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Systematic review on the association between respiratory virus real-time PCR cycle threshold values and clinical presentation or outcomes

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Objectives: It is unclear whether real-time (rt)-PCR cycle threshold (Ct) values can be utilized to guide clinical and infection-control decisions. This systematic review assesses the association between respiratory pathogen rt-PCR Ct values and clinical presentation or outcomes.

Methods: We searched MEDLINE, EMBASE and Cochrane library databases on 14–17 January 2020 for studies reporting the presence or absence of an association between Ct values and clinical presentation or outcomes, excluding animal studies, reviews, meta-analyses, and non-English language studies.

Results: Among 33 studies identified (reporting on between 9 and 4918 participants by pathogen), influenza ($n = 11$ studies; 4918 participants), human rhinovirus (HRV, $n = 11$; 2012) and respiratory syncytial virus (RSV, $n = 8$; 3290) were the most-studied pathogens. Low influenza Ct values were associated with mortality in 1/3 studies, with increased disease severity/duration or ICU admission in 3/9, and with increased hospitalization or length of hospital stay (LOS) in 1/6. Low HRV Ct values were associated with increased disease severity/duration or ICU admission in 3/10 studies, and with increased hospitalization or LOS in 1/3. Low RSV Ct values were associated with increased disease severity/duration or ICU admission in 3/6 studies, and with increased hospitalization or LOS in 4/4. Contradictory associations were also identified for other respiratory pathogens.

Conclusions: Respiratory infection Ct values may inform clinical and infection-control decisions. However, the study heterogeneity observed in this review highlights the need for standardized workflows to utilize Ct values as a proxy of genomic load and confirm their value for respiratory infection management.

Introduction

Common viral causes of respiratory tract infections in humans are influenza virus, respiratory syncytial virus (RSV), parainfluenza virus (PiV), metapneumovirus (HMPV), adenovirus (HAdV), rhinovirus (HRV), bocavirus (HBoV), and coronavirus (HCoV).[1–3] Most of these viruses demonstrate seasonal patterns of circulation, particularly in temperate regions.[1,4] During the development of this systematic review, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became a substantial pandemic; however, the long-term impact and whether this virus becomes a seasonal infectious disease is yet to be understood.[5]

Real-time PCR is increasingly used to detect and identify respiratory pathogens. The PCR cycle threshold (Ct) value represents the number of amplification cycles required for amplification of the amplicon to exceed a basal fluorescence threshold level. It is inversely related to the amount of copies of the target region in the sample, meaning that a low Ct value corresponds with a high

pathogen load. However, the use of Ct values as a proxy of genomic load is influenced by the assay (e.g. sample volume, chemical conditions and single copy or multi-copy region design) and factors within the sample (e.g. quality of sample, and inhibitors) that impact amplification efficiency. The absence of a standard curve in most assays means correction of most of these limitations is not possible. However, Ct values still provide semi-quantitative information. Results of diagnostic tests are usually reported as a positive or negative result using a specified cut-off, based on Ct value or integrated by an automatic algorithm interpreting different parameters of the potential amplification. Caution should be noted when interpreting Ct values, as they are an integer scale that represents a log result measurement of a log biological process.

Ct values are not normally reported, and it is unclear whether they can be utilized to guide clinical and infection-control decisions. Several studies have attempted to elucidate the clinical utility of Ct values, including contradictory reports that Ct values are associated with disease severity, ICU admission and length of hospital stay (LOS).[6–8] Ct values may also have utility in clarifying diagnostic uncertainty when several respiratory pathogens are identified, particularly when using multiplex PCR respiratory panels.[9,10]

Notably, the unprecedented challenges from the COVID-19 pandemic have raised the level of interest in the clinical and diagnostic utility of Ct values, particularly whether rapid understanding of Ct values could be leveraged as an early measure of disease severity or utilized to guide patient management or public health decisions.[11–14] A previous systematic review on the utility of Ct values in the management of patients with SARS-CoV-2 infection showed that lower Ct values are potentially associated with worse outcomes in patients with COVID-19, and correlate with increased probability of progression to severe disease, increased disease severity, increased mortality and presence of biochemical and haematological markers.[14] Furthermore, observed changes in population distributions of SARS-CoV-2 Ct values can potentially improve estimates of an epidemic's trajectory, enabling real-time outbreak management and response.[15]

This systematic review assesses the global medical literature for associations between Ct values of respiratory pathogens and clinical presentation or outcomes and therefore, whether they could provide valuable information to clinicians for more tailored decision-making. This systematic review does not include SARS-CoV-2 because the searches were carried out before COVID-19 and the literature on the relationship between SARS-CoV-2 Ct values and clinically relevant outcomes is still maturing.[14]

Methods

This review was undertaken according to the principles outlined in the Cochrane handbook and guidance published by the Centre for Reviews and Dissemination. The original protocol (PROSPERO CRD42020167239) included broad search terms, unrestricted by pathogen or disease type. For pragmatic purposes, this report focuses on respiratory pathogens.

Eligibility criteria

Between 14–17 January 2020, literature searches of MEDLINE, EMBASE and the Cochrane Library using search tools at ncbi.nlm.nih.gov/pubmed, embase.com and cochranelibrary.com were undertaken to identify studies reporting on the presence or absence of an association between real-time PCR Ct values and clinical presentation or outcomes (see Table S1, available as Supplementary data at *JAC* Online, for the PubMed search strategy). The search terms were specific for articles referring to quantitative real-time PCR Ct values. All randomized and non-randomized studies were eligible for inclusion; animal studies, systematic reviews, non-systematic reviews, and meta-analyses were excluded. When assessing the full text of articles, those reporting genomic load by measures other than Ct values were excluded unless a clear association between genomic load and Ct value was described. For reasons of feasibility, searches were limited to English language studies. We also carried out manual citation searches for additional articles.

Study selection and data extraction

Two reviewers independently screened titles and abstracts for inclusion, based on the eligibility criteria, and then independently assessed full texts of potentially relevant publications; a third reviewer resolved conflicts. Key data from all included studies were captured using a data extraction form. All extracted data were verified by an independent reviewer. Outcomes were reported in a narrative format and meta-analyses were deemed inappropriate due to a wide variation in reported outcomes. Outcomes were broadly divided into the following categories: mortality, severity of symptom, duration of symptoms, ICU admission, hospitalization, and LOS. We also captured information related to single versus co-infections and transmissibility.

The quality and risk of bias of each study was assessed using a tool relevant for each study design (Newcastle Ottawa Scale for cross-sectional, cohort and case–control studies and the Cochrane Risk of Bias tool for randomized controlled trials).

Results

Included studies

Literature searches identified 991 unique records. After screening and restricting to respiratory pathogens, 33 eligible studies were included (Figure 1). Detailed characteristics, methodology and variables are provided in Table S2. Influenza, HRV and RSV were the most studied pathogens (Figure 1). The total number of participants included by pathogen ranged from 9 to 4918; and 59 to 4918 when restricted to viral pathogens. The majority (18/33, 55%) of studies were clearly identifiable as being in a hospital setting, and a large percentage of studies (15/33, 45%) were solely in children. There was wide variation across the studies with regards to sampling, sample handling and analysis. Gaps in reported methodology were identified in 12/31 (39%) studies; excluding two studies that were congress abstracts only (Table S2). We inferred this to mean 19/31 (61%) studies provided clearly defined methodology.

Quality and bias

Using Newcastle-Ottowa scales, all cross-sectional studies and nearly all cohort studies were classed as being of low quality (Tables S3 and S4, respectively). This was generally due to a lack of comparability between groups, insufficient or unjustified sample sizes, the use of non-representative samples (often hospitalized patients or age-specific populations) and a lack of detail regarding patient follow-up or non-response; ascertainment of exposure and outcome was usually appropriate, although blinding was not often utilized. One cohort study (Clark *et al.*[16]) was considered to be of 'fair' quality and one (Kim *et al.*[17]) was

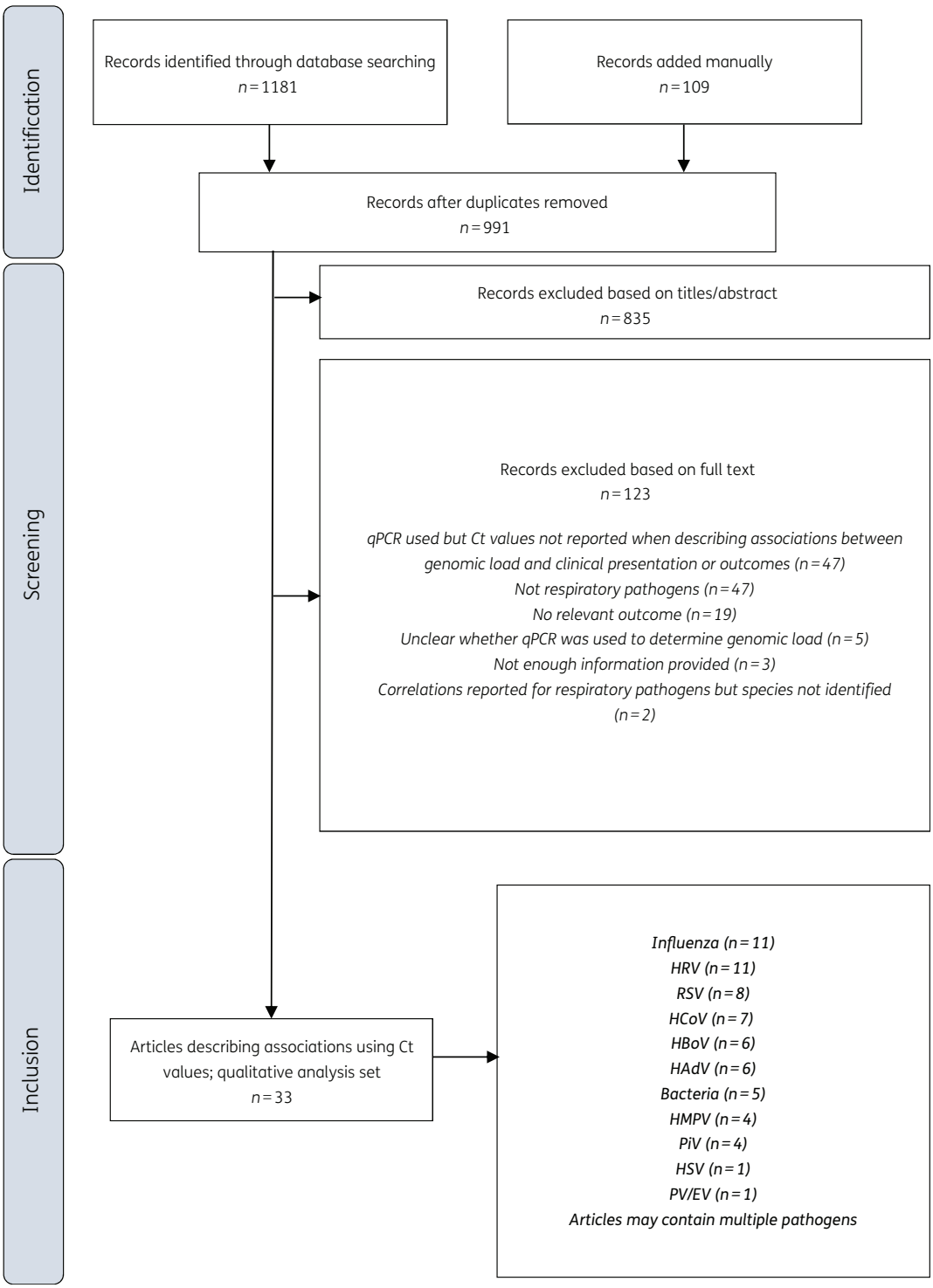

Figure 1. PRISMA flow diagram. Ct, cycle threshold; HAdV, human adenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, human rhinovirus; HSV, herpes simplex virus; PiV, parainfluenza virus; PV/EV, parechovirus/enterovirus; qPCR, quantitative PCR; RSV, respiratory syncytial virus.

considered to be of 'good' quality. Both case–control studies were considered of 'good' quality (Table S5),[6,18] as were the two randomized, controlled trials (Table S6).[19,20]

Influenza

Eleven studies investigated associations between Ct value of influenza infection and clinical presentation or outcomes,

summarized in Table 1. The total number of participants across studies was 4918.

Mortality, disease severity, disease duration and ICU admission

Three studies investigated the association of Ct value with mortality, of which two reported no significant associations,[21,22] and one reported significantly higher viral load in patients with radiographic evidence of pneumonia who died versus those who survived; however, this analysis included a small number of patients.[6]

Seven studies investigated association of Ct value with symptom severity or duration. In a study by Spencer *et al.*[23] in patients ≥3 years old ($n = 1660$ influenza A; $n = 806$ influenza B), low Ct values (≤25) compared with high Ct values (≥30) were associated with moderate to high self-rated illness severity for influenza A and to a lesser extent with influenza B. Furthermore, patients with influenza A and low Ct values were significantly more likely to report fever/feverishness. Two smaller studies reported significant associations between low Ct values and CURB65 score or duration of symptoms.[16,24] In contrast, 4/7 studies reported no significant associations of Ct value with symptom severity,[6,8,21,25] including two studies with >200 patients and clearly defined methodology (see Table S2).[21,25] In Lalueza *et al.*,[21] viral load was not significantly associated with multiple measures of disease severity, including pneumonia, respiratory failure, acute respiratory distress syndrome (ARDS), invasive ventilation, SOFA score and duration of invasive ventilation. However, patients with low Ct values (≤20) were more likely to have baseline abnormal chest X-rays compared with Ct values of >20–30 or >30 ($P = 0.004$), and more likely to record low lymphocyte counts (<1000 lymphocytes/μL; $P = 0.035$).

Three studies investigated the association of Ct value with ICU admission,[21,26,27] all of which reported no significant associations. In Reina *et al.*,[26] Ct values were lower for patients in the ICU, but this did not reach statistical significance.

Hospitalization and length of stay

Four studies investigated the association of Ct value with hospital admission, all of which showed no significant association between low Ct values and hospitalization.[8,18,26,27] The study by Reina *et al.*[26] reported no significant variation in Ct values between ICU, emergency department (ED) or hospital admission, and Zou *et al.*[27] reported no difference in Ct values between inpatients and outpatients. Conversely, the study by Fuller *et al.*,[18] which provided clearly defined methodology (Table S2), reported higher median Ct values for inpatients (Ct 35.0) than outpatients (Ct 29.8), with increasing Ct values increasing the odds of being an inpatient versus an outpatient.

Three studies investigated the association of Ct value and LOS.[8,16,21] Lalueza *et al.*[21] and Wishaupt *et al.*[8] reported no significant associations. In contrast, Clark *et al.*[16] reported LOS was longer in patients with high viral loads (Ct ≤20 versus other groups combined; $P = 0.005$). Notably, in comparison with Lalueza *et al.*[21] and Wishaupt *et al.*,[8] the study by Clark *et al.*[16] reported a more clearly defined standardized protocol and potentially lower risk of bias (Tables S2 and S4).

Human rhinovirus

Eleven studies investigated associations between Ct value of HRV infection and clinical presentation or outcomes, summarized in Table 2. The total number of participants across studies was 2012.

Mortality, disease severity, disease duration and ICU admission

Only one study investigated viral load and mortality, and found no association in paediatric patients with radiographic evidence of pneumonia.[6]

Ten studies investigated association of Ct value and symptom severity or duration. In a cohort study of 1421 paediatric patients by Feikin *et al.*,[6] with clearly defined and standardized methodology, lower Ct values were reported in patients with radiographic evidence of pneumonia compared with control participants with detectable HRV Furthermore, in patients with radiographic evidence of pneumonia, viral load was significantly higher for very severe versus severe pneumonia.[6] Two other studies also reported association between Ct value and symptom severity;[28,29] however, the remaining seven studies (all with <100 patients each) reported no association between Ct values and symptom severity, duration of symptoms and ICU admission.[8,16,19,24,30–32]

Hospitalization and length of stay

Three studies investigated association of the Ct value with hospital admission and/or LOS. Similar to the findings with influenza patients, Clark *et al.*[16] reported significant association between LOS and low Ct value. The other two studies[8,33] ($n \leq 32$ patients) reported no associations between Ct value and hospitalization or LOS; however, methodology for sampling and sample handling was less clear than for Clark *et al.*[16]

Respiratory syncytial virus

Eight studies investigated associations between Ct value of RSV infection and clinical presentation or outcomes, summarized in Table 3. Notably, all except one study (Fuller *et al.*,[18] which split cohorts into <5 years and ≥5 years) were in paediatric populations. The total number of participants across studies was 3290.

Mortality, disease severity, disease duration and ICU admission

Only one study investigated viral load and mortality, and found no association with radiographic evidence of pneumonia in paediatric patients.[6]

Six studies investigated the association of Ct value and symptom severity or duration. In a study of 1764 paediatric patients hospitalized with bronchiolitis by Hasegawa *et al.*,[7] which used standardized protocols for sampling and sample handling, there was no significant association between Ct values and vital signs, including oxygen saturation and severity of retractions. The same study reported that patients with low Ct values (<20.8) were at higher risk of needing ICU care compared with high Ct values (≥24.3); OR 1.43 (95% CI, 1.03–1.99), $P = 0.03$; however, significance was lost in a sensitivity analysis using a restrictive definition of bronchiolitis. Three other studies also investigated Ct values and disease severity and found no significant associations.[8,19,31]

Table 1. Summary of studies ($N = 11$) that assessed PCR Ct values for influenza infections against clinical presentation or outcomes ($N = 4918$ total participants)

Outcome (n/N studies with significant associations[a]) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
Mortality (1/3 studies) [307 participants]	Lalueza *et al.* 2019[21]	239 (A)	NPS	Viral load was not significantly associated with in-hospital mortality: 7.7% in patients with Ct ≤20; 4.5% Ct >20–30; 5.5% Ct >30 ($P = 0.63$ for Ct ≤20 versus other groups combined)	Yes
	Feikin *et al.* 2017[6] (influenza A)	56 (P)	NPS/OPS	**Among patients with radiographic evidence of pneumonia, mean viral load at enrolment was significantly higher in patients who died ($n = 4$) compared with patients who survived ($n = 52$); $P < 0.05$ after adjusting for age and site**	Yes
	Yu *et al.* 2013[22] (influenza A)	12	NPS/sputum	Viral load tended to be higher in sputum for fatal cases ($n = 6$), compared with survivors (median Ct, 23 versus 30.5; $P = 0.08$)	No
Severity of symptoms (2/6 studies) [3154 participants]	Spencer *et al.* 2016[23] (influenza A)	1660	NPS/OPS	**Low Ct values (≤25) compared with high Ct values (≥30) were associated with moderate to high self-rated illness severity [(0 worst health) to 100 (best health)]; adjusted multivariate model also controls for laboratory** **Influenza A [versus severity 76–100 ($n = 204$)] all categories had significantly increased odds** **Severity 0–25 ($n = 222$): OR (95% CI) 2.63 (1.53–4.52)** **Severity 26–50 ($n = 691$): OR (95% CI) 2.46 (1.56–3.88)** **Severity 51–75 ($n = 537$): OR (95% CI) 2.44 (1.54–3.86)** **Influenza B [versus severity 76–100 ($n = 93$)] only severity 26–50 category had significantly increased odds** Severity 0–25 ($n = 106$): OR (95% CI) 1.21 (0.59–2.48) **Severity 26–50 ($n = 332$): OR (95% CI) 1.92 (1.07–3.45)** Severity 51–75 ($n = 272$): OR (95% CI) 1.48 (0.82–2.68) **Additionally, for influenza A, patients with low Ct values were more likely to report fever/feverishness: OR (95% CI) 1.89 (1.28–2.78) versus no reported fever**	Yes
	Spencer *et al.* 2016[23] (influenza B)	806			Yes

Continued

Table 1. *Continued*

Outcome (*n*/*N* studies with significant associations[a]) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
	Lalueza *et al.* 2019[21]	239 (A)	NPS	Viral load was not significantly associated with disease severity (pneumonia, respiratory failure, ARDS, invasive ventilation, SOFA score, duration of invasive ventilation). However, patients with low Ct values (≤20) were more likely to have baseline abnormal chest X-rays compared with Ct values of >20–30 or >30 (76.9% versus 46.9% or 46.4%; $P = 0.004$). Additionally, high viral load (Ct ≤20) was associated with low lymphocyte count (<1000 lymphocytes/μL; $P = 0.035$) compared with other Ct groups (Ct >20–30 and Ct >30) combined	Yes
	Duchamp *et al.* 2010[25] (influenza A)	209 (P)	Nasal swabs	No significant difference in mean Ct values by clinical features (with versus without): Myalgia: 25.9 versus 26.6; $P = 0.46$ Digestive symptoms: 26.6 versus 26.3; $P = 0.76$ URTI: 26.9 versus 26.1; $P = 0.27$ LRTI: 25.2 versus 25.4; $P = 0.46$	Yes
	Feikin *et al.* 2017[6] (influenza A)	119 (P)	NPS/OPS	Ct values in patients with radiographic evidence of pneumonia were not significantly different from control participants with or without RTIs (adjusting for age and site). Mean Ct values (95% CIs) and OR (95% CIs) per $\log_{10}$ increase in viral copies/mL (approximately a 3.4 unit drop in Ct value) versus controls: Influenza A: 28.5 (27.7–29.4) versus 29.8 (28.4–31.2), $P = 0.31$; OR 1.21 (0.85–1.72) Influenza B: 27.6 (25.7–29.5) versus 28.5 (26.7–30.3), $P = 0.82$; OR 1.07 (0.63–1.83) Influenza C: 28.1 (24.8–31.4) versus 27.3 (25.3–29.3), $P = 0.14$; OR 0.44 (0.17–1.15) Among patients with radiographic evidence of pneumonia, viral load was not significantly different between patients with very severe and severe pneumonia, irrespective of influenza type	Yes
	Feikin *et al.* 2017[6] (influenza B)	47 (P)			Yes
	Feikin *et al.* 2017[6] (influenza C)	39 (P)			Yes

Continued

Table 1. *Continued*

Outcome (*n*/*N* studies with significant associations[a]) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
	Wishaupt *et al.* 2017[8]	22 (P)	Nasal wash	In patients with single infections, Ct values were not significantly associated with disease severity score (rho = 289; $P = 0.192$); however, there was moderate correlation between Ct value and length of oxygen use (rho = 0.550; $P = 0.027$)	Yes
	Clark *et al.* 2016[16]	13 (A)	NPS	**CURB65 score was higher in patients with Ct ≤20 ($P = 0.038$ across all Ct groups; $P = 0.011$ between Ct ≤20 and other groups combined). Median (IQR): Ct ≤20: 3 (2–3) Ct 21–25: 1 (0–2) Ct 26–30: 0 (0–0.8) Ct >31: 1 (0.25–1.8)**	Yes
Duration of symptoms (1/1 studies) [34 participants]	Brittain-Long *et al.* 2010[24] (influenza A)	24 (A)	NPS/OPS	**Ct values significantly correlated with duration of symptoms Influenza A: rho = 0.17, $P < 0.05$**	Yes
	Brittain-Long *et al.* 2010[24] (influenza B)	10 (A)		**Influenza B: rho = 0.65, $P < 0.01$**	Yes
ICU admission (0/3 studies) [1197 participants]	Zou *et al.* 2019[27]	658 samples	NPS	No difference in Ct values between ICU patients ($n = 61$ samples) and general ward patients	Yes
	Reina *et al.* 2018[26] (influenza A)	300: 150 (H1N1)pdm09 150 (H3N2)	NPA/smear	Mean Ct value was lower in ICU patients ($n = 21$ pdm09 Ct 29.86; $n = 16$ H3N2 Ct 31.01) compared with other settings, but the difference was not significant Adult ED: $n = 31$ pdm09 Ct 32.14; $n = 39$ H3N2 Ct 30.35 Admitted patients: $n = 38$ pdm09 Ct 31.59; $n = 32$ H3N2 Ct 32.25 Sentinel network: $n = 29$ pdm09 Ct 31.28; $n = 27$ H3N2 Ct 31.14	No
	Lalueza *et al.* 2019[21]	239 (A)	NPS	Viral load was not significantly associated with ICU admission: 7.4% in patients with Ct ≤20; 3.6% Ct >20–30; 8.1% Ct >30 ($P = 0.64$ for Ct ≤20 versus other groups combined)	Yes
Hospitalization (0/4 studies) [1680 participants]	Zou *et al.* 2019[27]	658 samples	NPS	No difference in Ct values between inpatients ($n = 512$) and outpatients ($n = 21$)	Yes
	Fuller *et al.* 2013[18]	187 ($P < 5$ years)	NPS/OPS	**Inpatients did not have significantly lower Ct values, in fact, outpatients with influenza had lower median Ct values (29.8) than inpatients (35.0, $P = 0.009$). In line with this observation, increasing**	Yes

Continued

Table 1. *Continued*

Outcome (*n*/*N* studies with significant associations[a]) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
				influenza Ct values increased the odds of being an inpatient versus being an outpatient OR 1.07, 95% CI (1.01–1.13)	
	Fuller *et al.* 2013[18]	363	NPS/OPS	**Inpatients did not have significantly lower Ct values, in fact, outpatients with influenza had lower median Ct values (24.1) than inpatients (33.4, $P < 0.001$). In line with this observation, increasing influenza Ct values increased the odds of being an inpatient versus being an outpatient OR 1.18, 95% CI (1.12–1.25). However, there was a strong association between increasing Ct values and decreasing odds of being an inpatient versus control groups [OR 0.85, 95% CI (0.75–0.97)]**	Yes
	Reina *et al.* 2018[26] (influenza A)	300: 150 (H1N1)pdm09 150 (H3N2)	NPA/smear	Mean Ct values did not vary significantly between settings Influenza A	No
	Reina *et al.* 2018[26] (influenza B)	150		Adult ED: $n = 31$ pdm09 Ct 32.14; $n = 39$ H3N2 Ct 30.35 Admitted patients: $n = 38$ pdm09 Ct 31.59; $n = 32$ H3N2 Ct 32.25 Sentinel network: $n = 29$ pdm09 Ct 31.28; $n = 27$ H3N2 Ct 31.14 Influenza B Adult ED: $n = 23$ Ct 29.32 Admitted patients: $n = 22$ Ct 31.00 Sentinel network: $n = 18$ Ct 27.48	No
	Wishaupt *et al.* 2017[8]	22 (P)	Nasal wash	In patients with single infections, median Ct values were not significantly different between patients admitted to hospital and those who were not (25.54 versus 34.77; $P = 0.065$)	Yes
Length of hospital stay (1/3 studies) [328 participants]	Lalueza *et al.* 2019[21]	239 (A)	NPS	Viral load was not significantly associated with length of hospital stay: median 9 days in patients with Ct ≤20; 7 days Ct >20–30; 8 days Ct >30 ($P = 0.34$ for Ct ≤20 versus other groups combined)	Yes
	Clark *et al.* 2016[16]	73 (A)	NPS	**Length of hospital stay was longer in patients with high viral load (Ct ≤20 versus other groups combined; $P = 0.005$), median (IQR) 7 days (2.5–13.5) in Ct ≤20; 2 (1–3.8) in Ct 21–25; 2 (1–4) in Ct 26–30;**	Yes

Continued

Table 1. *Continued*

Outcome (*n*/*N* studies with significant associations[a]) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
				2.5 (1–6) in Ct >30. Association was strongest in patients with exacerbation of COPD ($P = 0.007$) and pneumonia ($P = 0.028$)	
	Wishaupt *et al.* 2017[8]	16 (P)	Nasal wash	In patients with single infections, Ct values were not significantly associated with length of hospital stay (rho = 0.456; $P = 0.076$)	Yes

ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; Ct, cycle threshold; ED, emergency department; LRTI, lower respiratory tract infection; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; OPA, oropharyngeal swab; SOFA, sequential organ failure assessment; URTI, upper respiratory tract infection.
[a]Associations between low Ct values (high viral load) and worse clinical presentation or outcome.

In contrast, Feikin *et al.*[6] reported lower Ct values in paediatric patients with radiographic evidence of pneumonia compared with control participants with detectable RSV. However, unlike in patients with HRV, there was no significant difference in viral load between severe and very severe pneumonia. Houben *et al.*[30] also reported correlation between Ct values and disease severity, which was more pronounced in patients with RSV as the sole infection. Among two studies investigating Ct values and duration of illness, neither reported significant associations.[7,30]

Hospitalization and length of stay

Two studies (Wishaupt *et al.*[8] and Fuller *et al.*[18]) investigated the association of Ct value with hospital admission, both of studies reported significantly lower median Ct values in hospitalized versus non-hospitalized paediatric patients. In Fuller *et al.*,[18] no association was observed in patients ≥5 years old.

Three studies investigated association of Ct value with LOS. In the study by Hasegawa *et al.*,[7] a multivariable model indicated the risk of ≥3 days LOS was significantly higher in groups with intermediate (Ct 20.8–24.2) or high (Ct <20.8) viral loads, compared with low viral loads (Ct ≥24.3); OR 1.43 (95% CI, 1.20–1.69) and OR 1.58 (95% CI, 1.29–1.94), respectively, $P < 0.001$. A weak correlation between Ct value and LOS was also reported in the smaller study by Van Leeuwen *et al.*[33] ($n = 49$), but was contradicted by Wishaupt *et al.*[8] 2017 ($n = 163$).

Other respiratory pathogens

In line with the studies of influenza, HRV and RSV, evidence was mixed as to the association of Ct values and clinical presentation or outcomes for other respiratory pathogens (Tables S7–S12). In the paediatric study by Feikin *et al.*,[6] lower Ct values were associated with radiographic evidence of pneumonia compared with control participants for HBoV, HAdV, HMPV, PiV1 and PiV3. However, associations were not observed for HCoVs (229E, OC43, NL63 and HKU1), PiV2, PiV4 and parechovirus/enterovirus. None of these viruses had higher viral load with very severe versus severe pneumonia.[6] Across other HCoV studies (excluding Middle East respiratory syndrome coronavirus; MERS-CoV), three studies reported significant associations between low Ct values and symptom severity or duration,[8,24,34] but no associations between Ct value and hospitalization or LOS.[8] Two studies of MERS-CoV reported significant associations between low Ct values and mortality, symptom severity, ICU admission and transmissibility.[17,35] Among three HBoV studies, notably smaller in size than Feikin *et al.*,[6] none reported significant associations between Ct values and disease severity.[8,19,36] Across HAdV studies other than Feikin *et al.*,[6] Schjelderup Nilsen *et al.*[37] 2019 reported that Ct values <30 were associated with respiratory tract infection (RTI), adjusted for age, gender and presence of other viruses; however, three other studies reported no associations between Ct values and symptom severity, hospitalization or LOS.[8,18,28] Aside from Feikin *et al.*,[6] no further associations were reported across studies with HMPV and PiV, with exception of Fuller *et al.*,[18] in which hospitalized paediatric patients (<5 years old) with PiV reported lower Ct values than controls ($P = 0.047$), and conversely, HMPV infections had lower Ct values in outpatients versus inpatients ($P = 0.030$), similar to the outcome for influenza.[8,28,38] In one study of herpes simplex virus (HSV), higher viral load was associated with increased mortality, disease severity and ICU admission; however, there were no other HSV respiratory studies identified in this review.[39]

Among the limited number of bacterial studies identified, three studies investigated Ct values in patients with *Bordetella pertussis*, of which two reported significant associations between low Ct values and hospitalization or LOS.[40–42] There were too few studies in other bacterial pathogens to draw any conclusions.

Co-infections

Among the eligible studies in this review, nine investigated differences between Ct values between mono- and co-infections (Table S13). Associations were identified between lower Ct values in single infections versus co-infections; however no consistent trend

Table 2. Summary of studies ($N = 11$) that assessed PCR Ct values for HRV infections against clinical presentation or outcomes ($N = 2012$ total participants)

Outcome (*n*/*N* studies with significant associations) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
Mortality (0/1 studies) [330 participants]	Feikin *et al.* 2017[6]	330 (P)	NPS/OPS	Among patients with radiographic evidence of pneumonia, viral load at diagnosis was not significantly different between patients who died ($n = 27$) and those who survived ($n = 303$)	Yes
Severity of symptoms (3/9 studies) [1849 participants]	Feikin *et al.* 2017[6]	1421 (P)	NPS/OPS	**Patients with radiographic evidence of pneumonia had significantly lower mean Ct value than control participants with or without RTIs (adjusting for age and site): 31.7 (95% CI, 31.3–32.0) versus 32.4 (95% CI, 32.3–32.6), $P = 0.003$. The OR per $\log_{10}$ increase in viral copies/mL (approximately a 3.4 unit drop in Ct value) was 1.21 (95% CI, 1.08–1.35), $P < 0.05$. Among patients with radiographic evidence of pneumonia, mean viral load was significantly higher for very severe ($n = 123$) versus severe pneumonia ($n = 242$), $P < 0.05$ after adjusting for age and site**	Yes
	Waghmare *et al.* 2015[29]	128	Upper respiratory sample	**Higher Ct values were associated with presence of fewer symptoms at initial testing ($P = 0.036$). Patients with high initial Ct values ($\geq$35) were more likely to be asymptomatic at initial test than patients with lower initial Ct (25–30 and <25; 48% versus 26% versus 17%; $P = 0.064$) and were more likely to remain asymptomatic throughout viral shedding episodes ($P = 0.033$)**	N/A
	Moesker *et al.* 2016[31]	54 (P)	Respiratory tract samples	Ct values from ICU patients with severe acute RTI ($n = 8$) were not significantly different from those with non-severe acute RTI ($n = 46$; $P = 0.1861$). No significant difference was observed between acute ($n = 11$) and non-acute RTI ($n = 196$) in medium care settings ($P = 0.9157$)	No
	Tam *et al.* 2018[28]	43 (A)	Throat and nasal swabs	**Among military recruits, median Ct values for non-acute infections at the start of a 10 week training period ($n = 4$) were higher than for acute upper RTIs during follow-up**	Yes

Continued

Table 2. *Continued*

Outcome (*n*/*N* studies with significant associations) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
				(*n* = 39): 30.6 (IQR 28.6–31.8) versus 28.9 (IQR 26.9–31.4). Using a Tobit regression analysis, a significant difference was observed, coefficient 10.04 (95% CI 6.49–13.58); *P* < 0.001	
	Houben *et al.* 2010[30]	43 (P)	NPA	No association between Ct value and disease severity in patients with HRV as the primary pathogen (rho=–0.06; *P* = 0.71) or as HRV as the only detectable pathogen (*n* = 22; rho=–0.23; *P* = 0.31)	Yes
	Wishaupt *et al.* 2017[8]	32 (P)	Nasal wash	In patients with single infections, Ct values were not significantly associated with disease severity score (rho=−0.045; *P* = 0.809)	Yes
	Waghmare *et al.* 2017[32]	31	BAL	Among HRV positive patients who progressed from URTI to LRTI, HRV viral loads did not correlate with disease progression	N/A
	Clark *et al.* 2016[16]	27 (A)	NPS	CURB65 score was not significantly different across Ct groups (*P* = 0.799). Median (IQR): Ct ≤20: 1 (0–2) Ct 21–25: 2 (1–3) Ct 26–30: 1 (1–1) Ct >31: 1 (0–2.8)	Yes
	Jansen *et al.* 2010[19]	70 with ≥1 virus identified (P)	NPA	No correlation was observed between median Ct value and disease severity grouping (*P* = 0.3)	No
Duration of symptoms (0/1 studies) [19 participants]	Brittain-Long *et al.* 2010[24]	19 (A)	NPS/OPS	Ct values were not significantly correlated with duration of symptoms	Yes
ICU admission (0/1 studies) [19 participants]	Moesker *et al.* 2016[31]	19 (P)	Respiratory tract samples	No significant difference in median Ct values between patients with severe acute RTI in ICU (*n* = 8) and those with acute RTI in medium care (*n* = 11): 28.3 versus 26.4 (*P* = 0.44)	No
Hospitalization (0/1 studies) [32 participants]	Wishaupt *et al.* 2017[8]	32 (P)	Nasal wash	In patients with single infections, median Ct values were not significantly different between patients admitted to hospital and those who were not (29.85 versus 34.83; *P* = 0.126)	Yes
Length of hospital stay (1/3 studies) [194 participants]	Clark *et al.* 2016[16]	149 rhino/enterovirus (A)	NPS	**Length of hospital stay was longer in patients with high viral load (Ct ≤20 versus other groups combined; *P* = 0.004), median (IQR) 4 days (2–7.8) in Ct ≤20; 2 (1–4.3) in Ct 21–25; 2 (1–4) in Ct 26–30; 2**	Yes

Continued

Table 2. *Continued*

Outcome (*n*/*N* studies with significant associations) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
				(1–4) in Ct >30. Association was strongest in patients with asthma exacerbation (*P* = 0.006)	
	Wishaupt *et al.* 2017[8]	23 (P)	Nasal wash	In patients with single infections, Ct values were not significantly associated with length of hospital stay (rho = 0.298; *P* = 0.167)	Yes
	Van Leeuwen *et al.* 2012[33]	22 (P)	Nasal wash	Overall (*n* = 120 infants with LRTI) statistically significant, but very weak correlation between Ct value and length of hospital stay (mean Ct value 28.5 ± 4.9, median hospital stay 4 days, rho = 0.19, *P* = 0.04). In the HRV-LRTI group there was no correlation between Ct values and length of hospital stay (rho = 0.13; *P* = 0.58)	No

BAL, bronchoalveolar lavage; Ct, cycle threshold; HRV, rhinovirus; IQR, interquartile range; LRTI, lower respiratory tract infection; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; OPA, oropharyngeal swab; RTI, respiratory tract infection; URTI, upper respiratory tract infection.

among viruses was identified.[7,8,30,34,37,43–45] Both HCoV studies reported significant associations between lower Ct values and mono-infections; Heimdal *et al.*[34] $P < 0.001$ and Wishaupt *et al.*[8] $P = 0.015$. Two out of three RSV studies reported significantly lower Ct values in patients with sole infections, including a study of 1764 paediatric patients hospitalized with bronchiolitis, $P < 0.001$.[7,30]

Discussion

To the best of our knowledge, this is the first review to systematically assess and consolidate available evidence on associations between respiratory virus Ct values and clinical presentation or outcomes. Respiratory viruses were more commonly studied than bacterial infections, and among respiratory viruses, influenza, HRV and RSV were the most studied.

Across all studies included, there was contradictory evidence regarding the association between Ct value and clinical presentation or outcomes. However, for some pathogens, there are trends that warrant further investigation. In studies of RSV, several studies reported associations between low Ct values and clinically relevant outcomes, including hospitalization,[8,18] ICU requirement and LOS,[7] and radiographic evidence of pneumonia.[6] However, other studies of RSV viral load (Ct values not reported, hence not eligible for inclusion in this review) have failed to identify associations with disease severity or ICU admission.[46,47] Among HRV studies, despite numerous studies showing no associations, the three studies with cohorts >100 patients reported significant associations between low Ct values and symptom severity and hospital LOS.[6,16,29] A similar observation can be made with HBoV studies, particularly as other studies of HBoV viral load (Ct values not reported, hence not eligible for inclusion in this review) have shown significant associations with disease severity.[48,49] Although few HCoV studies showed significant associations, the two studies identified for MERS-CoV suggest low Ct values are associated with disease severity and transmissibility,[17,35] in line with reports of viral load studies for SARS-CoV-1 and SARS-CoV-2.[14,50]

Interestingly, influenza studies gave conflicting reports for associations between Ct values and hospitalization or ICU admission. However, the largest influenza study identified (Spencer *et al.*[23]) reported that patients with low Ct values were significantly more likely to self-report moderate to high disease severity and fever/feverishness. A similar observation was made in a study of influenza A viral load (Ct values not reported, hence not eligible for inclusion in this review) where patients with pneumonia had higher copies/mL than those with upper RTIs.[51]

When interpreting the studies presented in this literature review, consideration must be given to various factors.[8,52,53] Many of the studies included patients in hospital settings, which potentially corresponds to greater disease severity and a less-striking difference in viral load. Indeed, studies of RSV and PiV in this review reported significantly lower Ct values for paediatric inpatients versus outpatients or controls.[8,18]

Considering the fact that many studies identified significantly lower Ct values in single versus co-infections, the lack of clarity in studies as to whether reports are sole infections has potential implications. Future studies investigating Ct values should consider the quantification of all pathogens present to obtain more detailed and consistent information on the impact of co-infections. The use

Table 3. Summary of studies ($N = 8$) that assessed PCR Ct values for RSV infections against clinical presentation or outcomes ($N = 3290$ total participants)

Outcome (*n*/*N* studies with significant associations) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
Mortality (0/1 studies) [412 participants]	Feikin *et al.* 2017[6]	412 (P)	NPS/OPS	Among patients with radiographic evidence of pneumonia, viral load at enrolment was not significantly different between patients who died ($n = 13$) and those who survived ($n = 399$)	Yes
Severity of symptoms (2/6 studies) [2718 participants]	Hasegawa *et al.* 2015[7]	1764 (P)	NPA	Based on low genomic load (≥24.3 Ct values), intermediate (20.8–24.2) and high (<20.8), no significant difference in vital signs across the groups were observed, including respiratory rate (median 48 breaths/min in all groups), oxygen saturation (<90% saturation in 11%, 9% and 9%, respectively) and retractions (moderate or severe in 29%, 31% and 30%, respectively)	Yes
	Feikin *et al.* 2017[6]	601 (P)	NPS/OPS	**Patients with radiographic evidence of pneumonia had significantly lower mean Ct value than control participants with or without RTIs (adjusting for age and site): 22.2 (95% CI, 21.8–22.5) versus 27.0 (95% CI, 26.1–28.0); $P < 0.001$. The OR per $\log_{10}$ increase in viral copies/mL (approximately a 3.4 unit drop in Ct value), was 2.02 (95% CI, 1.71–2.37; $P < 0.05$). Notably, mean viral load was significantly higher in RTI controls ($n = 49$) versus non-RTI controls ($n = 45$); $P < 0.05$ after adjusting for age and site**. Among patients with radiographic evidence of pneumonia, viral load was not significantly different between patients with very severe ($n = 123$) and severe ($n = 338$) pneumonia	Yes
	Wishaupt *et al.* 2017[8]	202 (P)	Nasal wash	In patients with single infections, Ct values were not significantly associated with disease severity score (rho=−0.029; $P = 0.678$)	Yes
	Moesker *et al.* 2016[31]	51 (P)	Respiratory tract samples	Ct values from ICU patients with severe acute RTI ($n = 10$) were not significantly different from those with non-severe acute RTI ($n = 41$; $P = 0.1771$). No significant difference was observed between acute	No

Continued

Table 3. *Continued*

Outcome (*n*/*N* studies with significant associations) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
				($n = 13$) and non-acute RTI ($n = 63$) in medium care settings ($P = 0.0815$)	
	Houben *et al.* 2010[30]	30 (P)	NPA	**Using a disease severity scoring system, there was a positive correlation between viral load and disease severity (rho=−0.52; *P* = 0.003). Stronger correlation was observed in 11 patients with RSV as the sole infection (rho=−0.68; *P* = 0.02)**	Yes
	Jansen *et al.* 2010[19]	70 with ≥1 virus identified (P)	NPA	No correlation was observed between median Ct value and disease severity grouping ($P = 0.6$)	No
Duration of illness (0/2 studies) [1780 participants]	Hasegawa *et al.* 2015[7]	1764 (P)	NPA	Based on low genomic load (≥24.3 Ct values), intermediate (20.8–24.2) and high (<20.8), no significant difference in the duration of symptoms (Spearman correlation for duration of difficulty breathing, $P = 0.98$)	Yes
	Houben *et al.* 2010[30]	16 (P)	NPA	No association between duration of illness and Ct value (rho = 0.16; $P = 0.56$)	Yes
ICU requirement (1/2 studies) [1787 participants]	Hasegawa *et al.* 2015[7]	1764 (P)	NPA	**In a multivariable model, patients with high genomic loads (Ct values <20.8) were at higher risk of needing ICU care compared with lower genomic loads (≥24.3 Ct values) OR 1.43 (95% CI, 1.03–1.99) *P* = 0.03.** However, significance was lost in a sensitivity analysis using a restrictive definition of bronchiolitis (<12 months old with gestational age ≥37 weeks; $n = 1223$), OR 1.36 (95% CI, 0.91–2.03) $P = 0.14$	Yes
	Moesker *et al.* 2016[31]	23 (P)	Respiratory tract samples	No significant difference in median Ct values between patients with severe acute RTI in ICU ($n = 10$) and those with acute RTI in medium care ($n = 13$): 20.6 versus 21.4 ($P = 0.94$)	No
Hospitalization (2/2 studies) [725 participants]	Fuller *et al.* 2013[18]	370 ($P < 5$ years)	NPS/OPS	**Median Ct value was significantly lower for inpatients (27.2) than controls (35.8, *P* = 0.008) and outpatients (34.7, *P* < 0.001). Increasing Ct value was associated with decreased odds of being an inpatient versus an outpatient: OR 0.96; 95% CI 0.93–0.99**	Yes

Continued

Table 3. *Continued*

Outcome (*n*/*N* studies with significant associations) [Total number of participants]	Study	Number of PCR+ patients (A, adult; P, paediatric)	Sample type	Outcome measure (statistically significant results in bold)	Clearly defined methodology (see Table S2)
	Fuller *et al.* 2013[18]	153		No significant associations between Ct values and inpatients versus outpatients or controls	Yes
	Wishaupt *et al.* 2017[8]	202 (P)	Nasal wash	**In patients with single infections, median Ct values were significantly lower in hospitalized patients (23.17 versus non-hospitalized 24.57; *P* = 0.04)**	Yes
Length of hospital stay (2/3 studies) [1976 participants]	Hasegawa *et al.* 2015[7]	1764 (P)	NPA	**In a multivariable model, the risk ≥3 days length of stay was significantly higher in the groups with intermediate (Ct 20.8–24.2; OR 1.43; 95% CI, 1.20–1.69) and high (Ct <20.8; OR 1.58; 95% CI, 1.29–1.94) genomic loads compared with low (≥24.3 Ct values); *P* < 0.001**	Yes
	Wishaupt *et al.* 2017[8]	163 (P)	Nasal wash	In patients with single infections, Ct values were not significantly associated with length of hospital stay (rho=−0.036; *P* = 0.652)	Yes
	Van Leeuwen *et al.* 2012[33]	49 (P)	Nasal wash	**Overall (*n* = 120 infants with LRTI) statistically significant, but very weak correlation between Ct value and length of hospital stay (mean Ct value 28.5 ± 4.9, median hospital stay 4 days, rho = 0.19, *P* = 0.04). In the RSV-LRTI group, this correlation was significant but weak (rho = 0.28, *P* = 0.05)**	No

Ct, cycle threshold; IQR, interquartile range; LRTI, lower respiratory tract infection; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; OPA, oropharyngeal swab; RSV, respiratory syncytial virus; RTI, respiratory tract infection.

of multiplex PCR panels would be advantageous in this regard, particularly where data on Ct values are reported.

There is evidence that genomic load in respiratory infections is affected by age and comorbidities.[23,41,46,54] It is therefore important to consider the age and comorbidity profile of each of the studies when making comparisons, and future studies should consider adjusting comparisons to account for the impact of age and comorbidities.

Differences in study procedures and PCR workflow are likely to impact viral load measurement, including: specimen source, collection method, transport media type and volume; stability, quality of the sample and time of sampling versus onset of infection; inter-and intra-variability in assay platforms, and whether they were single or multiplex systems. Study procedures varied widely between studies. Many studies had gaps in reported methods relating to sampling, sample handling and analysis, and most studies did not mention the use of standardized protocols and procedures; therefore within-study variability may have limited the ability to detect associations. Consistent approaches to standardize a given workflow (e.g. sample collection, pre-treatment, real-time PCR methodology) should be implemented to utilize Ct values as a proxy of genomic load linked to a given methodology. Alternatively, normalized genomic load/copies in the test media, using reference material or international standards when available, should be attempted,[55] and cross study comparisons where this is not clear should be avoided without normalization. Importantly, raw Ct values across studies should not be interpreted as a unit of genomic load as standard curves are needed to calculate the link between Ct values and genomic load in any given study.[52] Furthermore, trends in Ct values reported in many studies, including those within this review, are population data and caution should be taken if applying this to individual patients.

This systematic review has several limitations. The protocol was restricted to consideration only of articles referring to Ct values as a measure of genomic load, meaning numerous studies that define genomic load other than using Ct values were excluded. Furthermore, it is inevitable that some studies investigating Ct values as secondary or post-hoc analyses were missed in the screening process. However, considering no aggregated outcomes were generated in this review, and no definitive conclusions made, the absence of these studies is unlikely to be a major consideration. Additionally, the high heterogeneity of reported outcomes meant it was not possible to conduct aggregated/meta-analyses, reducing the clinical utility of this review. A single reviewer conducted the data extraction and a second reviewer checked all the data points. Whilst this approach is generally accepted as adequate, it is acknowledged the optimal approach is double independent reviewer data extraction with a third reviewer resolving any discrepancies. However, given the large number of studies and outcomes identified, this was a pragmatic approach to ensure feasibility. Despite these limitations, the studies included provide insights into the potential clinical utility of respiratory pathogen Ct values, and future areas of research.

In summary, Ct values for some respiratory tract infections could be clinically useful in guiding treatment, hospital, or wider healthcare decisions. However, large-scale clinical trials, designed to minimize the limitations and disparities highlighted, are required to draw definitive conclusions.

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Author contributions

All authors contributed to protocol development, data interpretation and writing of this report.

Supplementary data

Tables S1 to S13 are available as Supplementary data at *JAC* Online.

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