

Comparison of RT-PCR Cycle Threshold Values from Respiratory Specimens in Symptomatic and Asymptomatic Children with SARS-CoV-2 Infection

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Summary: Of 728 children who tested positive for SARS-CoV-2 by RT-PCR, mean cycle threshold value was lower in asymptomatic children. Children less than 5 years had the lowest mean cycle threshold value. 71.2% of children were symptomatic.

Abstract

Background: Understanding viral kinetics of SARS-CoV-2 is important to assess risk of transmission, manage treatment, and determine the need for isolation and protective equipment. The impact of viral load in asymptomatic infected children is important to understand transmission potential. We sought to determine whether children deemed to be asymptomatic had a difference in the PCR cycle threshold (Ct) value of respiratory samples from symptomatic children with SARS-CoV-2 infection.

Methods: This was a retrospective cross-sectional study to compare PCR Ct values of children who tested positive for SARS-CoV-2 by respiratory samples collected over a 4-month period at a large tertiary care children's hospital.

Results: We analyzed 728 children who tested positive for SARS-CoV-2 by RT-PCR from a respiratory sample over a 4-month period and for whom data was available in the electronic medical record. Overall, 71.2% of infected children were symptomatic. The mean Ct value for symptomatic patients (Ct mean 19.9, SD 6.3) was significantly lower than asymptomatic patients (Ct mean 23.5, SD 6.5) (P value < 0.001, CI^{95th} 2.6 - 4.6). The mean PCR Ct value was lowest in children less than 5 years of age.

Conclusions: In this retrospective review of children who tested positive by RT-PCR for SARS CoV-2, the mean Ct was significantly lower in symptomatic children and was lowest in children under 5 years of age, indicating that symptomatic children and younger children infected with SARS-CoV-2 may have a higher viral load in the nasopharynx compared to asymptomatic children. Further studies are needed to assess the transmission potential from asymptomatic children.

Key words: SARS-CoV-2, PCR, cycle threshold, children

SARS-CoV-2, responsible for the COVID-19 pandemic, has severely impacted societal function [1,2].

Understanding viral kinetics of SARS-CoV-2 is of vital importance, both for determining appropriate therapy at different points in the disease course and in assessing risk of transmission. Asymptomatic individuals are thought to be significant contributors to transmission, though not to the extent of symptomatic individuals [3]. Studies have shown that infected children may have a similar viral load in the nasopharynx as adults, but few studies have examined if the viral load in asymptomatic children is less than symptomatic children, which may correlate with less transmissibility [4–6]. Quantification of viral load in respiratory samples is challenging, and some studies have attempted to infer viral load from the polymerase chain reaction (PCR) cycle threshold (Ct) value [7]. We sought to determine whether there is a difference in the PCR Ct values of respiratory samples between symptomatic and asymptomatic children with SARS-CoV-2 infection.

Methods

This is a retrospective cross-sectional chart review examining hospitalized and ambulatory pediatric patients ages 0-18 years who had positive SARS-CoV-2 PCR from nasopharyngeal (NP) or anterior nares (AN) testing from April 1st, 2020 to August 1st, 2020 at the Rady Children's Hospital San Diego (RCHSD) lab. The protocol for this study was reviewed and approved by the institutional review board of the University of California San Diego. RCHSD is a 524 bed tertiary children's hospital in southern California with upwards of eighteen thousand inpatient admission annually. Demographics of the region reflect a diverse racial mix of 45% White, 34.1% Hispanic, 5.5% Black, 12.6% Asian/Pacific Islander, and 1.3% American Indian/Alaska Native[8]. Of note, every patient admitted to RCHSD and every patient having a procedure in the operating room is tested for SARS-CoV-2. In addition, since mid-June 2020, as part of a San Diego County wide effort to increase testing, SARS-CoV-2 testing has been offered at select primary care sites and the RCHSD emergency department to all patients regardless of symptoms. For children tested more than once, only the first positive PCR test was included from each child. Children were excluded if they had incomplete electronic medical record (EMR) data to determine if they were symptomatic or asymptomatic. Children were determined to be symptomatic if they had any of the following new (or worsening from baseline) symptoms in the seven days prior to SARS-CoV-2 PCR testing: cough, fever, chills, rhinorrhea, congestion, sore throat, shortness of breath, abdominal pain, nausea, vomiting, diarrhea, rash, headache, myalgia, loss of taste or smell, fatigue or malaise. Children were determined to be asymptomatic if none of the above symptoms were documented in the EMR. Data regarding comorbidities were collected from the EMR problem list. Obesity, defined as a BMI equal to or greater than the

95th percentile for age and sex, was assessed by problem list or as documented in the vital signs of the medical progress notes. Chart review was audited by a second reviewer to ensure consistency in data extraction. Three PCR platforms were used: (1) Simplexa® COVID-19 Direct kit on the Liaison MDX Cycler (DiaSorin, Saluggia, Italy), (2) BioGX SARS-CoV-2 Reagent kit with the BD MAX™ System (Becton, Dickinson and Company, Sparks, Maryland, USA), and (3) Lyra® Direct SARS-CoV-2 assay (Quidel, San Diego, CA, USA) using the Applied Biosystems™ 7500 Real-Time PCR System (ThermoFisher, Waltham, MA, USA). The Simplexa® COVID-19 Direct kit uses 2 targets: the S gene (Ct 1) and ORF1ab (Ct 2). The BioGX SARS-CoV-2 Reagent kit uses 2 targets: The N1 region (Ct 1) and the N2 region (Ct2) of the SARS-CoV-2 virus nucleocapsid phosphoprotein gene. The Simplexa® COVID-19 Direct kit and the BioGX SARS-CoV-2 Reagent kit report a positive result for Ct value less than 40.0. The Lyra® Direct SARS-CoV-2 assay detects the non-structural polyprotein pp1ab and reports a positive result for Ct values between 5.0 and 30.0. Ct means were compared between symptomatic and asymptomatic children in the following age groups: less than 5 years, 5-12 years, and 13-18 years.

Statistics:

A student t-test was used to compare Ct means between the groups. Means were reported with standard deviation (SD) and medians with interquartile range (IQR). For platforms with 2 targets, the mean was calculated from Ct 1. If a value was not available for Ct 1, then the value from Ct 2 was imputed in its place. Statistical calculations were made using Microsoft® Excel® 2010 (14.0.7249.5000).

Results

We identified 916 respiratory samples that were positive for SARS-CoV-2 by PCR from respiratory specimens at RCHSD during the study period. Twenty-six samples were excluded given they were not the child's first positive PCR sample. One hundred sixty-two children were excluded due to incomplete information in the EMR. Seven hundred twenty-eight children were left for analysis. Two hundred sixty-eight children were under age 5 years, 241 were age 5-12 years, and 219 were age 13-18 years. Symptomatology is described in Table 1. Overall, 71.2% of children were symptomatic and 28.8% were asymptomatic. Respiratory symptoms were present in 67.4% of all children. By age group, 76.9% (206/268) of children under 5 years of age, 62.2% (150/241) of the children 5-12 years of age, and 74.0% (162/219) of the children 13-18 years of age were symptomatic. Comorbid conditions were represented similarly in symptomatic and asymptomatic patients (Table 1). Using a

Fischer's exact test to compare comorbidities, a significant difference between symptomatic and asymptomatic children was not observed.

A NP specimen was collected for 26.6% of children and an AN specimen was collected on 73.4% of children. Two samples were improperly labeled and unable to be differentiated as NP or AN samples and were not included in subset analysis. Of included children, 6.5% were hospitalized and 93.5% were outpatient. The mean Ct value for symptomatic patients (Ct mean 19.9, SD 6.3) was significantly lower than asymptomatic patients (Ct mean 23.5, SD 6.5) (P value < 0.001, CI^{95th} 2.6 - 4.6). This finding was consistent across PCR platforms and age groups (Table 2). The overall mean Ct value was significantly lower in the less than 5 year group when compared to the other age groups. This finding held true in the symptomatic children, but not for asymptomatic children. The mean Ct value for NP samples was not statistically different than that of AN samples (Table 3). The mean Ct value for children with respiratory symptoms was not significantly different from the mean Ct value for symptomatic children without respiratory symptoms (supplement Table 1). Comparison of the mean Ct values between Ct 1 and Ct 2 for both the BioGX SARS-CoV-2 Reagent kit and the Simplexa[®] COVID-19 Direct kit demonstrated low variance (supplement Table 2).

Discussion

In this a single center retrospective review of 728 children who tested positive by RT-PCR from a respiratory specimen for SARS-CoV-2 at a large tertiary children's hospital, Ct means were lower among symptomatic children compared to asymptomatic children. Correlation of Ct value with viral load has been established in prior studies, and a recent publication by Kociolek et al also made a similar observation that children with symptomatic disease had lower Ct values from respiratory specimens [6,7,9]. Lower Ct values for symptomatic compared to asymptomatic children suggests symptomatic children have a higher viral load in the anterior nares and nasopharynx, which has been previously described by Han et al [10]. The overall difference in mean Ct value between asymptomatic and symptomatic children was 3.6, and since PCR is a doubling process, a difference of 3.3 between Ct values correlates with an approximate 10-fold difference in viral load. This is similar to the data collected by Kociolek et al. and in contrast to recently published data from Hurst et al. where no significant difference in viral load was seen between symptomatic and asymptomatic children [6,11]. The smaller number of children in the study by Hurst et al., may have limited the ability to detect a difference in Ct values between asymptomatic and symptomatic children [11]. A difference in viral load between

symptomatic and asymptomatic children may have important implications for transmissibility. In a recent epidemiologic analysis of transmission in Wuhan, China, asymptomatic subjects were noted to have reduced transmission potential [3]. A decreased viral load in addition to the lack of normal modes of transmission (e.g. coughing or sneezing) may account for this finding. Transmission from asymptomatic children is an important consideration for bringing children back to school and group childcare. Children are unlikely to receive vaccination against SARS-CoV-2 in the immediate future and characterizing the transmissibility of asymptomatic children may have an impact on mitigation measures in the classroom and for extracurricular activities.

Through a detailed chart review of the EMR, we observed that 28.8% of children in our cohort were asymptomatic, a finding that is consistent with other published studies [12]. The largest proportion of asymptomatic children was in the 5-12 year group at 37.8 %. Respiratory symptoms were present in 67.4% of all children, and 19.5% of all children had gastrointestinal symptoms. Just 45.1% of all children and 63.3% of symptomatic children had fever. The broad range of symptoms and large proportion of asymptomatic and afebrile patients may have important implications for screening practices in both healthcare and public settings.

Interestingly, similar to data recently published by Heald-Sargent et al., Ct values were lower for children under 5 years of age.[5] Some studies have indicated that children are less likely to be infected by an index case [13,14]. It has also been suggested that, when children are infected, they are less likely to contribute to secondary cases [15]. Additionally, when children are infected, they are less likely than adults to experience severe disease [16]. One would therefore expect a lower viral load and consequently higher Ct values in younger children. However, this is belied by the lower Ct value seen in the younger age group in our cohort [7]. Indeed, in a recent study by L'Hullier et al, SARS-CoV-2 viral load of culture-competent virus in symptomatic children resembled the findings in infected adults [17]. It is possible that, while the Ct value was lower for children, that a less robust immune response in this age group spares them from the more severe disease manifestations noted in older patients [18]. Weisberg et al. showed that children had reduced breadth of anti-SARS-CoV-2 specific antibodies and reduced neutralizing activity compared to adults [19]. It is also possible that the pathophysiologic mechanisms required by the virus to transmit to the lower respiratory tract are not as readily available in young children [20]. Another possibility is that this age group has had less exposure to

other strains of non-SARS-CoV-2 coronavirus, thus lacking cross immune recognition and producing a less robust immune response [21]. Viral load may also be higher in younger children because they present to care and are tested earlier in the illness course. Transmissibility may be blunted by the decreased ability of young children to generate the aerosols and droplets that are required for optimal transmission of virus from an infected host, explaining why they may be less likely to transmit infection despite higher viral loads. This may also explain why it may be more likely for household contacts (e.g. parents) to acquire SARS-CoV-2 infection from young children [22].

An additional finding in our data was the similarity in Ct values between NP and AN specimens, which may suggest similar capability in detection (Table 3). We also found that Ct values were fairly similar between platforms. While a comparison of Ct values, especially between different platforms and their various PCR targets as well as different sample types, does not translate directly to efficacy in terms of sensitivity and specificity, there are studies demonstrating comparative utility of AN versus NP samples [23,24]. As mentioned above, since viral load correlates with Ct value, the similarity in mean Ct value for AN and NP samples may indicate a correspondingly similar burden of viral load from both sample sites.

This study has a number of limitations. The retrospective nature of the study and inconsistencies in documentation in the EMR may have led to the inclusion of incomplete or inaccurate information. One hundred sixty-two charts were not included for analysis due to incomplete information. Furthermore, comorbidities were generated from problem lists, which may not be frequently updated by clinicians. Samples were collected when children presented, thus samples were likely collected at various time points during illness, which may affect Ct values.[25] Given that Ct values are expected to rise over time as the illness progresses, the lack of granular data regarding day of illness is an important limitation. Furthermore, some children characterized as asymptomatic in the EMR may have been in a pre-symptomatic period of the infection. Testing was conducted on different platforms and while this does provide a diverse real-world examination of testing at a tertiary facility, it does hamper the overall generalizability of the data set. That said, Ct values were fairly consistent between platforms (Table 2) and preformed using standardized validation practices at a single center. Similar analysis with Ct values between platforms has been reported in the literature as well [6]. A Ct 2 value was substituted for a Ct 1 value if it was not available, which is problematic given that the targets are different. However, comparison of Ct value between Ct 1 and Ct 2 for both the BioGX

SARS-CoV-2 Reagent kit and the Simplexa® COVID-19 Direct kit demonstrated low variance (supplement Table 2). The Ct values were also subject to user error during sample collection, and it was not possible to verify that all samples, especially the NP samples, were collected consistently. Finally, while Ct values are a surrogate for viral load (as opposed to the preferred standardized logarithmic curve in RNA/mL), this is a substandard method of quantifying virus in the upper respiratory tract and may not correlate with viable virus [26,27].

Conclusion

In this retrospective review of children who tested positive by RT-PCR for SARS-CoV-2, the mean Ct value was significantly lower for symptomatic children. Children under 5 years of age were found to have the lowest mean Ct value. Lower Ct in symptomatic children suggests higher viral loads and transmission potential, compared to asymptomatic children. The mean Ct value was similar between samples from anterior nares and nasopharyngeal swabs. Further studies are required to assess potential of children with asymptomatic infection.

Notes:

None of the authors has a conflict of interest to report.

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Table 1. Demographics and clinical characteristics.

Total	728 (100%)	
Median age in years (IQR)	7.5 (2.0-14.0)	
Collection from anterior nares	533 (73.4%)	
Collection from nasopharynx	193 (26.6%)	
Inpatient	47 (6.5%)	
Outpatient	681 (93.5%)	
Asymptomatic	210 (28.8%)	
Symptomatic	518 (71.2%)	
% of Symptomatic Cases		
Fever	63.3%	
Cough	36.7%	
Headache	27.6%	
Congestion	27.6%	
Rhinorrhea	25.5%	
Sore throat	24.1%	
Diarrhea	13.5%	
Fatigue/Malaise	10.8%	
Loss of taste or smell	8.9%	
Abdominal pain	8.5%	
Vomiting	7.9%	
Myalgia	7.7%	
Shortness of breath	7.3%	
Nausea	6.0%	
Rash	4.6%	
Chills	4.2%	
Comorbidities	Symptomatic %	Asymptomatic %
Cardiac	4.1%	1.0%

Pulmonary	13.9%	12.9%
Gastrointestinal	2.1%	2.9%
Renal	1.4%	2.4%
Oncologic	1.0%	1.9%
Obesity	10.6%	11.0%
Other	15.3%	19.0%

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Table 2. Ct means for symptomatic and asymptomatic children by platform and in the following age ranges (in years): <5, 5-12, 13-18, and all children. Platforms include the Simplexa® COVID-19 Direct kit on the Liaison MDX Cycler (DiaSorin, Saluggia, Italy), the BioGX SARS-CoV-2 Reagent kit on the BD MAX™ System (Becton, Dickinson and Company, Sparks, Maryland, USA), and the Lyra Direct SARS-CoV-2 assay (Quidel, San Diego, CA, USA) on the Applied Biosystems™ 7500 Real-Time PCR System (ThermoFisher, Waltham, MA, USA).

Age (years)	N (% Total)	Symptomatic Children Ct Mean (SD)	Asymptomatic Children Ct Mean (SD)	P value
< 5				
All Platforms	268 (36.8)	18.7 (6.4)	22.5 (7.7)	<0.001
Simplexa® COVID-19 Direct kit	83 (38.8)	19.3 (7.2)	24.8 (7.6)	0.04
BioGX SARS-CoV-2 Reagent kit	84 (38.9)	19.6 (7.5)	26.4 (9.3)	0.007
Lyra Direct SARS-CoV-2 assay	101 (33.9)	17.3 (3.9)	19.6 (5.0)	0.04
5-12				
All Platforms	241 (33.1)	21.0 (6.3)	24.0 (6.7)	<0.001
Simplexa® COVID-19 Direct kit	68 (31.8)	22.1 (6.0)	25.0 (8.0)	0.15
BioGX SARS-CoV-2 Reagent kit	65(30.1)	22.7 (8.5)	28.1 (6.1)	0.004
Lyra Direct SARS-CoV-2 assay	108 (36.2)	19.1 (4.5)	21.2 (4.9)	0.02
13-18				
All Platforms	219 (30.1)	20.2 (5.9)	23.7 (6.5)	< 0.001
Simplexa® COVID-19 Direct kit	43 (29.4)	21.8 (6.4)	24.4 (8.6)	0.59
BioGX SARS-CoV-2 Reagent kit	51 (23.6)	21.1 (7.3)	27.2 (5.1)	0.002
Lyra Direct SARS-CoV-2 assay	86 (28.9)	18.4 (3.8)	21.0 (4.3)	0.006
Total				
All Platforms	728 (100)	19.9 (6.3)	23.5 (6.9)	< 0.001
Simplexa® COVID-19 Direct kit	214 (100)	20.9 (6.7)	24.6 (7.9)	0.007
BioGX SARS-CoV-2 Reagent kit	216 (100)	20.7 (7.6)	27.7 (7.1)	< 0.001
Lyra Direct SARS-CoV-2 assay	124 (100)	18.4 (3.9)	20.6 (3.9)	<0.001

Table 3. Mean Ct value for nasopharyngeal (NP) and anterior nares (AN) samples in the following age groups: <5 years, 5-12 years, 13-18 years, and all ages.

Age (years)	N (% Total)	NP Ct Mean (SD)	AN Ct Mean (SD)	P value
< 5	269 (37.1)	19.1 (6.4)	21.3 (7.8)	0.03
5-12	242(33.3)	22.2 (6.6)	22.1 (6.6)	0.3
13-18	215 (29.6)	21.0 (5.9)	21.2 (7.1)	0.8
All	726 (100)	21.6 (7.2)	20.6 (6.4)	0.13

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