); (2) SARS-CoV-2 infection confirmed by RT-qPCR performed in either of the 2 participating tertiary centers; (3) patients admitted or treated in an out-patient basis; (4) SARS-CoV-2 infection diagnosed between March 3rd, 2020 (when the first case of COVID-19 was confirmed in Argentina) to March 31st, 2022. Exclusion criteria: incomplete data.

We extracted epidemiologic and clinical data, and the Ct value of each patient, from the medical records.

The laboratories of the participating centers used different RT-qPCR SARS-CoV-2 kits. Thirty (30) nasal swabs from one center were analyzed using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (Integrated DNA Technologies – IDT, Iowa, USA), based on N1 and N2 probes for detecting SARS-CoV-2, and the human RNaseP (RP) as RNA extraction quality control. Five (5) nasal swabs from the other tertiary center were analyzed using cobas® SARS-CoV-2 Test (Roche Molecular Systems, Inc – South Branchburg, NJ, USA), which targets the ORF1ab and E genes, and uses internal control RNA (RNA IC) molecules. A mean Ct was obtained from both targeted genes in each case.

We compared the mean Ct value of the deceased patients with the survivors. We evaluated mortality due to COVID-19 rather than mortality due to the hematologic neoplasm or all-cause mortality. For those we were able to follow up

Table 1 Characteristics of SARS-CoV-2 infected patients.

Patient	Gender	Age (years)	Hematologic neoplasm	Comorbidities	Date of diagnosis ^a	Ct (cycles)	Gene ^b	Deceased	# vaccine doses at diagnosis
1	F	34	ALL	DM, HT, T, Ob	6/29/2021	11.8	N1/N2	No	0
2	M	22	ALL	Ob	1/12/2021	22.9	N1/N2	No	0
3	M	49	Lymphoma	T, Ob, HIV2	7/27/2020	19.9	N1/N2	Yes	0
4	M	61	Lymphoma	HT, CKD	5/15/2021	10.3	N1/N2	Yes	0
5	F	39	ALL	Ob	8/11/2020	15.8	N1/N2	No	0
6	F	60	AML	DM, HT	9/6/2020	20.4	N1/N2	Yes	0
7	M	42	AML	Ob	3/31/2021	17.1	N1/N2	Yes	0
8	M	20	ALL	Ob	6/9/2020	11.5	N1/N2	No	0
9	M	23	AML	Ob	4/15/2021	28.0	N1/N2	No	0
10	M	19	ALL	Ob	20/7/2020	37.0	N1/N2	No	0
11	F	52	Lymphoma	HT, Ob, HIV1	5/26/2021	9.9	N1/N2	Yes	0
12	M	66	CML	T, Ob	5/15/2021	18.7	N1/N2	No	1
13	F	70	Lymphoma	Ob	7/28/2021	28.1	N1/N2	No	1
14	F	57	Lymphoma	None	3/22/2021	8.8	N1/N2	No	0
15	F	32	Lymphoma	None	5/8/2021	22.3	N1/N2	No	0
16	F	32	Lymphoma	None	8/26/2021	34.5	N1/N2	No	0
17	F	50	Lymphoma	T	5/24/2021	19.7	N1/N2	No	0
18	M	51	Lymphoma	None	9/29/2020	28.5	N1/N2	No	0
19	M	19	ALL	None	12/21/2021	17.1	N1/N2	No	2
20	M	22	ALL	Ob	1/10/2022	17.4	N1/N2	No	0
21	F	82	Lymphoma	HT, Ob	1/12/2022	21.1	N1/N2	No	2
22	F	49	ALL	None	1/14/2022	26.5	N1/N2	No	2
23	M	77	Lymphoma	None	1/26/2022	29.6	N1/N2	No	3
24	F	19	ALL	None	2/23/2022	27.8	N1/N2	No	0
25	M	63	MM	None	1/13/2022	17.0	N1/N2	Yes	3
26	M	66	Lymphoma	HIV2	1/11/2022	17.9	N1/N2	No	2
27	F	26	ALL	None	3/14/2022	35.0	N1/N2	No	2
28	F	50	Lymphoma	DM, HT, Card	3/14/2022	36.0	N1/N2	No	1
29	M	33	Lymphoma	HIV1	9/1/2021	35.8	N1/N2	No	0
30	M	42	AML	Ob	4/27/2021	32.8	N1/N2	No	0
31	F	24	Lymphoma	CKD	3/23/2022	26.2	ORF1ab/E	No	3
32	M	39	Lymphoma	Ob	10/13/2020	20.6	ORF1ab/E	No	0
33	F	19	Lymphoma	None	6/2/2021	33.7	ORF1ab/E	Yes	0
34	M	63	MM	T	6/1/2021	16.1	ORF1ab/E	Yes	0
35	M	39	Lymphoma	Ob	2/7/2022	22.8	ORF1ab/E	No	0

Gender: F: female; M: male.

Hematologic neoplasm: ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MM: multiple myeloma.

Comorbidities: DM: diabetes mellitus; HT: hypertension; Ob: obesity; CKD: chronic kidney disease; T: tobacco use; HIV1: HIV with <200 CD4⁺/μl; HIV2: HIV with >200 CD4⁺/μl.

^a Date of diagnosis expressed as month/day/year.

^b Mean Ct value obtained from both genes.

with sequential testing, we calculated the time to SARS-CoV-2 clearance, understood as the time elapsed between the first positive and the first negative result. The patients were not subclassified into the sequential COVID-19 waves but analyzed as a whole.

The identities of the patients included were preserved. This study was approved by the Ethics Committees of the participating institutions (*Comité de Ética del Hospital de Clínicas "José de San Martín"* and *Comité de Ética del Sanatorio Sagrado Corazón*). The procedures followed were in accordance with the Helsinki Declaration of 1975, revised

in 2013 of the World Medical Association. Informed consent was obtained.

A total of 35 patients were eligible. No patient was excluded. [Table 1](#) describes the clinical characteristics and disease denouement of the patients, their comorbidities, Ct values and vaccination status at diagnosis.

Twenty seven (27) patients (77.1%) survived, and 8 died (22.9%). Only 1 of the deceased patients had been vaccinated. Two (2) HIV+ patients died: an unvaccinated 49-year-old male with CD4⁺ count >200/μl and a lymphoma (patient 3), and an unvaccinated 52-year-old female – also

with a lymphoma – with less than <200 CD4+/ μ l, among other comorbidities also shown in [Table 1](#).

The global mean Ct was 22.8 cycles with a median of 21.7. Among the survivors, the mean Ct was 24.2, and the median was 22.9 cycles. In regard to the deceased patients, the mean Ct was 18.0 and the median Ct value was 17.0 cycles. Since we could not assume a normal distribution of Ct, we analyzed the existing difference between the survivors and deceased using the Wilcoxon Rank Sum test. This difference was significant ($p=0.035$). Among the patients that we were able to follow-up, the mean SARS-CoV-2 time to clearance was 60 days (range = 19–158 days).

This retrospective analysis of patients with hematologic malignancies infected with SARS-CoV-2, resulted in a relatively small population ($n=35$). One hypothesis is that uninfected cancer patients limited their hospital visits and stayed safe at home during lockdown. Some of the patients who had been diagnosed and treated for their hematologic malignancies could have attended other institutions for medical care. Notwithstanding this small number, we did find a statistically significant difference between the SARS-CoV-2 Ct value in the nasal swabs from the patients who died, compared with those who survived.

Ct values could be useful in several clinical scenarios. First, it could help clinicians identify patients at high risk of mortality from COVID-19 at the time of diagnosis¹⁶. Second, it may serve as a guide to end isolation precautions in convalescent patients¹⁴, since Ct values above 33–34 are considered noncontagious, at least from what has been observed in vitro using Vero E6 cell cultures⁶. Lastly, it could help estimate the risk that could result from administering chemotherapy in patients infected with SARS-CoV-2, whose decision on whether to proceed or delay the treatment is complex due to the nature of their hematologic neoplasms. This, however, has not been universally validated and can only be used on an individual basis and with caution.

SARS-CoV-2 viral load appears to peak in the upper respiratory tract during the first week after symptom onset, at least in immunocompetent patients¹. Immunocompromised patients may have prolonged viral shedding and even clinical relapses^{5,10}. In this subpopulation, which includes patients with hematologic malignancies, viral shedding has been described as long as 238 days¹. In the group of patients that we were able to follow up, our results are compatible with these reports (mean 60 days, range = 19–158 days).

This study is not without limitations. First, we were not able to perform a multivariate analysis, and, therefore, our results were not adjusted for possible confounders. Of note, the COVID-19 vaccination campaign in Argentina began on December 29th, 2020. Moreover, the small sample size and the high variability of Ct could condition an artifactual result. Although Ct values are inversely proportional to the viral load, this correlation is nonlinear and should be used with caution⁴. It should be borne in mind that the patients' immune status was variable and depended on numerous factors that we were not able to assess completely. The decision to compare the Ct results obtained using different RT-PCR kits was grounded on our intention to explore the clinical utility of Ct, without ignoring its limitations. In other words, when considered at the time of making a clinical decision, the gene the Ct value targets is not taken into account. We were unable to normalize the viral Ct to

the internal control Ct. Finally, further studies should be conducted before adopting Ct as a universal prediction tool.

In conclusion, we found that SARS-CoV-2 Ct measured in nasal swabs obtained at diagnosis from patients with hematologic malignancies is associated with mortality. This may constitute an interesting line of investigation for clinical decision-making in this subpopulation.

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Conflict of interests

None declared.

References

1. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe*. 2021;2, e13–e22.
2. Chu CM, Poon LL, Cheng VC, Chan KS, Hung IF, Wong MM, Chan KH, Leung WS, Tang BS, Chan VL, Ng WL, Sim TC, Ng PW, Law KI, Tse DM, Peiris JS, Yuen KY. Initial viral load and the outcomes of SARS. *CMAJ*. 2004;171:1349–52.
3. Dahdouh E, Lázaro-Perona F, Romero-Gómez MP, Mingorance J, García-Rodríguez J. Ct values from SARS-CoV-2 diagnostic PCR assays should not be used as direct estimates of viral load. *J Infect*. 2021;82:414–51.
4. Faico-Filho KS, Passarelli VC, Bellei N. Is higher viral load in SARS-CoV-2 associated with death? *Am J Trop Med Hyg*. 2020;103:2019–21.
5. Helleberg M, Niemann CU, Moestrup KS, Kirk O, Lebech AM, Lane C, Lundgren J. Persistent COVID-19 in an immunocompromised patient temporarily responsive to two courses of remdesivir therapy. *J Infect Dis*. 2020;222:1103–7.
6. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, Gautret P, Raoult D. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *Eur J Clin Microbiol Infect Dis*. 2020;39:1059–61.
7. Lee LYW, Cazier JB, Starkey T, Briggs SEW, Arnold R, Bisht V, Booth S, Campton NA, Cheng VWT, Collins G, Curley HM, Earwaker P, Fittall MW, Gennatas S, Goel A, Hartley S, Hughes DJ, Kerr D, Lee AJX, Lee RJ, Lee SM, McKenzie H, Middleton CP, Murugaesu N, Newsom-Davis T, Olsson-Brown AC, Palles C, Powles T, Protheroe EA, Purshouse K, Sharma-Oates A, Sivakumar S, Smith AJ, Topping O, Turnbull CD, Várnai C, Briggs ADM, Middleton G, Kerr R, UK Coronavirus Cancer Monitoring Project Team. COVID-19 prevalence and mortality in patients with cancer and the effect of primary tumour subtype and patient demographics: a prospective cohort study. *Lancet Oncol*. 2020;21:1309–16.
8. Ng EK, Hui DS, Chan KC, Hung EC, Chiu RW, Lee N, Wu A, Chim SS, Tong YK, Sung JJ, Tam JS, Lo YM. Quantitative analysis and prognostic implication of SARS coronavirus RNA in the plasma and serum of patients with severe acute respiratory syndrome. *Clin Chem*. 2003;49:1976–80.
9. Rabaan AA, Tirupathi R, Sule AA, Aldali J, Mutair AA, Alhumaid S, Muzahheed, Gupta N, Koritala T, Adhikari R, Bilal M, Dhawan M, Tiwari R, Mitra S, Emran TB, Dhama K. Viral dynamics and

- real-time RT-PCR Ct values correlation with disease severity in COVID-19. *Diagnostics (Basel)*. 2021;11:1091.
10. Sepulcri C, Dentone C, Mikulska M, Bruzzone B, Lai A, Fenoglio D, Bozzano F, Bergna A, Parodi A, Altosole T, Delfino E, Bartalucci G, Orsi A, Di Biagio A, Zehender G, Ballerini F, Bonora S, Sette A, De Palma R, Silvestri G, De Maria A, Bassetti M. The longest persistence of viable SARS-CoV-2 with recurrence of viremia and relapsing symptomatic COVID-19 in an immunocompromised patient – a case study. *Open Forum Infect Dis*. 2021;8, ofab217.
 11. Shah S, Singhal T, Davar N, Thakkar P. No correlation between Ct values and severity of disease or mortality in patients with COVID 19 disease. *Indian J Med Microbiol*. 2021;39:116–7.
 12. Shah VP, Farah WH, Hill JC, Hassett LC, Binnicker MJ, Yao JD, Murad MH. Association between SARS-CoV-2 cycle threshold values and clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. *Open Forum Infect Dis*. 2021;8, ofab453.
 13. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. *J Clin Microbiol*. 2020;58, e00512–e00520.
 14. Taramasso L, Sepulcri C, Mikulska M, Magnasco L, Lai A, Bruzzone B, Dentone C, Bassetti M. Duration of isolation and precautions in immunocompromised patients with COVID-19. *J Hosp Infect*. 2021;111:202–4.
 15. Westblade LF, Brar G, Pinheiro LC, Paidoussis D, Rajan M, Martin P, Goyal P, Sepulveda JL, Zhang L, George G, Liu D, Whittier S, Plate M, Small CB, Rand JH, Cushing MM, Walsh TJ, Cooke J, Safford MM, Loda M, Satlin MJ. SARS-CoV-2 viral load predicts mortality in patients with and without cancer who are hospitalized with COVID-19. *Cancer Cell*. 2020;38: 661–71.
 16. Wright J, Achana F, Diwakar L, Semple MG, Carroll WD, Bailie K, Thompson C, Alcock A, Kemp TS. Cycle threshold values are inversely associated with poorer outcomes in hospitalized patients with COVID-19: a prospective, observational cohort study conducted at a UK tertiary hospital. *Int J Infect Dis*. 2021;111:333–5.