Children Have Similar Reverse Transcription Polymerase Chain Reaction Cycle Threshold for Severe Acute Respiratory Syndrome Coronavirus 2 in Comparison With Adults

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Background: The viral dynamics and the role of children in the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are not completely understood. Our aim was to evaluate reverse transcription polymerase chain reaction (RT-PCR) cycle threshold (Ct) values among children with confirmed SARS-CoV-2 compared with that of adult subjects.

Methods: Patients (from 2 months to ≤18 years of age and adults) with signs and symptoms of acute SARS-CoV-2 infection for less than 7 days were prospectively enrolled in the study from May to November 2020. All participants performed RT-PCR assay for SARS-CoV-2 detection; Ct values of *ORF1ab*, *N* and *S* gene targets and the average of all the 3 probes were used as surrogates of viral load.

Results: There were 21 infants (2 months to <2 years), 40 children (≥2 to <12 years), 22 adolescents (≥12 to <18 years) and 293 adults of 376 participants with confirmed SARS-CoV-2 infections. RT-PCR Ct values from all participants less than 18 years of age, as well as from all childhood subgroups, were not significantly different from adults, comparing *ORF1ab*, *N*, *S* and all the gene targets together ($P = 0.453$).

Conclusions: Ct values for children were comparable with that of adults. Although viral load is not the only determinant of SARS-CoV-2 transmission, children may play a role in the spread of coronavirus disease 2019 in the community.

Key Words: severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019, children, cycle threshold, viral load

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In December 2019, an outbreak of a new viral pneumonia was identified in Wuhan, China.[1] Phylogenetic analysis of the new virus, classified initially as 2019 novel coronavirus (nCoV), pointed it as a member of the *Betacoronavirus* genus. This virus presented typical features of the coronavirus family previously identified in humans, bats and other wild animals. By February 2020, the Coronavirus Study Group of the International Committee on Virus Taxonomy suggested a nomenclature change to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) because of phylogenetic analysis relating this new virus to coronaviruses.[2]

All countries have been dealing with this pandemic with an unprecedented number of severe and also nonsevere patients. Still, children have somehow been spared from these severe outcomes. An important issue that still needs clarification relates to the understanding of the impact of viral load at different levels of disease, because these could be associated not only with disease severity but also to its impact on infectivity and viral transmission in the community. The difference on viral load levels between infected children and adults is at this stage of knowledge yet a matter of some debate.[3,4] A study in the United States of 145 subjects including children younger than 5 years of age, 5–17 and adults with mild-to-moderate illness has found that there was no difference in RT-PCR cycle threshold (Ct) values between older children and adults. Interestingly, children younger than 5 years of age presenting even lower Ct levels than the other age groups.[3] It is important to note that this analysis did not take into account time of symptom onset (SO) to the actual testing of the subjects, a possible confounder that also needs to be addressed. An even larger sample size study in Switzerland has shown similar results.[5]

The viral load of SARS-CoV-2 can be indirectly measured using the Ct value of automated RT-PCR techniques, and its value is inversely proportional to the viral load. Ct value is defined as the number of cycles necessary to amplify viral RNA to reach a detectable level, which is a positive fluorescent amplification signal in a real-time reverse transcription polymerase chain reaction (RT-PCR) assay.[6,7] Pujadas et al[8] suggested the use of viral load to identify severe patients and to develop predictive algorithms.

Although the role of children in SARS-CoV-2 transmission is not completely understood, it is clear that they have been spared of severe presentations of the disease.[3,9] However, many differences among different age groups remain unclear.[10] In this study, we report the viral RNA Ct values patterns observed during the early phase of infection in a cohort of patients with confirmed SARS-CoV-2 infection and have these Ct values compared

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between children and adults, also taking into account time from SO to polymerase chain reaction testing.

MATERIALS AND METHODS

This was a prospective cross-sectional multicenter study with data collected in 2 hospitals in Brazil. From May to November 2020, we assessed outpatient subjects seen at these emergency rooms, or those hospitalized, presenting with at least one sign or symptom suggestive of coronavirus disease 2019 (cough, fever or sore throat) within 7 days of SO. Eligible patients with confirmed SARS-CoV-2 infection were included in the study. All children (from ≥2 months to <18 years of age) were compared with adults (≥18 years). To explore possible differences among childhood age groups, participants less than 18 years of age were subgrouped as infants (2 months to <2 years), children (≥2 to <12 years) and adolescents (≥12 to <18 years).

All participants performed RT-PCR assay for SARS-CoV-2 detection, and samples were analyzed in the Molecular Biology Laboratory at Hospital Moinhos de Vento. Procedure for sample collection involved 1 bilateral nasopharyngeal and 1 oropharyngeal swab collection. Both swabs were placed in the same transport medium with saline solution and RNAlater, RNA Stabilization Solution (Catalog number AM7021; Life Technologies, Carlsbad, CA). RNA was extracted using MagMax Viral/Pathogenic Nucleic Acid Isolation (Catalog number A48310; Applied Biosystems, Austin, TX) in the KingFisher Duo Prime System platform (ThermoFisher Scientific, Waltham, MA). The RT-PCR assay was performed using Path 1-Step RT-qPCR Master Mix, CG (catalog number A15299; AppliedBiosystems, Frederick, MD) and TaqMan 2019-nCoV Assay Kit v1 (catalog number A47532; ThermoFisher Scientific, Pleasanton, CA) in 10 μL total reaction, of which 5 μL were RNA. For reaction control, we used 5 μL (200 copies/μL) of TaqMan 2019-nCoV Control Kit v1 (catalog number A47533; ThermoFisher Scientific, Pleasanton, CA). Due to the possibility of the S dropout, since October 7, the RT-PCR assays were performed using only for *N* and *ORF1ab* SARSCoV-2–specific targets.

We have excluded participants with clinical conditions that compromised the immune system that could potentially interfere with viral load, such as type 1 or 2 diabetes, previous organ transplant, cancer diagnosis or subjects who had chemotherapy in the 2 weeks previous to enrollment.

We included in these analyses only participants within the first 7 days of SO, because the peak of infectivity largely occurs in the first week of illness.[11] All the 3 probes (*ORF1ab*, *N*, *S* gene targets) and the median of these were compared as surrogates of viral load. Data normality assumptions were verified for continuous variables. Percentages were used to describe categorical variables; continuous variables were summarized in terms of median and interquartile range (IQR). Two-tailed Mann–Whitney–Wilcoxon test or two-tailed Kruskal–Wallis test followed by Benjamini–Hochberg correction for multiple comparisons was used to compare Ct values of *ORF1ab, S* and *N* SARS-CoV-2–specific targets between groups. The main analysis considered the Ct values between all children versus adults, and a secondary analysis compared the Ct values among infants, children, adolescents and adults. A sensitivity analysis was performed excluding hospitalized participants. All data preprocessing and analyses were performed in R 3.5.0 statistical software (R Core Team, 2017, https://www.R-project.org).[12]

The study was performed in accordance with the declaration of Helsinki and Good Clinical Practice Guidelines, after approval by the Hospital Moinhos de Vento Institutional Review Board (IRB number 30749720.4.1001.5330) submitted April 14, 2020 and a decision made April 17, 2020 (decision number 3.977.144) with approval. All participants included in this study provided either a written informed consent or a legal responsible provided written informed consent.

RESULTS

In the study, 1823 subjects were originally screened, 1113 were excluded because of a negative SARS-CoV-2 RT-PCR result and 334 excluded for other reasons, as shown in Figure 1. There were 376 participants with confirmed SARS-CoV-2 infection; among these, 83 (22.1%) were children (from ≥2 months to

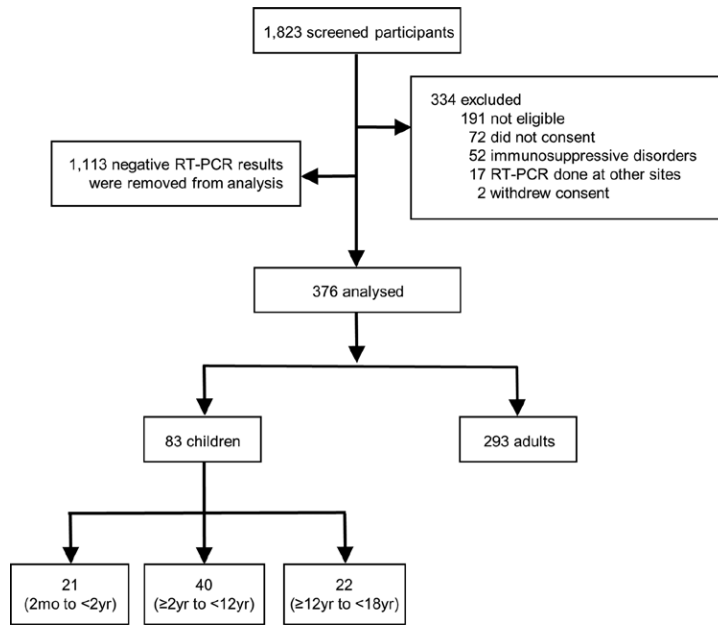

Figure 1. Study subjects' flowchart. yr indicates years.

<18 years of age) and 293 (77.9%) were adults. The median age and IQR were 6.8 (2.0–12.5) and 38.0 (29.9–46.6) years, respectively. No difference was found between the Ct values compared with these age groups, as shown in Figure 2. Regarding the age of the participants were enrolled 21 infants (5.6%), 40 children (10.6%), 22 adolescents (5.9%) and 293 adults (77.9%). The median age and IQR of infants, children, adolescents and adults were 1.2 (0.6–1.7), 6.7 (3.4–9.7), 14.3 (13.1–15.8) and 38.0 (29.9–46.6), respectively. Among adults, 10.2% (30/293) required hospitalization, while almost all infants and children had only mild clinical symptoms with only 1 child needing admission without need of supplemental oxygen and not progressing for a severe outcome. All infants (21/376, 5.6%) were outpatients, whereas among children, adolescents and adults, 324 of 376 (86.2%) participants were seen as outpatients; 31 of 376 (8.2%) were hospitalized (including adults and 1 child). From these 31 participants, 28 were admitted to general ward and 3 to ICU (all adults), respectively.

For the initial analysis, participants were split in 2 groups, of <18 and ≥18 years of age, to compare overall children and adult Ct values. The Ct values for children (<18 years) and adults were 20.73 and 20.83 ($P = 0.453$), respectively, for all gene targets (Fig. 2). When we stratified infants (2 months to <2 years), children (≥2 to <12 years), adolescents (≥12 to <18 years) and adults (≥18 years), the Ct values considering all the gene targets were 18.11, 20.61, 22.40 and 20.84, respectively ($P = 0.239$), as shown in Figure 3, indicating that those under 18 years have equal Ct values than adults.

Median of SO to the time of sample collection was lower in infants (2 days; IQR, 1.0–3.0) and children (2 days; IQR, 1.0–3.2) when compared with adolescents (3 days; IQR, 1.2–3.0) and adults (3 days; IQR, 2.0–4.0) with $P = 0.001$. Demographic and baseline clinic details are presented in Supplementary Table 1 (Supplemental Digital Content 2, http://links.lww.com/INF/E508).

A sensitivity analysis was performed excluding hospitalized participants, and no difference was observed regarding Ct values among all age groups ($P = 0.285$, for all gene targets) (Supplementary Fig. 1, Supplemental Digital Content 1, http://links.lww.com/INF/E507).

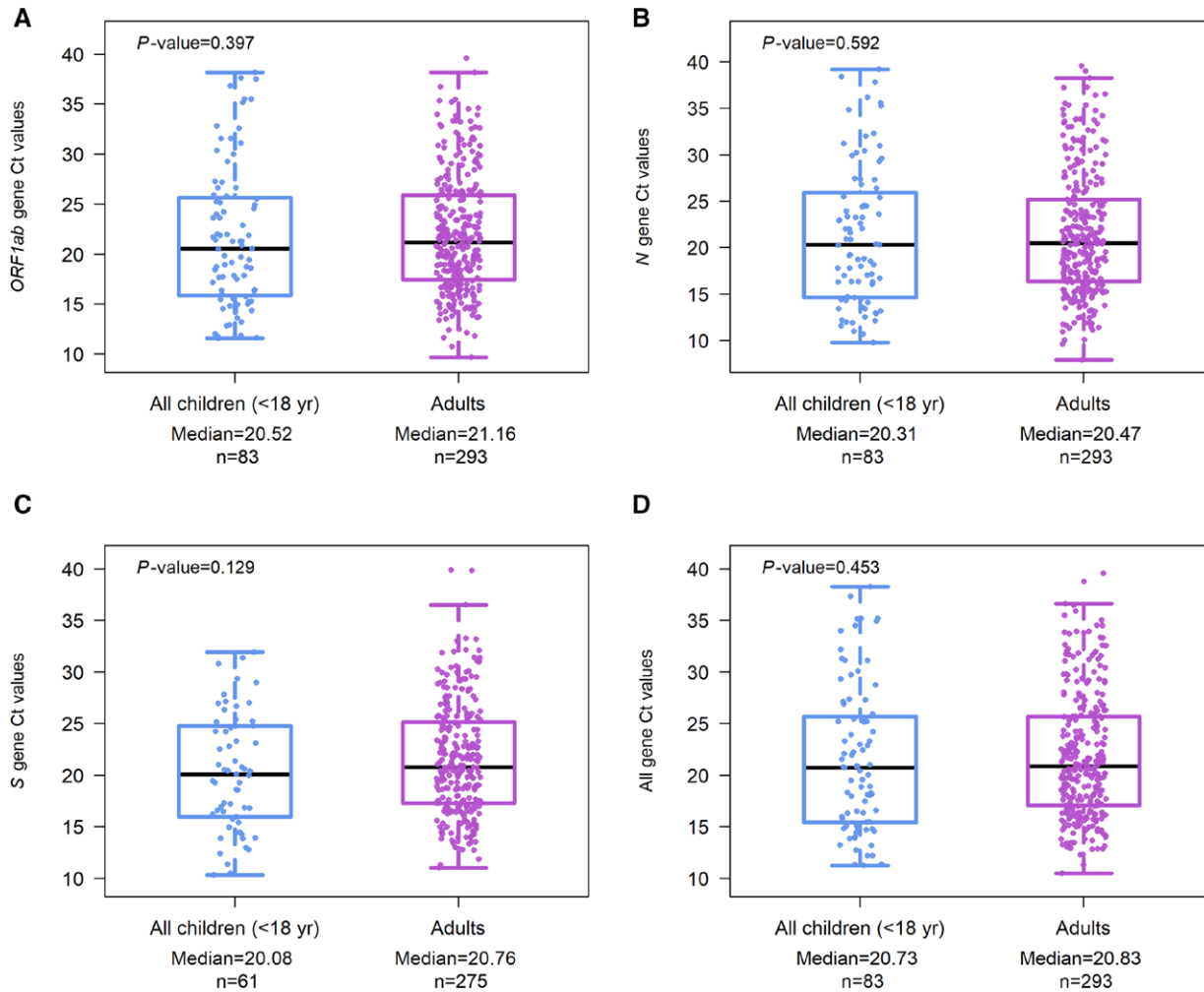

Figure 2. Comparison of Ct values in those of 2 months to <18 years versus adults with confirmed SARS-CoV-2 infection. A Ct values of *ORF1ab* gene. B: Ct values of *N* gene. C: Ct values of *S* gene. D: Ct values of the mean of *ORF1ab, N* and *S* genes. Median is represented as a solid black line, interquartile ranges are represented by boxes, upper and lower adjacent values are represented by whiskers, and outliers are represented by isolated points. yr indicates years.

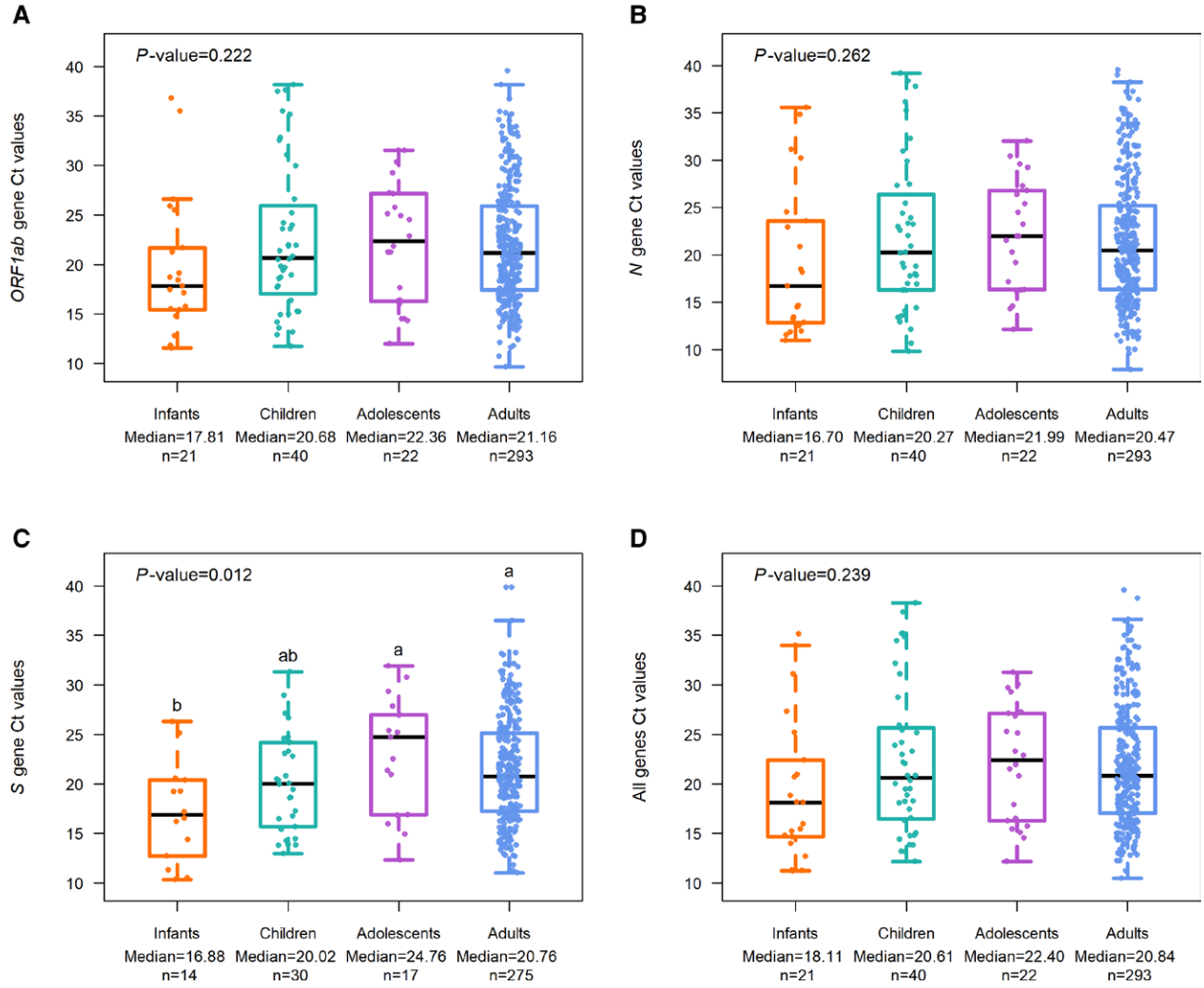

Figure 3. Comparison of Ct values according to age stratification among participants with confirmed SARS-CoV-2 infection. A: Ct values of *ORF1ab* gene. B: Ct values of *N* gene. C: Ct values of S gene. D: Ct values of the mean of *ORF1ab, N* and *S* genes. Median is represented as a solid black line, interquartile ranges are represented by boxes, upper and lower adjacent values are represented by whiskers, and outliers are represented by isolated points.

DISCUSSION

Our findings reinforce previous findings suggesting that children do not have lower SARS-CoV-2 viral loads compared with adults.[13] Furthermore, no differences were found among different childhood age groups.

As suggested by Yonker et al,[4] children could carry higher viral loads even with mild symptoms when a sample is obtained in an early infection phase. Another study also pointed out to the fact that children younger than 5 years of age presented higher viral load when compared with older children and adults.[3] However, recent viral load comparisons across age groups have yielded inconsistent conclusions.[5,13] Some studies evaluating adults showed only show a positive correlation between higher SARS-CoV-2 viral load with disease severity, need of mechanical ventilation and/or higher mortality rates, which is not the case in children.[14] The reasons for such differences in the relation of viral load and severity across age groups are not yet fully understood[14] but could well be related to different testing times in relation to SO.

Although viral load may not be the only determinant of transmission of SARS-CoV-2, it is probably one of the most important factors. On the other hand, as children usually present with a milder form of disease, the lower frequency of cough and the generation of droplets and aerosols may have some impact in reducing the risk of aerosolized transmission. However, despite the usual milder disease in children, our findings highlight that efforts to mitigate transmission should include this age group, because we did not find any difference in Ct levels between overall children and adults. The finding of (at least) similar viral load in infants is of some concern, because most current guidelines do not recommend that this age group should wear face masks in public places.[15,16]

In the context of SARS-CoV-2 variants, the B.1.1.28 and P.1 lineages are highly found in Southern Brazil[17,18] and harbor important mutations in the virus spike protein. The emergence of B.1.1.28 lineage was described in November 2020[18], while the P.1 (which derives from B.1.1.28) was first described in late January 2021[19]. Although our enrollment was completed in early November, it is unknown if these variants were present in our samples.

Our study has some limitations worth mentioning. We have included only symptomatic participants during the early stage of SARS-CoV-2 infection, meaning we cannot draw conclusions about asymptomatic individuals or those presenting later in the course of the disease. Another limitation was a restricted sample size of infected children. Social distancing, closed schools and day-care centers, together with the concern to take children to emergency rooms, have led to a sharp reduction in the overall number of pediatric consultations. Therefore, our sample could be underpowered to detect greater differences between age groups. It also raises an important issue of undiagnosed pauci symptomatic or asymptomatic children spreading the virus in the community.[5,20,21] Also, the quality and volume of viral RNA on collected swabs could have varied depending on how the sample collection was conducted.[22] We have not corrected our samples for the amount of viral RNA, because it would only add another step to the diagnostic routine, which was already overstretched at that point of the pandemic. But, it is well known that Ct values can be affected by a batch effect,[23] because variations among different runs can occur.

Despite these limitations, our findings suggest that symptomatic children may play a role in SARS-CoV-2 transmission because they harbor viral loads that are not lower than adults. Our findings highlight that in earlier stages of disease, there is a greater chance of higher viral load and possible higher risk of transmission. Our data indicate that the return of school for children and day-care activities for the younger ones must be made with caution because of the impact in spreading the infection by these groups, who do not yet have a vaccination perspective.

Our findings suggest that symptomatic children have equivalent Ct values when compared with adults, and even more interestingly higher viral loads seem to be great very early in the disease, even in the presence of mild clinical presentation.

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