Open Forum Infectious Diseases

REVIEW ARTICLE

Association Between SARS-CoV-2 Cycle Threshold Values and Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis

Vishal P. Shah,[1] Wigdan H. Farah,[1] James C. Hill,[1] Leslie C. Hassett,[2] Matthew J. Binnicker,[3] Joseph D. Yao,[3] and M. Hassan Murad[1]

[1]Division of Preventive, Occupational and Aerospace Medicine, Mayo Clinic, Rochester, Minnesota, USA, [2]Mayo Clinic Libraries, Mayo Clinic, Rochester, Minnesota, USA, and [3]Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota, USA

Cycle threshold (C_T) values are correlated with the amount of viral nucleic acid in a sample and may be obtained from some qualitative real-time polymerase chain reaction tests used for diagnosis of most patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, C_T values cannot be directly compared across assays, and they must be interpreted with caution as they are influenced by sample type, timing of sample collection, and assay design. Presently, the correlation between C_T values and clinical outcomes is not well understood. We conducted a systematic review and meta-analysis of published studies through April 19, 2021, that reported an association between C_T values and hospitalization, disease severity, and mortality in patients ≥18 years old with SARS-CoV-2. A meta-analysis of 7 studies showed no significant difference in mean C_T values between hospitalized and nonhospitalized patients. Among hospitalized patients, those with C_T values <25 had a high risk of more severe disease and mortality than patients with C_T values >30 (odds ratio [OR], 2.31; 95% CI, 1.70 to 3.13; and OR, 2.95; 95% CI, 2.19 to 3.96; respectively). The odds of increased disease severity and mortality were less pronounced in patients with C_T values of 25–30 compared with >30.

Keywords. clinical outcomes; COVID-19; cycle threshold; meta-analysis; prognosis; SARS-CoV-2.

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may lead to a spectrum of disease, ranging from asymptomatic infection to severe symptomatic coronavirus disease 2019 (COVID-19). As of May 5, 2021, there have been over 32 million confirmed cases of COVID-19 in the United States, resulting in >5 million hospitalizations [1, 2]. Among hospitalized patients with COVID-19, roughly one-third of patients have required intensive care unit (ICU) admission, and 1 in 9 patients have died [3–6].

Current known host risk factors for progression to severe COVID-19 include advanced age, male sex, and certain comorbidities including obesity and heart failure [7–9]. Laboratory values such as interleukin-6 level, C-reactive protein level, and peripheral blood lymphocyte count have also been correlated with disease severity [10–12].

There has also been interest in assessing the impact of viral load on clinical outcomes. Most patients with COVID-19 are diagnosed with real-time polymerase chain reaction (rtPCR) assays, which are most commonly qualitative tests (ie, providing a positive or negative result). Many rtPCR assays can provide a cycle threshold (C_T) value, which refers to the number of PCR cycles required to generate target amplification (as measured by fluorescence) that is distinguishable from baseline fluorescence [13]. Using a standard curve correlating C_T values to different known concentrations of SARS-CoV-2 virions, a quantitative viral load can be determined in a given clinical specimen.

While C_T value is inversely proportional to viral load, this correlation is nonlinear, and many factors influence this association, including sample collection and rtPCR assay [14]. Additional limitations in the use of C_T values in patients with SARS-CoV-2 include the impact of the timing of sample collection, as generally earlier in the disease course individuals will have a higher viral load. Despite these limitations, there is widespread interest among clinicians in how the C_T value can be used to better manage patients infected with SARS-CoV-2.

However, a gap remains in the knowledge of the clinical utility of C_T values to aid in prognostication of patients with COVID-19. An early systematic review evaluated the clinical utility of C_T values in patients with COVID-19, but this analysis only included 1 study on disease progression and another study on patient mortality [15]. Several studies have reported noncorrelative results between clinical outcomes in patients with COVID-19 and both SARS-CoV-2 viral load and C_T values [16–21]. These discrepancies may be due, in part, to different technologies used, timing of testing,

Received 30 June 2021; editorial decision 25 August 2021; accepted 30 August 2021.

Correspondence: Vishal Shah, MD, MPH, Department of Preventive, Occupational, and Aerospace Medicine, Mayo Clinic, 200 First St. SW, Rochester, MN 55905 (shah.vishal1@mayo.edu).

Open Forum Infectious Diseases®2021

https://doi.org/10.1093/ofid/ofab453

and differing criteria for assessing clinical outcomes at varying institutions across the globe. Given this uncertainty, we conducted a systematic review and meta-analysis to assess the association between C_T values and clinical outcomes, including the risk of hospitalization among patients with COVID-19 and the risk of disease severity and death in such patients.

METHODS

This study was registered in PROSPERO (CRD42021235617), and findings were reported according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guideline (Supplementary Data).

Outcomes of Interest

We sought to identify published studies evaluating the association between CT values and 3 distinct outcomes among patients with a confirmed SARS-CoV-2 infection: (1) need for hospitalization; and among hospitalized patients, (2) disease severity (WHO Severity scale grade 5 or higher, specifically invasive or noninvasive ventilation and/or ICU need); (3) in-hospital and 30-day mortality.

Data Sources and Search Strategies

A comprehensive search of several databases from January 1, 2019, through January 28, 2021, limited to the English language and excluding animal studies, was conducted. Given the rapid pace of publications, the search was repeated on April 19, 2021. The databases included Ovid MEDLINE and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily, Ovid Embase, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, Web of Science, and Scopus. The search strategy was designed and conducted by a medical reference librarian (L.C.H.) with input from the study investigators. Controlled vocabulary supplemented with keywords was used to search for studies describing the association between SARS-CoV-2 C_T values and clinical outcomes. The actual strategy listing all search terms used and how they are combined is described in the Appendix (Supplementary Data).

Eligibility Criteria and Study Selection

We included cohort studies, randomized controlled trials, and case reports and series that met the following criteria: (1) adults ≥18 years, (2) publication in English, (3) reported C_T value data, (4) specified sample source (eg, nasopharyngeal swab), (5) specified rtPCR assay, (6) minimum 5 study subjects with specified outcome of interest, and (7) full manuscript available.

Each study was assessed for inclusion by 2 independent reviewers, first by screening the publication title and abstract and subsequently by analyzing content in the full-text articles (V.P.S., W.H.F., or J.C.H.). Discordance of study data was resolved by evaluation by a third reviewer or discussion on eligibility and consensus agreement.

Data Collection

Two reviewers abstracted data from each included study (V.P.S. and W.H.F.). Disagreements were resolved by discussion. When multiple studies from the same data set were reported, we included only the largest data set. If a study reported the use of multiple rtPCR assays, data were abstracted and synthesized separately for each assay.

For each outcome of interest, adjusted odds ratios (ORs) for low (C_T <25) and medium (C_T 25–30) compared with high (C_T >30) C_T values were collected. If unavailable or not reported, data from tables were abstracted and unadjusted odds ratios were calculated. If data were reported but were insufficient for meta-analysis (eg, graphic data), authors were contacted for more details.

Additionally, the mean C_T value and SD for each outcome were collected if available (eg, survivor vs nonsurvivor mean C_T values). If mean C_T values and SD data were not available, information was imputed from interquartile ranges. Sample population, sample source, and rtPCR platform data were also collected.

Risk of Bias Assessment

Risk of Bias assessment was performed using a modified Newcastle-Ottawa scale by 2 independent reviewers (V.P.S. and W.H.F.) (Supplementary Data). Disagreements were resolved by discussion and consensus. We assessed the representativeness of the study population, selection of the nonexposed cohort, comparability, and outcome assessment. A quantitative score for risk of bias was not used, but we focused on the most critical element of bias in this specific context, which was adjustment for confounders [22].

Data Synthesis

Studies that reported ORs or reported data from which odds ratios could be calculated were analyzed separately from studies that reported C_T values as continuous variables for outcomes of interest. If studies reported C_T values both as categorical and continuous variables, the study was evaluated as part of the synthesis that had the larger data set.

Because of heterogeneity across study settings and populations, the DerSimonian-Laird random effect model as implemented in the OpenMeta Analyst software package was used [23]. Heterogeneity was assessed using the I^2 statistic, with low heterogeneity being <50%, moderate 50% to 75%, and high >75%. Heterogeneity was explored using subgroup analyses by sample source, rtPCR assay, use of adjusted vs unadjusted ORs, and risk of bias. We were unable to statistically evaluate the presence of publication bias due to the small number of studies included per analysis.

RESULTS

Study Selection and Characteristics

The search yielded 459 potentially relevant articles, of which 21 studies met inclusion criteria (Figure 1). Study characteristics are listed in Tables 1–3 for each outcome. A total of 18

studies contributed data to the meta-analysis. Overall, 8 and 10 studies reported C_T values as categorical and continuous variables, respectively, in relation to outcomes of interest and were synthesized collectively for each outcome.

Risk of Bias Assessment

Overall, 15 studies had a high risk of bias (Supplementary Data). Three studies were deemed to have a moderate risk of bias.

Meta-analysis

For the outcome of hospitalization, 1 study reported only categorical C_T values, and thus we were not able to perform an analysis [21]. Seven studies (n = 3291 patients) were analyzed, and 4 studies reported higher C_T values in hospitalized patients, 1 of which did not reach statistical significance. Three studies reported lower mean C_T values among hospitalized patients, 1 of which did not reach statistical significance. Meta-analysis found no difference in the mean C_T value between hospitalized and nonhospitalized patients with SARS-CoV-2 with high heterogeneity (0.062; 95% CI, −1.933 to 2.056; I^2 = 92.71%) (Figure 2).

For disease severity among hospitalized patients, 4 studies (n = 2347 patients) reported categorical C_T values. Hospitalized patients with C_T values <25 or 25–30 had an increased risk of

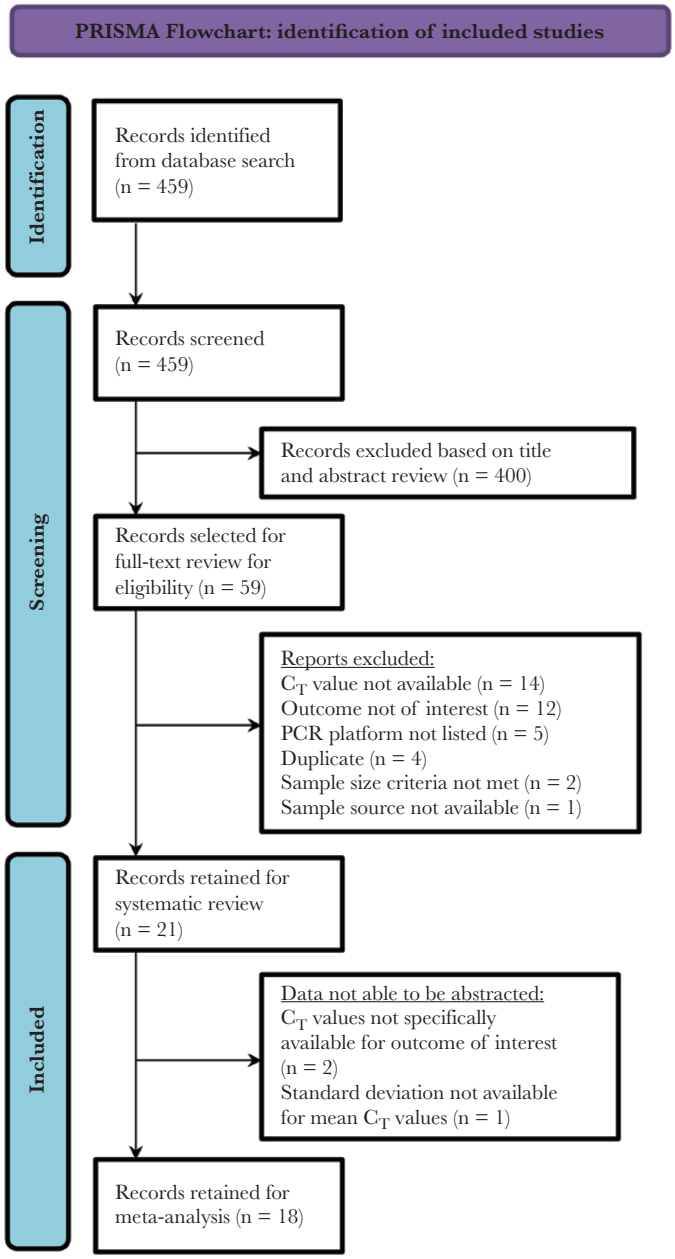

Figure 1. PRISMA flowchart. Abbreviations: C_T, cycle threshold; PCR, polymerase chain reaction.

Table 1. Summary of Studies Evaluating Association Between CT Values and Hospitalization

Author	Year	City, State, Country	Study Enrollment Period	Sample Size	Sample Source	rtPCR Assay	Comparator	Reference Group	Lower C_T Values Associated With Hospitalization	Summary
Ade et al. [37]	2021	Western Germany	March 15–September 2020	1077	NP	Qiagen QIAgility	Hospitalized patients	Nonhospitalized patients	No	There was no significant difference in mean C_T values between hospitalized and nonhospitalized patients with SARS-CoV-2.
Amodio et al. [38]	2020	Palermo, Italy	February 24–April 8, 2020	381	Nasal, pharyngeal, or NP	Thermo Fisher Scientific TaqMan (custom)	Hospitalized patients	Nonhospitalized patients	Yes	After adjusting for age and sex, authors found a lower risk of hospitalization with higher C_T values (aOR, 0.95; 95% CI, 0.91 to 0.99).
Faico-Filho et al. [39]	2020	São Paulo, Brazil	March 17–June 17, 2020	875	NP	Thermo Fisher Scientific AgPath-ID	Hospitalized patients	Nonhospitalized patients and health care workers	No	Study participants who did not require hospitalization had lower mean C_T values than patients who required hospitalization.
Koureas et al. [40]	2021	Thessaly, Greece	April 8–June 4, 2020	142	OP	AriaMx Real-time PCR System; Roche Cobas; Qiagen Rotor-Gene Q analyzer	Hospitalized patients	Nonhospitalized patients	Yes	During an outbreak investigation, index cases were evaluated for their C_T values and followed up for need for hospitalization. Cases who required hospitalization had lower mean C_T values.
Maltezou et al. [21]	2021	Athens and Thessaloniki, Greece	February 26–May 3, 2020	518	NP or OP	Genesig COVID-19 CE-IVD real-time RT-PCR kit or Thermo Fischer Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	No	In univariate analyses, compared with patients with C_T >30 or 25–30, patients with C_T <25 were older, more often had comorbidities, developed symptomatic COVID-19, were intubated, and died. In multivariate analysis controlling for age, sex, and comorbidities, patients with C_T <25 had a higher likelihood of having symptomatic disease, but did not have increased odds of hospitalization, ICU admission, or mortality.
McEllistrem et al. [41]	2021	Pennsylvania, USA	June 24–August 23, 2020	53	NP	Cepheid Xpert Xpress	Hospitalized patients	Nonhospitalized patients and health care workers	No	At a Veterans Affairs Hospital, patients who were hospitalized had a higher C_T value than both outpatients and nonhospitalized health care workers.
Seeni et al. [42]	2021	Nevada, USA	April 1–October 30, 2020	134	NP	Cepheid Xpert Xpress	C_T <34	C_T >34	NR	Adjusting for age, diabetes, heart disease, kidney disease, and lung disease, patients with a C_T <34 had a higher odds of a composite of hospitalization requiring supplemental oxygen, ICU need, mechanical ventilation, and death compared with patients with a C_T value >34 (aOR, 4.0; 97.5% CI, 1.69 to 10.10).
Yagci et al. [43]	2020	Istanbul, Turkey	March 22–May 20, 2020	730	NP	Biospeedy COVID-19 qPCR detection kit, version 2	Hospitalized patients	Nonhospitalized patients	No	Among patients with a positive SARS-CoV-2 rtPCR and a chest CT performed, nonhospitalized patients had a lower mean C_T value compared with hospitalized patients.

Abbreviations: aOR, adjusted odds ratio; COVID-19, coronavirus disease 2019; CT, cycle threshold; CT, computed tomography; ICU, intensive care unit; NP, nasopharyngeal; NR, not reported; OP, oropharyngeal; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; rtPCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 2. Summary of Studies Evaluating the Association Between CT Values and Disease Severity Among Hospitalized Patients

Author	Year	City, State, Country	Study Enrollment Period	Sample Size	Sample Source	rtPCR Assay	Comparator	Reference Group	Lower C_T Values Associated With Disease Severity	Summary
de la Calle et al. [44]	2021	Madrid, Spain	March 1–March 18, 2020	455	NP	Thermo Fisher Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes	In a multivariate analysis adjusted for age, sex, cardiovascular disease, chronic lung disease, immunosuppression, smoking status, presence of dyspnea, abnormal chest x-ray findings on admission, severe lymphopenia (≤0.7 × 103 cells/μL), LDH ≥350 U/L, and C-reactive protein ≥6 mg/dL, C_T value <25 was independently associated with increased risk of respiratory failure (aOR, 2.99; 95% CI, 1.57 to 5.69) compared with C_T >30 among hospitalized patients. Similarly, this association was significant for patients with C_T 25–30 compared with >30 (aOR, 1.81; 95% CI, 1.02 to 3.22).
Faico-Filjo et al. [39]	2020	São Paulo, Brazil	March 17–June 17, 2020	376	NP	Thermo Fisher Scientific AgPath-ID	Patients requiring the ICU	Hospitalized patients	Yes	Among hospitalized patients with SARS-CoV-2, patients who required ICU-level care had lower mean C_T values compared with other hospitalized patients.
Fukushima et al. [45]	2021	Tokyo, Japan	March 24–May 14, 2020	19	NP	LDT	Patients who required the ICU, mechanical ventilation, or died	Hospitalized patients	Yes	Among hospitalized patients with a chest CT confirming COVID pneumonia who were initially hospitalized in a non–intensive care unit, patients who subsequently died or needed mechanical ventilation or ICU care had lower mean C_T values compared with other hospitalized patients.
Gaston et al. [46]	2020	Connecticut, USA	March 1–May 25, 2020	25	NP or OP + NP	LDT; Cepheid Xpert Xpress	Patients who required noninvasive positive pressure ventilation, mechanical ventilation, ICU, or who died	Hospitalized patients	No	Among a cohort of patients with solid organ transplant, mean C_T scores did not significantly differ between patients who required ICU-level care and other hospitalized patients.
Guo et al. [47]	2020	Guangdong Province, China	January 13–February 28, 2020	195	NP	LDT	Patients requiring mechanical ventilation or ICU, or with shock	Hospitalized patients	Yes	Patient requiring ICU-level care had lower mean C_T values compared with other hospitalized patients.
Magleby et al. [20]	2020	New York, USA	March 30–April 30, 2020	678	NP	Roche Cobas	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes	After adjusting for BMI, use of steroids as an outpatient, fever, dyspnea, infiltrates on chest x-ray, patients with C_T <25 had a higher odds of intubation compared with other hospitalized patients with C_T >30 (aOR, 2.73; 95% CI, 1.68 to 4.44). This finding did not reach significance for patients with C_T 25–30 compared with >30 (aOR, 1.59; 95% CI, 0.96–2.63).
Maltezou et al. [21]	2021	Athens and Thessaloniki, Greece	February 26–May 3, 2020	518	NP or OP	Genesig COVID-19 CE-IVD real-time RT-PCR kit; Thermo Fischer Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	No	In univariate analyses, compared with patients with C_T >30 or 25–30, patients with C_T value <25 were older, more often had comorbidities, developed symptomatic COVID-19, were intubated, and died. In multivariate analysis controlling for age, sex, and comorbidities, patients with C_T <25 had a higher likelihood of having symptomatic disease, but not increased odds of hospitalization, ICU admission, or mortality. Multivariate analysis described in the article included hospitalized and nonhospitalized patients. Data were abstracted to evaluate disease severity outcomes among hospitalized patients.
Seeni et al. [42]	2021	Nevada, USA	April 1–October 30, 2020	88	NP	Cepheid Xpert Xpress	C_T <34	C_T >34	NR	Adjusting for age, diabetes, heart disease, kidney disease, and lung disease, patients with C_T <34 had a higher odds of a composite of hospitalization requiring supplemental oxygen, ICU need, mechanical ventilation, and death compared with patients with C_T >34 (aOR, 4.0; 97.5% CI, 1.69 to 10.10).

Abbreviations: aOR, adjusted odds ratio; COVID-19, coronavirus disease 2019; C_T, cycle threshold; CT, computed tomography; ICU, intensive care unit; NP, nasopharyngeal; NR, not reported; OP, oropharyngeal; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; rtPCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 3. Summary of Studies Evaluating the Association Between CT Values and Mortality Among Hospitalized Patients

Author	Year	City, State, Country	Study Enrollment Period	Sample Size	Sample Source	rtPCR Assay	Comparator	Reference Group	Lower C_T Values Associated With Mortality	Summary
Bryan et al. [48]	2020	Washington, USA	NR	109	NP	Hologic Panther Fusion; LDT	Patients with C_T <22	Patients with C_T >22	Yes	After adjusting for SARS-CoV-2 antibody status, age, and sex, hospitalized patients with C_T <22 had higher odds of 30-day mortality compared with patients with C_T >22 (aOR, 4.20; 95% CI, 1.62 to 10.86).
Choudhuri et al. [49]	2020	New York, USA	March 26–August 5, 2020	1044	NP	Hologic Panther Fusion	Patients with C_T <22.9 and 23–27.9	Patients with C_T >32.9	Yes	After adjusting for age, sex, BMI, hypertension, and diabetes, patients with C_T <22.9 or 23–27.9 had higher odds of mortality compared with patients with C_T >32.9 (aOR, 3.85; 95% CI, 2.3 to 6.2; and aOR, 2.63; 95% CI, 1.6 to 4.2; respectively).
de la Calle et al. [44]	2021	Madrid, Spain	March 1–18, 2020	455	NP	Thermo Fisher Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes (No for C_T 25–30 compared with >30)	Adjusting for age, cardiovascular disease, chronic renal disease, smoking history, tachypnea, LDH, CRP, patients with C_T <25 had higher odds of mortality compared with patients with C_T >30 (aOR, 2.04; 95% CI, 1.44–4.00). These increased odds of mortality were not found in patients with C_T 25–30 compared with >30.
Faico-Filjo et al. [39]	2020	São Paulo, Brazil	March 17–June 17, 2020	376	NP	Thermo Fisher Scientific AgPath-ID	Nonsurvivors	Survivors	Yes	Among hospitalized patients with SARS-CoV-2, patients who did not survive had lower mean C_T values at the time of diagnosis compared with survivors. Compared with patients with C_T >24, hospitalized patients with a C_T <25 had increased odds of mortality (OR, 2.93; 95% CI, 1.87 to 4.60).
Maltezou et al. [21]	2021	Athens and Thessaloniki, Greece	February 26–May 3, 2020	518	NP or OP	Genesig COVID-19 CE-IVD real-time RT-PCR kit OR Thermo Fischer Scientific TaqMan	Patients with C_T <25 and 25–30	Patients with C_T >30	No	In univariate analyses, compared with patients with C_T >30 or 25–30, patients with C_T <25 were older, more often had comorbidities, developed symptomatic COVID-19, were intubated, and died. In multivariate analysis controlling for age, sex, and comorbidities, patients with C_T <25 had higher likelihood of having symptomatic disease, but without increased odds of hospitalization, ICU admission, or mortality. Multivariate analysis described in the article included hospitalized and nonhospitalized patients. Data were abstracted to evaluate mortality among hospitalized patients.
Seeni et al. [42]	2021	Nevada, USA	April 1—October 30, 2020	88	NP	Cepheid Xpert Xpress	Patients with C_T <34	Patients with C_T >34	NR	Adjusting for age, diabetes, heart disease, kidney disease, and lung disease, patients with C_T <34 had higher odds of a composite of hospitalization requiring supplemental oxygen, ICU need, mechanical ventilation, and death, compared with patients with C_T >34 (aOR, 4.0; 97.5% CI, 1.69 to 10.10). Authors were contacted and provided data on mortality outcomes among hospitalized patients.
Shah et al. [29]	2021	Mumbai, India	March 23–June 30, 2020	111	NP	Altona Diagnostics Real Star SARS-CoV-2 kit	C_T of 11–20	C_T of 31–40	Yes	Among hospitalized patients with a pulse oximetry ≤93%, patients with C_T 11–20 had a higher percentage of inpatient mortality (66.7%) compared with patients with C_T 31–40 (39.1%). Notably, the duration of illness before testing in patients who died was shorter (3 days) than in those who survived (5 days).
Westblade et al. [24]	2020	New York, USA	March 15–May 14, 2020	3914	NP	Roche Cobas and Cepheid Xpert Xpress	Patients with C_T <25 and 25–30	Patients with C_T >30	Yes	Among patients with cancer, after adjusting for age and need for supplemental O2 within 3 hours of ED presentation, a C_T value <25 compared with a C_T value >30 was associated with inpatient mortality (aOR, 4.71; 95% CI, 1.44 to 15.44), but not for patients with a C_T value 25–30 compared with >30. These results were similar to a comparative cohort without cancer.
Yagci et al. [43]	2020	Istanbul, Turkey	March 22–May 20, 2020	284	NP	Biospeedy COVID-19 qPCR detection kit, version 2	Nonsurvivors	Survivors	No	Among patients with a positive SARS-CoV-2 RT-PCR and a chest CT performed on hospital admission, mean C_T values did not significantly differ between survivors and nonsurvivors. Mortality was not associated with C_T value, but was associated with older age, CRP positivity, and worsened CT chest total severity score.
Zhao et al. [50]	2021	New Jersey, USA	March 12–April 8, 2020	722	NPa	LDT	Nonsurvivors	Survivors	Yes	Among hospitalized patients with SARS-CoV-2, patients who did not survive had lower mean C_T values at the time of diagnosis compared with survivors for both the E and N2 gene targets.

Abbreviations: aOR, adjusted odds ratio; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; C_T, cycle threshold; CT, computed tomography; ICU, intensive care unit; LDH, lactate dehydrogenase; LDT, laboratory-developed test; NP, nasopharyngeal; NR, not reported; OP, oropharyngeal; OR, odds ratio; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; rtPCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]99.6% of samples were NP, and 0.42% were OP.

more severe disease compared with patients with C_T values >30 (OR, 2.31; 95% CI, 1.70 to 3.13; and OR, 1.45; 95% CI, 1.06 to 1.97, respectively) (Figure 3). There was low heterogeneity for these outcomes (I^2 = 0%). Analysis of 4 studies (n = 675 patients) found a mean C_T difference of −5.22 (95% CI, −7.11 to −3.32) in patients with severe disease compared with nonsevere disease among hospitalized patients, also with low heterogeneity (I^2 = 42.07%) (Figure 3C).

For the outcome of mortality, 7 studies (n = 6053 patients) reported categorical C_T values. While Magleby et al. reported on the relationship between C_T values and mortality, this data set was also included in the report by Westblade et al., which was a larger data set [20, 24]. Thus, the synthesis did not include data from Magleby et al. for the mortality outcome to avoid duplication of results. Hospitalized patients with C_T values <25 had an increased risk of mortality compared with those with C_T values >30 (OR, 2.95; 95% CI, 2.19 to 3.96) (Figure 4A). There was moderate heterogeneity (I^2 = 53.25%), which did not change significantly during a subgroup analysis by risk of bias or rtPCR assay (data not shown). In subgroup analysis by sample source, the 6 studies that utilized only nasopharyngeal swab had low heterogeneity (I^2 = 28.9%) (Figure 4B).

Hospitalized patients with C_T values of 25–30 compared with >30 also had an increased mortality risk (OR, 1.59; 95% CI, 1.19 to 2.14) with low heterogeneity (I^2 = 41.19%), though this finding was driven by a single large study (Figure 4C) [24]. Three additional studies (n = 1382 patients) reported on the relationship between mean C_T values and mortality in hospitalized patients and found a lower mean C_T value among nonsurvivors than survivors (OR, −4.27; 95% CI, −6.38 to −2.16) with high heterogeneity (I^2 = 83.88%).

Three studies did not provide sufficient data for meta-analysis and are summarized narratively. Piubelli et al. reported 373 patients from a single center in Italy and reported C_T values by month. C_T values decreased from March 2020 through April 2020 with decreased ICU need, consistent with a waning epidemic trajectory, but the C_T values for patients who required ICU-level care did not change [25, 26]. Young et al. reported a prospective observational study of 100 patients from Singapore in which 20 patients had pneumonia and hypoxia and found no difference in C_T values compared with patients without pneumonia [27]. However, there was no separate analysis for the 12 patients who required ICU care. Yu et al. reported a study from China of 92 patients comparing baseline C_T values in patients with severe disease with C_T values in those with mild or moderate disease on admission [28]. They found that patients with more severe disease on admission, as well as patients who went on to have severe disease during their hospitalization, had lower admission C_T values compared with those with mild or moderate disease. However, disease severity was not defined.

DISCUSSION

This systematic review and meta-analysis did not find an association between C_T values and hospitalization of persons with SARS-CoV-2. Four studies reported higher C_T values in hospitalized patients, while 3 studies reported lower C_T values. The single study that reported only OR for the outcome of hospitalization also found no association between low C_T value and risk of hospitalization [21]. There was high heterogeneity in the data, which did not significantly decrease in subgroup analysis by sample source (data not shown). These 7 studies from 6 different countries utilized 6 different rtPCR assays, which may account for the difference in results. Additionally, the different study periods and local disease dynamics may contribute to the heterogeneity in the reported data. If testing was limited or delayed, this could also have an impact on the comparator group and may, in part, account for some of the observed heterogeneity. The certainty of a lack of association is also limited by different standards for hospitalization globally, particularly early in the COVID-19 pandemic when many institutions were admitting all patients with SARS-CoV-2 infection regardless of symptoms.

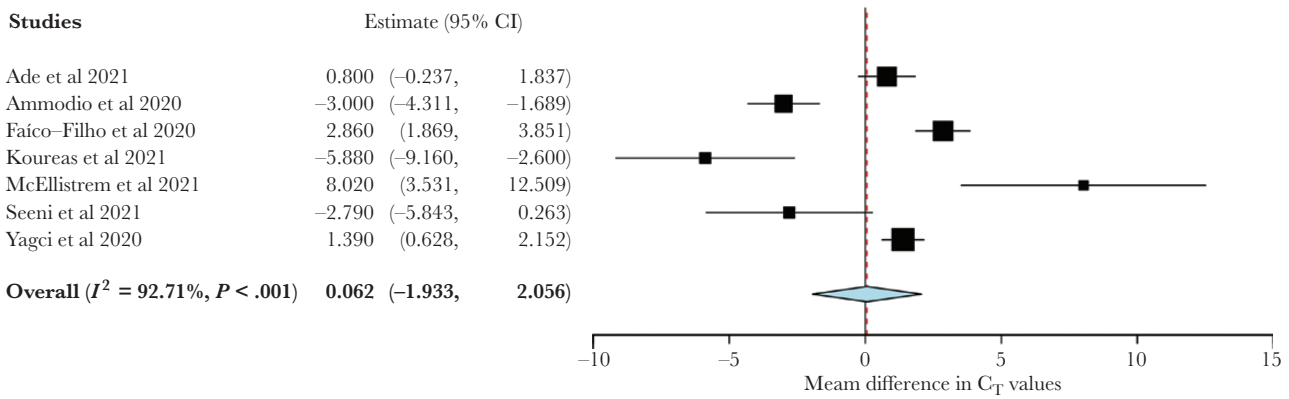

Figure 2. Forest plot of mean C_T value difference between hospitalized and nonhospitalized patients. Abbreviation: C_T, cycle threshold.

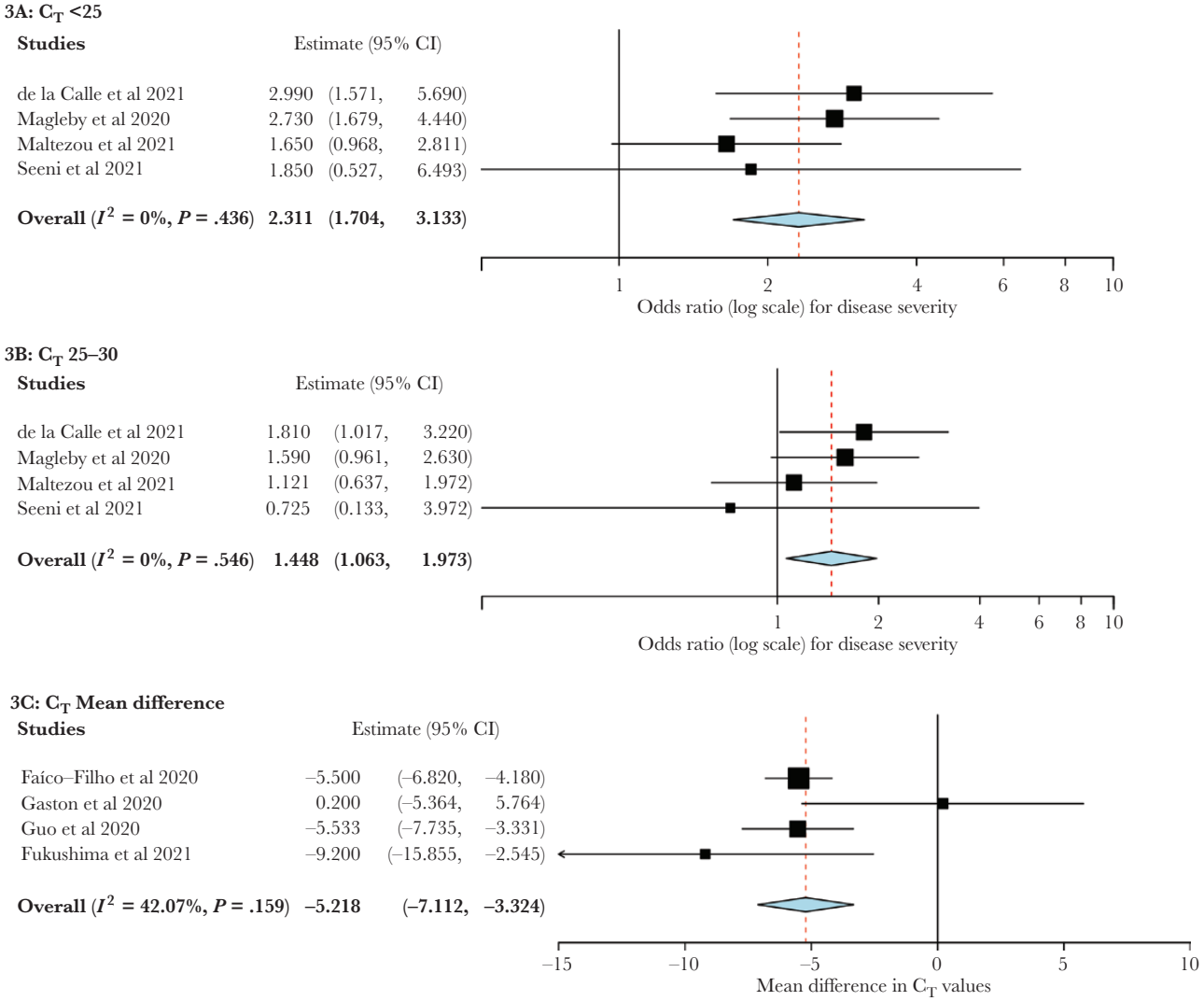

Figure 3. Forest plots of disease severity outcome among hospitalized patients. A, C_T value <25 vs >30. B, C_T value 25–30 vs >30. C, Mean C_T value difference between patients with severe and nonsevere disease in subgroup analysis by sample source. Abbreviation: C_T, cycle threshold.

For the disease severity and mortality outcomes, C_T value data were evaluated both as a numerical difference between outcomes and as a categorical variable depending upon how individual studies reported data. Comparing outcomes across studies using categorical C_T values is challenging due to variations in sample collection and the rtPCR platform utilized between studies. Evaluating mean differences in C_T values has the advantage of canceling out systematic differences within studies such as testing availability and the rtPCR platform, which allows for more robust comparisons between studies.

Among patients hospitalized with COVID-19, those with lower C_T values had more severe disease necessitating noninvasive ventilation, mechanical ventilation, or ICU admission. This association was most notable when comparing patients with C_T values <25 with patients with C_T values >30 and was also noted among patients with C_T values of 25–30. Consistent with this finding, our analyses also revealed a lower mean C_T value among those with more severe disease (Figure 3C). Contrary to the other 3 studies, Gaston et al. found no mean difference in those with more severe outcomes, though confidence intervals overlapped with other studies. However, this study was in patients with a solid organ transplant, representing a unique patient population. Overall, we observed low heterogeneity in the data.

Among patients hospitalized with COVID-19, lower C_T values, particularly C_T values <25, were associated with higher mortality compared with those with C_T values >30. This analysis included the study by Shah et al., which evaluated mortality among patients with severe disease, defined as having pulse oximetry readings of <93% on room air [29]. This cohort is similar to other hospitalized patients and was thus included in the pooled analysis. There was moderate heterogeneity that decreased during

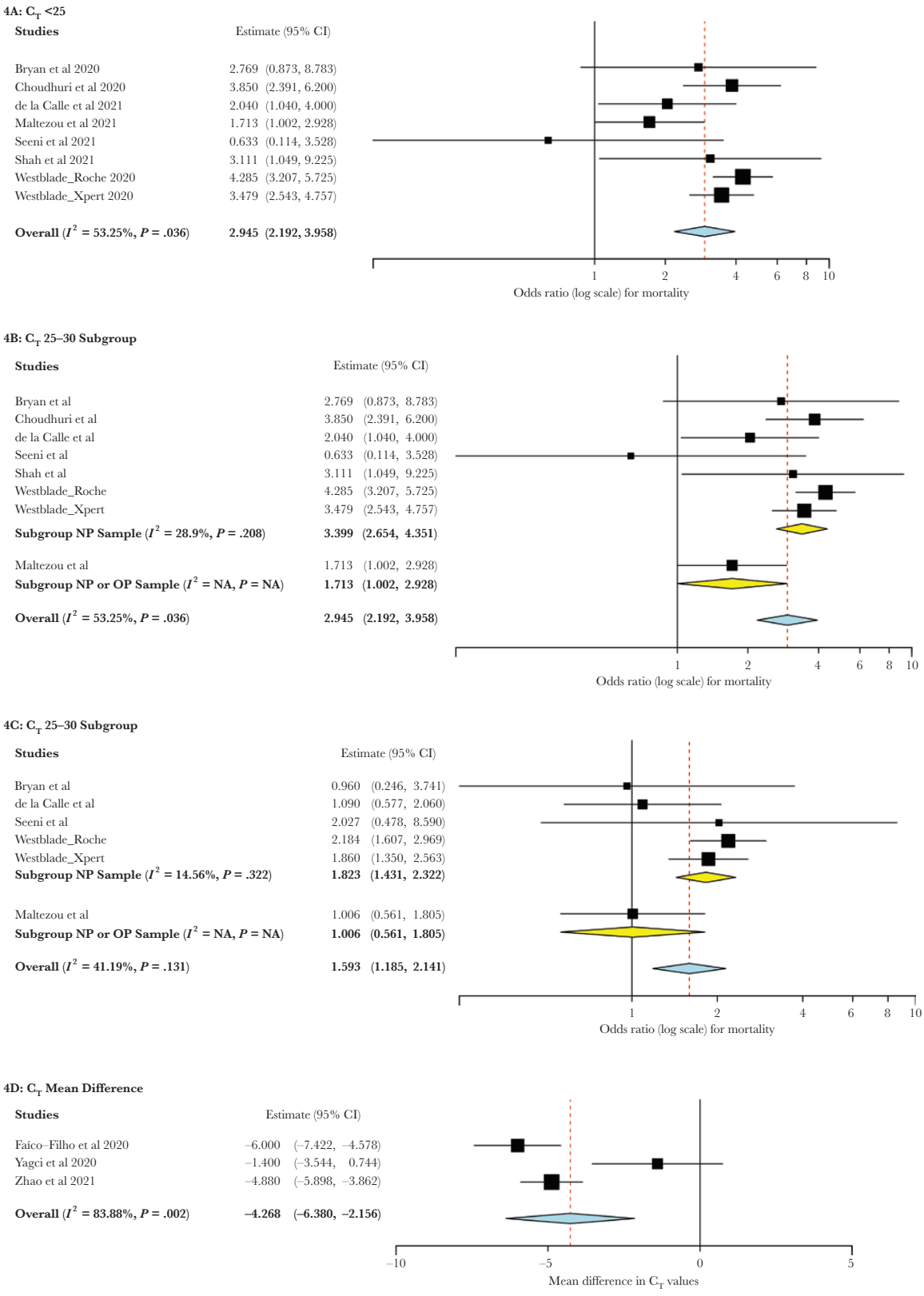

Figure 4. Forest plots of mortality outcome among hospitalized patients. A, C_T value <25 vs >30. B, C_T <25 vs >30, subgroup analysis by sample source. C, C_T value 25–30 vs >30. D, Mean C_T value difference between survivors and nonsurvivors. Abbreviations: C_T, cycle threshold; NP, nasopharyngeal.

subgroup analysis by sample source (Figure 4B). The association between C_T values and mortality was less pronounced when comparing hospitalized patients with C_T values of 25–30 with patients with C_T values of >30, driven largely by a single study (Figure 4C). Our analysis also revealed higher mean C_T values among survivors compared with nonsurvivors.

Interestingly, there have been mixed reports of the association between viral load and outcomes in patients with other respiratory illnesses. A low C_T was not associated with worsened outcomes in patients with influenza [30]. Duncan et al. evaluated adults with respiratory syncytial virus and showed that higher viral loads were not independent predictors of hospitalization, but peak viral load was a predictor for mechanical ventilation [31]. Hung et al. performed a prospective study of 154 patients infected with the original SARS-CoV in 2003 and found that higher viral load later in the disease course was associated with increased rates of mechanical ventilation and death [32]. These mixed reports have been described in patients with SARS-CoV-2, and the heterogeneity in the data may be, in part, due to different sample populations, sample sources, and rtPCR assays.

The timing of clinical specimen collection is critical and may impact the C_T value as well. A study by Hu et al. suggested that viral load peaks shortly after symptom onset then declines in a steady manner [33]. Early in the disease course, patients infected with SARS-CoV-2 generally have low C_T values, with no discernable difference between those who require hospitalization and those who do not. However, as symptoms progress and those who require hospitalization present for medical care, patients with persistently high viral loads may have a worsened prognosis, which may be predicted using the C_T value as a surrogate marker. This correlation may also be age-dependent. Faes et al. evaluated a cohort of patients in Belgium and found age to be correlated with time from symptom onset to hospitalization, with younger patients having the shortest duration [34]. The time at which patients get tested may impact the C_T value, particularly for those with less severe disease.

Limitations

There are several limitations to our review. As highlighted by Rhoads et al., the use of C_T values for clinical decision-making is a challenging proposal for several reasons [35]. First, sample source, collection method, volume, and storage may impact the C_T value. Additionally, C_T values can vary widely based on the rtPCR assay used.

This meta-analysis was limited to studies published in English. However, patients from many countries are represented in this evaluation. Due to the small number of studies per outcome, the presence of publication bias could not be evaluated; nonetheless, reporting and publication bias remain a concern as the overall large number of publications related to COVID-19 may have resulted in studies with null results that may not have been reported or published. In addition, time from symptom onset to sample collection or testing was not considered in this evaluation as such data were not widely reported in published studies. Most studies were conducted earlier in the pandemic, when rtPCR testing was more limited and individuals were immune-naive. Findings from this analysis may not be applicable to those with immunity through vaccination or prior infection. Furthermore, most studies were found to have a high risk of bias, largely due to not adjusting for potential confounding variables that are known to affect outcomes assessed in this study, such as age, gender, and use of therapeutics. Additionally, study population was mostly done by convenience sampling, which can lead to significant selection bias. Therefore, using the GRADE approach to evaluate certainty in the meta-analytic estimates, we judged this certainty to be very low due to risk of bias and heterogeneity [36].

Future Directions

Further prospective research that takes into account confounding factors such as age, gender, comorbidities, and duration from symptom onset to testing would further add to the knowledge base on the clinical utility of the C_T value. A prospective serial evaluation of C_T values in patients with multiple risk factors for severe disease could aid in determining whether persistently high levels of viral RNA early in the disease course are related to worse outcomes and whether patients who are able to mount an immunologic response and clear more virus have improved outcomes. Additional evaluation of viral load and C_T value dynamics in emerging variants and in populations with immunity would also be valuable. Development and availability of quantitative rtPCR assays would allow for standardization and more direct comparison of the prognostic utility of viral load of SARS-CoV-2. To date, no quantitative SARS-CoV-2 assay has received Emergency Use Authorization by the US Food and Drug Administration.

Despite limitations on the interpretation of individual C_T values, they may aid in prognostication of patients, along with other demographic, clinical, and laboratory findings. The C_T value may allow clinicians to better triage certain patients admitted to the hospital to provide appropriate interventions in a timely manner. Another major benefit of the C_T value is that it may be obtained without need for additional testing, assuming the test for SARS-CoV-2 is performed on rtPCR assays that provide this value.

CONCLUSIONS

This systematic review suggests a role for C_T values in the prognostication of hospitalized individuals for the outcomes of disease severity and mortality, with lower C_T values (ie, higher levels of viral RNA) correlating with increased disease severity

and mortality. However, C_T results must be interpreted with caution given the limitations and lack of assay standardization.

Acknowledgments

Financial support. The authors received no specific funding for this work.

Potential conflicts of interest. M.J.B. is an advisory board member for DiaSorin Molecular and Mammoth Biosciences. J.D.Y. received research grants from Roche Molecular Systems, Inc., and Abbott Molecular, Inc. All remaining authors have no reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Data availability. Data abstracted from this research are available by contacting the corresponding author.

Patient consent. This systematic review and meta-analysis is not human subjects research and conforms to the ethical standards within the United States.

References

1. Centers for Disease Control and Prevention. COVID data tracker weekly review. Available at: https://www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/index.html. Accessed 10 May 2021.
2. Centers for Disease Control and Prevention. Estimated disease burden of COVID-19. Updated 29 April **2021**. Available at: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/burden.html. Accessed 11 May 2021.
3. Asch DA, Sheils NE, Islam MN, et al. Variation in US hospital mortality rates for patients admitted with COVID-19 during the first 6 months of the pandemic. JAMA Intern Med **2021**; 181:471–8.
4. Kompaniyets L, Goodman AB, Belay B, et al. Body mass index and risk for COVID-19-related hospitalization, intensive care unit admission, invasive mechanical ventilation, and death - United States, March-December 2020. MMWR Morb Mortal Wkly Rep **2021**; 70:355–61.
5. Nguyen NT, Chinn J, Nahmias J, et al. Outcomes and mortality among adults hospitalized with COVID-19 at US medical centers. JAMA Netw Open **2021**; 4:e210417.
6. Roth GA, Emmons-Bell S, Alger HM, et al. Trends in patient characteristics and COVID-19 in-hospital mortality in the United States during the COVID-19 pandemic. JAMA Netw Open **2021**; 4:e218828.
7. Garg S, Kim L, Whitaker M, et al. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019 - COVID-NET, 14 States, March 1-30, 2020. MMWR Morb Mortal Wkly Rep **2020**; 69:458–64.
8. Ioannou GN, Locke E, Green P, et al. Risk factors for hospitalization, mechanical ventilation, or death among 10 131 US veterans with SARS-CoV-2 infection. JAMA Netw Open **2020**; 3:e2022310.
9. Peckham H, de Gruijter NM, Raine C, et al. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. Nat Commun **2020**; 11:6317.
10. Henry BM, de Oliveira MHS, Benoit S, et al. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med **2020**; 58:1021–8.
11. Huang I, Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. J Intensive Care **2020**; 8:36.
12. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet **2020**; 395:1054–62.
13. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 test, consider the cycle threshold value. Clin Infect Dis **2020**; 71:2252–4.
14. Dahdouh E, Lázaro-Perona F, Romero-Gómez MP, et al. 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.
15. Rao SN, Manissero D, Steele VR, Pareja J. A systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther **2020**; 9:573–86.
16. Argyropoulos KV, Serrano A, Hu J, et al. Association of initial viral load in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients with outcome and symptoms. Am J Pathol **2020**; 190:1881–7.
17. Le Borgne P, Solis M, Severac F, et al. SARS-CoV-2 viral load in nasopharyngeal swabs in the emergency department does not predict COVID-19 severity and mortality. Acad Emerg Med **2021**; 28:306–313.
18. Pujadas E, Chaudhry F, McBride R, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. Lancet Respir Med **2020**; 8:e70.
19. Tsukagoshi H, Shinoda D, Saito M, et al. Relationships between viral load and the clinical course of COVID-19. Viruses **2021**; 13:15.
20. Magleby R, Westblade LF, Trzebucki A, et al. Impact of SARS-CoV-2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. Clin Infect Dis **2020**; 30:30.
21. Maltezou HC, Raftopoulos V, Vorou R, et al. Association between upper respiratory tract viral load, comorbidities, disease severity and outcome of patients with SARS-CoV-2 infection. J Infect Dis **2021**; 3:3.
22. Viswanathan M, Patnode CD, Berkman ND, et al. Recommendations for assessing the risk of bias in systematic reviews of health-care interventions. J Clin Epidemiol **2018**; 97:26–34.
23. Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, Schmid CH. Closing the Gap between methodologists and end-users: R as a computational back-end. J Stat Softw **2012**; 49:15.
24. Westblade LF, Brar G, Pinheiro LC, et al. 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.e2.
25. Piubelli C, Deiana M, Pomari E, et al. Overall decrease in SARS-CoV-2 viral load and reduction in clinical burden: the experience of a hospital in Northern Italy. Clin Microbiol Infect **2021**; 27:131.e1–3.
26. Hay JA, Kennedy-Shaffer L, Kanjilal S, et al. Estimating epidemiologic dynamics from cross-sectional viral load distributions. Science **2021**; 373:eabh0635.
27. Young BE, Ong SWX, Ng LFP, et al. Viral dynamics and immune correlates of COVID-19 disease severity. Clin Infect Dis **2020**; 28:28.
28. Yu X, Sun S, Shi Y, et al. SARS-CoV-2 viral load in sputum correlates with risk of COVID-19 progression. Crit Care **2020**; 24:170.
29. 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.
30. Lalueza A, Folgueira D, Muñoz-Gallego I, et al. Influence of viral load in the outcome of hospitalized patients with influenza virus infection. Eur J Clin Microbiol Infect Dis **2019**; 38:667–73.
31. Duncan CB, Walsh EE, Peterson DR, et al. Risk factors for respiratory failure associated with respiratory syncytial virus infection in adults. J Infect Dis **2009**; 200:1242–6.
32. Hung IF, Cheng VC, Wu AK, et al. Viral loads in clinical specimens and SARS manifestations. Emerg Infect Dis **2004**; 10:1550–7.
33. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med **2020**; 26:672–5.
34. Faes C, Abrams S, Van Beckhoven D, et al. Time between symptom onset, hospitalisation and recovery or death: statistical analysis of Belgian COVID-19 patients. Int J Environ Res Public Health **2020**; 17:7560.
35. Rhoads D, Peaper DR, She RC, et al. College of American Pathologists (CAP) Microbiology Committee perspective: caution must be used in interpreting the cycle threshold (Ct) value. Clin Infect Dis **2021**; 72:e685–6.
36. Murad MH. Clinical practice guidelines: a primer on development and dissemination. Mayo Clin Proc **2017**; 92:423–33.
37. Ade C, Pum J, Abele I, et al. Analysis of cycle threshold values in SARS-CoV-2-PCR in a long-term study. J Clin Virol **2021**; 138:104791.
38. Amodio E, Pipitone RM, Grimaudo S, et al. SARS-CoV-2 viral load, ifnlambda polymorphisms and the course of COVID-19: an observational study. J Clin Med **2020**; 9:15.
39. Faíco-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.
40. Koureas M, Speletas M, Bogogiannidou Z, et al. Transmission dynamics of SARS-CoV-2 during an outbreak in a Roma Community in Thessaly, Greece—control measures and lessons learned. Int J Environ Res Public Health **2021**; 18.
41. McEllistrem MC, Clancy CJ, Buehrle DJ, et al. SARS-CoV-2 is associated with high viral loads in asymptomatic and recently symptomatic healthcare workers. PLoS One **2021**; 16:e0248347.
42. Seeni R, Firzli T, Riddle MS, et al. Using COVID-19 cycle threshold and other lab values as predictors of hospitalization need. J Med Virol **2021**; 93:3007–14.
43. Karahasan Yagci A, Sarinoglu RC, Bilgin H, et al. Relationship of the cycle threshold values of SARS-CoV-2 polymerase chain reaction and total severity score of computerized tomography in patients with COVID 19. Int J Infect Dis **2020**; 101:160–6.
44. de la Calle C, Lalueza A, Mancheno-Losa M, et al. Impact of viral load at admission on the development of respiratory failure in hospitalized patients with SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis **2021**; 7:7.
45. Fukushima T, Kabata H, Yamamoto R, et al; Keio Donner Project Team. The real-time reverse transcription-polymerase chain reaction threshold cycle values for

severe acute respiratory syndrome coronavirus 2 predict the prognosis of coronavirus disease 2019 pneumonia. Respir Investig **2021**; 59:360–3.
46. Gaston DC, Malinis M, Osborn R, et al. Clinical implications of SARS-CoV-2 cycle threshold values in solid organ transplant recipients. Am J Transplant **2020**; 12:12.
47. Guo X, Jie Y, Ye Y, et al. Upper respiratory tract viral ribonucleic acid load at hospital admission is associated with coronavirus disease 2019 disease severity. Open Forum Infect Dis **2020**; 7:XXX–XX.
48. Bryan A, Fink SL, Gattuso MA, et al. SARS-CoV-2 viral load on admission is associated with 30-day mortality. Open Forum Infect Dis **2020**; 7:XXX–XX.
49. Choudhuri J, Carter J, Nelson R, et al. SARS-CoV-2 PCR cycle threshold at hospital admission associated with patient mortality. PLoS One **2020**; 15:e0244777.
50. Zhao Y, Cunningham MH, Mediavilla JR, et al. Diagnosis, clinical characteristics, and outcomes of COVID-19 patients from a large healthcare system in Northern New Jersey. Sci Rep **2021**; 11:4389.