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Virological Characteristics of Hospitalized Children With SARS-CoV-2 Infection

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Abbreviations: SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2, VL – viral load, RNA – ribonucleic acid, RT-PCR – reverse transcriptase polymerase chain reaction, MIS-C - multi-system inflammatory disorder, KD - Kawasaki disease, TSS - toxic shock syndrome, ACE – angiotensin converting enzyme, NP – nasopharyngeal, N – nasal, S – saliva, R – rectal, COA – Children's of Alabama, COVID-19 – coronavirus disease 2019, EMR – electronic medical record, Ct – cycle threshold, CXR – chest X ray, DKA – diabetic ketoacidosis

Table of Contents Summary: SARS-CoV-2 viral load in the respiratory tract is significantly higher in children < 1 year and in those with symptomatic disease.

What is known on this subject: Data on clinical characteristics of children with SARS-CoV-2 is widely available compared to limited information on virological characteristics, particularly in children with asymptomatic and mild infections.

What this study adds: Children with COVID-19 are predominantly asymptomatic or have mild illness despite high viral load (VL) levels in the respiratory tract and at other sites, irrespective of age, severity of illness and underlying co-morbidities.

Contributors' Statement Page:

Dr. Pinninti designed the study, carried out the initial data analysis, drafted the initial manuscript, and reviewed and revised the manuscript.
Dr. Boppana conceptualized and designed the study, analyzed the data and critically reviewed the manuscript for important intellectual content.
Misty Latting and Drs. Pinninti, Arora and Boppana were responsible for patient enrollment in the study.
April Yarbrough and Drs. Pinninti and Poole were responsible for data collection and database management.
Drs. Britt, Seleme and Pati were responsible for laboratory assay development, validation and performance.

All authors reviewed and revised the manuscript and approve of the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Background and Objectives: In children with SARS-CoV-2 infection, virological characteristics and correlation with disease severity has not been extensively studied. The primary objective of this study is to determine the correlation between SARS-CoV-2 viral load (VL) in infected children with age, disease severity and underlying co-morbidities.

Methods: Children <21 years, screened for SARS-CoV-2 at the time of hospitalization, who tested positive by polymerase chain reaction (PCR) were included in this study. VL at different sites was determined and compared between groups.

Results: Of the 102 children included in this study, 44% of the cohort had asymptomatic infection and children with >1 co-morbidities were most at-risk for severe disease. VL in children with symptomatic infection was significantly higher than in children with asymptomatic infection ($3.0x10^5$ copies/ml vs $7.2x10^3$ copies/mL; $p = 0.001$). VL in the respiratory tract was significantly higher in children <1 year compared to older children (($3.3x10^7$ copies/mL vs $1.3x10^4$ copies/mL respectively; $p < 0.0001$) despite most infants presenting with milder illness. Besides the respiratory tract, SARS-CoV-2 RNA was also detectable in samples from the gastrointestinal tract (saliva and rectum) and blood. In 13 children for whom data on duration of PCR positivity was available, 12/13 tested positive 2 weeks after initial diagnosis and 6/13 continued to test positive 4 weeks after initial diagnosis.

Conclusions: In hospitalized children with SARS-CoV-2, those with >1 co-morbid condition experienced severe disease. SARS-CoV-2 VL in the respiratory tract is significantly higher in children with symptomatic disease and in children <1 year of age.

Background

Infections caused by SARS-CoV-2 have been reported from 192 countries with > 107 million individuals infected worldwide and responsible for > 2 million deaths to date[1]. The spectrum of illness in adults has ranged from mild upper respiratory tract symptoms to multi-system involvement (severe lower respiratory tract, cardiac, renal, thrombotic and neurologic) with significant morbidity and mortality[2-7], particularly in individuals with underlying risk factors[8-11]. Interestingly, most infected children either lack symptoms (asymptomatic infection) or experience mild disease[12-15] while few experience either a severe lower respiratory tract infection or, multi-system inflammatory disorder (MIS-C), with overlapping features of Kawasaki disease (KD) and toxic shock syndrome (TSS)[16-19].

The reasons for the distinctly different clinical presentations and outcomes between adults and children with SARS-CoV-2 infection are largely unknown. Some of the hypotheses proposed to explain these differences include: 1) underexpression of ACE-2, the binding receptor for SARS-CoV-2 spike (S) protein in children, 2) lower respiratory tract viral load (VL) levels in children compared to adults, and 3) pre-existing cross-reactive immunity conferred by exposure to seasonal coronaviruses[20-22]. Pediatric data so far has focused predominantly on the description of demographic and clinical characteristics of hospitalized children with SARS-CoV-2 [14, 23, 24], with very limited information on virological characteristics[25, 26], particularly in children with asymptomatic and mild infections. Defining the virological characteristics of children with SARS-CoV-2 infection is important to facilitate identification of biomarkers of severe infection and adverse outcomes, understand transmission dynamics within families and communities and for development of effective management and prevention strategies. The objectives of this study

are to describe the virological characteristics of hospitalized children with SARS-CoV-2 and examine the relationship of VL with age, disease severity and underlying co-morbidities.

Methods

Subjects and Specimens: The study cohort consists of 102 children < 21 years evaluated and/or admitted to the Children's of Alabama (COA) and tested positive for SARS-CoV-2 RNA by reverse transcription polymerase chain reaction (RT-PCR) performed on nasopharyngeal (NP) samples between March 24 and August 20, 2020. Between March 24 and April 26, 2020, SARS-CoV-2 testing was only performed on hospitalized children suspected to have COVID-19 based on symptoms and exposure. Screening of all hospitalized children and those scheduled for elective procedures was initiated on April 27, 2020. NP swabs for SARS-CoV-2 PCR were collected by trained personnel and children who tested SARS-CoV-2 PCR positive were approached for collection of additional swabs (mid-turbinate nasal, saliva, rectal) and whole blood. Of the 102 children included in this study, 61.7% (63/102) consented for additional sample collection.

Specimen processing and laboratory analysis: NP, nasal, saliva, rectal swabs and blood were collected by trained medical staff (respiratory therapists/nurses/physicians/phlebotomists) as described in the Supplement. The swabs were placed in viral transport media (VTM) and processed within 24 hours or stored at -80ºC. The details of collection, processing, and analysis of samples and data extraction from EMR are provided in the Supplement and in a previous publication[27]. Briefly, RT-PCR was performed for the detection of SARS-CoV-2 RNA and a specimen was considered positive if one or more copies per reaction were detected before 40 PCR cycles. Quantitation of VL was accomplished by generating a standard curve based on dilutions of known SARS-CoV-2 genomic RNA and results expressed as copies/ml of VTM. A

strong inverse correlation between cycle threshold (Ct) and VL was observed ($r = -1$, $p < 0.0004$; Figure 1). The study was approved by the Institutional Review Board (IRB) for Human Use and an informed consent was obtained from all study participants or their legally authorized representatives.

The cohort was categorized based on age, disease severity, and comorbidities as follows:

Age: 1) < 1 year, 2) 1-5 years, 3) 6-17 years, 4) 18-21 years.

Disease Severity: Based on published data[28-30], the cohort was categorized as: 1) Asymptomatic (no clinical signs or symptoms attributable to COVID-19) 2) Mild (fever or chills, cough, nasal congestion or runny nose, new loss of taste or smell, sore throat, difficulty breathing ± non-invasive supplemental oxygenation (nasal cannula), diarrhea, nausea or vomiting, abdominal pain, fatigue, headache, myalgias, poor appetite or poor feeding), 3) Moderate-severe illness (pneumonia with hypoxemia requiring ventilatory support, ± abnormal chest imaging, respiratory failure, shock or multi-organ dysfunction). Children who tested negative for SARS-CoV-2 RNA at the time of hospital admission with a COVID-related illness (MIS-C) were excluded from this study.

Co-morbidities: The cohort was divided into groups with zero, one or >1 co-morbid condition for comparing VL and disease severity.

Statistical Analysis: The frequency of PCR positivity for each sample type was determined and compared among study children of different age groups (< 1yr, 1-5 years, 6-17 years and 18-21 years) and with varying degrees of disease severity. Continuous variables were compared by Kruskal-Wallis test. Fisher's exact test was used to compare categorical variables. Statistical significance between outcomes was assessed by Mann-Whitney U test and Spearman rank test

was used to determine correlation between variables. GraphPad Prism 8 was used for statistical analysis and create figures.

Results

Demographic Characteristics: Between March 24 and August 20, 2020, 102 patients < 21 years, who tested positive for SARS-CoV-2 by PCR and were either hospitalized (91%, 93/102) or screened prior to procedures (8.8%, 9/102) were included. The mean age of the study children was 9.8 years (±6.6 years) and about half were female (49/102). Race and ethnicity composition of the study cohort consisted of 41% black non-Hispanic, 32% white non-Hispanic, and 27% white Hispanic children (Table 1).

Clinical Findings: Forty four percent (45/102) were categorized as asymptomatic while 44% (45/102) and 11.7% (12/102) as mild and moderate-severe disease, respectively (Table 1). Of those with symptoms, 72% (41/57) reported fever and 40.3% (23/57) reported cough at initial presentation while most reported non-specific symptoms like abdominal pain, headache and fatigue. Of the 93 hospitalized children, more than a third (34/93, 36.5%) reported close contact with an individual diagnosed with COVID-19, and 19.3% (18/93) required invasive or non-invasive ventilatory support. Radiological imaging of chest was not routinely obtained in all infected children, but more than half of the children in whom a chest X ray was obtained had bilateral patchy opacities suggestive of multi-focal pneumonia (Table 1).

Of the 53% children with underlying co-morbidities, 35.3% reported one co-morbid condition and 17.6% had >1 co-morbidity with obesity most frequently reported in 19.6% (20/102) of children. Other notable co-morbidities were type-1 or type-2 diabetes mellitus in 13.7% and an underlying hematological disorder (HbSS, HbSC or Fanconi's anemia) in 7.8%. Children with

co-morbidities were no more at-risk of developing moderate-severe disease compared to those without underlying co-morbidities (p=0.14). The median hospital stay was significantly longer for children with moderate-severe infection compared to children with asymptomatic or mild infection (15 days vs 1-day vs 3 days respectively; p<0.0001).

Virological Characteristics:

Viral load by age: Comparison of NP VL between different age groups showed significantly higher VL levels in children <1 year than all other age groups (p=0.0004). The median VL in children <1 year was $3.3x10^7$ copies/mL (median Ct - 17) compared to $9.3x10^3$ copies/mL (median Ct - 33) in the 1-5 years age group, $1.2x10^4$ copies/mL (Ct - 33) in the 6-17 years group and $1.4x10^5$ copies/mL (Ct - 28) in the 18-21 years group (Figure 2, panel A). Children <1 year had significantly higher median VL than the rest of the cohort ($3.3x10^7$ copies/mL (Ct - 17) vs $1.3x10^4$ copies/mL (Ct - 33) respectively; p<0.0001; Figure 2, panel B). Of note, except for two neonates with severe illness, remaining children in the <1 year age group presented with either mild illness or were asymptomatic.

VL by disease severity: Asymptomatic children ($7.2x10^3$ copies/mL; median Ct - 34) had significantly lower median VL than those with symptomatic infection ($3.0x10^5$ copies/mL; median Ct -26; p=0.001) (Figure 3, panel A). This difference in VL between the groups persisted even after exclusion of children <1 year, who predominantly presented with asymptomatic or mild disease but had high VL in the respiratory tract (p=0.02; Supplement Fig S1). Among children with symptomatic infection, there was no significant difference in the median VL between those with mild or moderate-severe disease ($2.9x10^5$ copies/mL (Ct - 26) vs $5.5.0x10^5$ copies/mL (Ct -25) respectively, p=0.5; Figure 3, panel B).

VL based on underlying co-morbidities: There was no significant difference in median VL between children with zero, one or >1 co-morbid condition ($1.5x10^4$ copies/ml (Ct - 32) vs $4.6x10^4$ copies/ml (Ct - 29) vs $6.2x10^4$ copies/ml (Ct -30), respectively; p=0.8) as shown in figure 4. Although children receiving chemotherapy or immunomodulatory treatment had higher median VL than those not receiving such treatments, the difference was not statistically significant ($2.3x10^5$ copies/ml (Ct - 27) vs $2.3x10^4$ copies/mL (Ct - 31); p=0.33).

Sites of detection and VL: In addition to NP swabs, nasal, saliva, rectal and blood samples were available from 52, 53, 26 and 24 children, respectively. Of those, 65% (34/52) of nasal swabs, 38% (20/53) of saliva swabs, 61% (16/26) of rectal swabs and 50% (12/24) of blood samples were positive for SARS-CoV-2 RNA. While samples from the respiratory tract had higher VL, there were no significant differences between the compartments (Figure 5). A comparison between paired NP and nasal samples, available in 51 children, showed no significant difference in VL (p = 0.5) with strong correlation between the two sample types (r=0.81; Figure 6, Panel A). A similar comparison between NP and saliva swabs in 53 children revealed significantly higher VL in NP swabs (p<0.001) with only a modest correlation (r=0.59; Figure 6, Panel B). A comparison of VL between paired NP and rectal swabs available in 26 children showed a significantly higher VL in NP swabs (p < 0.0001) with only a modest correlation between these sample types (r=0.61; Figure 6, Panel C), suggesting viral replication in the gastrointestinal tract may be independent of the respiratory tract involvement. A similar comparison between saliva and rectal swabs, available in 25 children, did not reveal significant differences in VL between the sample types (p=0.35; Figure6, Panel D).

Of the 63 children with multiple sample types, ≥3 samples were available in 52 (82.5%) children and the presence of SARS-CoV-2 RNA in ≥ 1 site was not associated with symptomatic

infection or disease severity (p=0.18). In 24 children from whom blood samples were available, 50% (12 children) were viremic; two with asymptomatic infection, one with mild and nine with mod-severe infection with no significant difference in VL between the groups.

Duration of Shedding: Data for shedding duration beyond 2-weeks and 4-weeks from the initial diagnosis was only available in 13 children from this cohort who were hospitalized beyond 2 weeks or followed as outpatients. All except one newborn had underlying co-morbid conditions with half of this cohort (6/12) receiving chemotherapy/immunomodulatory treatment. Most children (12/13) continued to test positive for SARS-CoV-2 RNA in NP swabs at 2 weeks and 6/13 (46%) were PCR positive beyond 4 weeks with decreasing VL in samples obtained serially (figure S2). The detection of viral RNA at 2 and 4 weeks was not associated with symptomatic disease or severity of infection at initial presentation.

Discussion

In this cohort of mostly hospitalized children with SARS-CoV-2 infection, 44% of the study children were asymptomatic and the majority of those with symptomatic infection had non-specific findings at the time of presentation. We document high VL in the respiratory tract in children with symptomatic infection and in infants. While children with >1 co-morbid condition had a higher risk for development of severe disease, we did not see an association between SARS-CoV-2 VL in the respiratory tract and the severity of illness. In half of the children from whom blood samples were available, we document SARS-CoV-2 viremia, suggesting disseminated infection. The finding of viral RNA in samples from the gastrointestinal tract (saliva and rectal), suggesting independent viral replication at these sites in children who were tested has implications for the spread of infection through routes other than respiratory tract. Additionally, in a smaller group of children with underlying hematological disorders or in those

receiving chemotherapy or immunomodulatory treatment, it is not uncommon for the persistence of SARS-CoV-2 RNA beyond 2 weeks.

Despite worldwide spread of SARS-CoV-2, the incidence rates in children have continued to be low with substantially lower morbidity and mortality compared to adults. While the initial reports of COVID-19 from China included limited pediatric data, subsequent reports have focused on clinical and demographic factors of SARS-CoV-2 infections in children, however, with limited information on virological characteristics[13-15, 28, 31-33].

Contrary to the belief that children are less likely to spread the infection, we document high VL in the respiratory tract in children, with significantly higher levels in children with symptoms compared to asymptomatic children, similar to recently published reports[34]. Data from small cohorts of adults admitted to ICU's have suggested an association between high respiratory tract SARS-CoV-2 VL and the severity of illness or risk of progression in severe COVID-19[35-37]. However, we did not find an association between VL in the respiratory tract and severity of illness, possibly due to the inclusion of more children with asymptomatic and milder illnesses in this study. A major strength of this study is that almost half of the cohort is asymptomatic and identified by screening of all hospitalized children for SARS-CoV-2, thus providing a better description of virologic characteristics in children who were underrepresented in previous studies. Another strength of our study is the availability of samples other than NP swabs in about two-thirds of the study children.

An intriguing finding in this study is the presence of significantly high VL in the respiratory tract of children <1 year with predominantly asymptomatic or mild disease corroborating findings from a recent report that documented high VL in children <5 years compared to older children and adults[25]. However, that study did not include VL information in infants, or VL as a correlate

of disease severity. We speculate that passively transferred maternal antibodies against seasonal coronaviruses may provide cross-reactive protective immunity, leading to a lower severity of illness in infants despite high VL.

Our findings, together with other reports contradict the belief that young children are less susceptible to SARS-CoV-2 infection or do not significantly contribute to SARS-CoV-2 transmission[38-40]. However, one of the shortfalls of this study is that we did not examine the transmission dynamics of SARS-CoV-2 within the families or communities of children enrolled in this study. While the discordance between VL levels and disease severity needs further study, this observation suggests that a vigorous immune response and the resulting hyper inflammatory state in older children and adults may play a role in the severity of SARS-CoV-2 infection.

In this study, we also present evidence of gastrointestinal involvement with detection of SARS-CoV-2 RNA in saliva and rectal swabs in majority of children from whom samples were available with VL comparable to that in the respiratory tract. While the testing of NP swabs for SARS-CoV-2 RNA is currently considered the standard for diagnosis, we show a strong correlation between NP and nasal swab VL during acute infection. However, the finding of significantly lower VL in saliva and rectal samples suggests that viral replication in the gastrointestinal tract is independent of the respiratory tract. However, the lower VL in the gastrointestinal tract suggests that saliva and rectal swabs are not appropriate samples for the identification of children with COVID-19. Although we did not carry out cell culture experiments to recover infectious SARS-CoV-2 in these specimens (NP, nasal, saliva and rectal), higher RNA levels have been associated with an ability to recover the virus[41], suggesting that young children in the acute phase of infection could spread the virus to contacts through

activities such as feeding and diaper changes with implications for infection control practices not just during hospitalization, but also at home, childcare settings, and school settings.

Viremia, suggesting systemic infection/dissemination, has been reported in patients with SARS-CoV-2 but the significance of this finding remains unclear[42]. Although blood samples were obtained from only 25% of the children in this cohort, we document viremia in 50% of samples analyzed. There was no association between viremia and severity of illness at initial presentation, but the small sample size limits the value of this finding. The documentation of viremia during acute infection could be of significance because of the emergence of MIS-C in some infected children during the convalescent phase[17-19] and findings of adults with cardiac involvement on follow-up after COVID-19[43, 44], highlighting the need for prospective follow-up to examine the role of viremia during acute infection and long-term adverse outcomes, irrespective of severity of initial illness.

While most individuals with SARS-CoV-2 infection are believed to be infectious for 10-14 days from diagnosis, studies have documented shedding duration beyond 14 days[33, 45]. We document shedding beyond 4 weeks in children receiving chemo/immunomodulatory therapy. Although it is not clear whether these children continue to shed infectious virus for prolonged periods, this finding does raise questions for infection control practices during hospitalization. However, consistent with published data, we did not document increased morbidity in children with underlying hematological and oncological conditions[46, 47].

This study is one of the few to focus on virological characteristics of SARS-CoV-2 infection in children. Since our cohort predominantly includes hospitalized children, this data might not be representative of SARS-CoV-2 infected children in the community. However, nearly half the cohort was identified because of screening of all hospitalized children, suggesting that the

findings of this study are generalizable. A major limitation of this study is the inability to correlate VL to time from infection due to inclusion of children with asymptomatic infection.

In conclusion, the findings of this study suggest that children with SARS-CoV-2 are predominantly asymptomatic or have mild illness with high VL in the respiratory tract and at other sites. In addition, significantly high VL was documented in the respiratory tract of infants compared to all other age groups. There remain a number of unanswered questions about long-term outcomes and the association between virologic characteristics and adverse clinical outcomes in children with SARS-CoV-2 infection, highlighting the need for prospective follow-up studies.

References

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis*. 2020;20(5):533-534.
2. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323(13):1239-1242.
3. Liu PP, Blet A, Smyth D, Li H. The Science Underlying COVID-19: Implications for the Cardiovascular System. Circulation. 2020;142(1):68-78.
4. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential Effects of Coronaviruses on the Cardiovascular System: A Review. *JAMA Cardiol*. 2020;5(7):831-840.
5. Pei G, Zhang Z, Peng J, et al. Renal Involvement and Early Prognosis in Patients with COVID-19 Pneumonia. *J Am Soc Nephrol*. 2020;31(6):1157-1165.
6. Bikdeli B, Madhavan MV, Jimenez D, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2020;75(23):2950-2973.
7. Paniz-Mondolfi A, Bryce C, Grimes Z, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). J Med Virol. 2020;92(7):699-702.
8. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-1720.
9. 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(10229):1054-1062.

10. Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180(7):934-943.
11. Price-Haywood EG, Burton J, Fort D, Seoane L. Hospitalization and Mortality among Black Patients and White Patients with Covid-19. *N Engl J Med*. 2020;382(26):2534-2543.
12. Shekerdemian LS, Mahmood NR, Wolfe KK, et al. Characteristics and Outcomes of Children With Coronavirus Disease 2019 (COVID-19) Infection Admitted to US and Canadian Pediatric Intensive Care Units. *JAMA Pediatr*. 2020;174(9):868-873.
13. Tagarro A, Epalza C, Santos M, et al. Screening and Severity of Coronavirus Disease 2019 (COVID-19) in Children in Madrid, Spain [published online ahead of print, 2020 Apr 8]. *JAMA Pediatr*. 2020;e201346.
14. Götzinger F, Santiago-García B, Noguera-Julián A, et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. *Lancet Child Adolesc Health*. 2020;4(9):653-661.
15. Parri N, Magistà AM, Marchetti F, et al. Characteristic of COVID-19 infection in pediatric patients: early findings from two Italian Pediatric Research Networks. *Eur J Pediatr*. 2020;179(8):1315-1323.
16. Belhadjer Z, Méot M, Bajolle F, et al. Acute Heart Failure in Multisystem Inflammatory Syndrome in Children in the Context of Global SARS-CoV-2 Pandemic. *Circulation*. 2020;142(5):429-436.
17. Dufort EM, Koumans EH, Chow EJ, et al. Multisystem Inflammatory Syndrome in Children in New York State. *N Engl J Med*. 2020;383(4):347-358.
18. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. *N Engl J Med*. 2020;383(4):334-346.
19. Moraleda C, Serna-Pascual M, Soriano-Arandes A, et al. Multi-Inflammatory Syndrome in Children related to SARS-CoV-2 in Spain [published online ahead of print, 2020 Jul 25]. *Clin Infect Dis*. 2020;ciaa1042.
20. Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science*. 2020;370(6522):1339-1343.
21. Williams PCM, Howard-Jones AR, Hsu P, et al. SARS-CoV-2 in children: spectrum of disease, transmission and immunopathological underpinnings. *Pathology*. 2020;52(7):801-808.
22. Mehta NS, Mytton OT, Mullins EWS, et al. SARS-CoV-2 (COVID-19): What Do We Know About Children? A Systematic Review. *Clin Infect Dis*. 2020;71(9):2469-2479.
23. Kainth MK, Goenka PK, Williamson KA, et al. Early Experience of COVID-19 in a US Children's Hospital. *Pediatrics*. 2020;146(4):e2020003186.
24. Goyal MK, Simpson JN, Boyle MD, et al. Racial and/or Ethnic and Socioeconomic Disparities of SARS-CoV-2 Infection Among Children. *Pediatrics*. 2020;146(4):e2020009951.
25. Heald-Sargent T, Muller WJ, Zheng X, Rippe J, Patel AB, Kociolek LK. Age-Related Differences in Nasopharyngeal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Levels in Patients With Mild to Moderate Coronavirus Disease 2019 (COVID-19). *JAMA Pediatr*. 2020;174(9):902-903.

26. Bahar B, Jacquot C, Mo YD, DeBiasi RL, Campos J, Delaney M. Kinetics of Viral Clearance and Antibody Production Across Age Groups in Children with Severe Acute Respiratory Syndrome Coronavirus 2 Infection. *J Pediatr*. 2020;227:31-37.e1.
27. Pinninti S, Trieu C, Pati SK, et al. Comparing Nasopharyngeal and Mid-Turbinate Nasal Swab Testing for the Identification of SARS-CoV-2 [published online ahead of print, 2020 Jun 29]. *Clin Infect Dis*. 2020;ciaa882.
28. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 Among Children in China. *Pediatrics*. 2020;145(6):e20200702.
29. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed February 2, 2021.
30. World Health organization. Clinical management of COVID-19. Available at https://www.who.int/publications-detail-redirect/clinical-management-of-covid-19. Accessed February 2, 2021.
31. Parri N, Lenge M, Buonsenso D; Coronavirus Infection in Pediatric Emergency Departments (CONFIDENCE) Research Group. Children with Covid-19 in Pediatric Emergency Departments in Italy. *N Engl J Med*. 2020;383(2):187-190.
32. DeBiasi RL, Song X, Delaney M, et al. Severe Coronavirus Disease-2019 in Children and Young Adults in the Washington, DC, Metropolitan Region. *J Pediatr*. 2020;223:199-203.e1.
33. Han MS, Choi EH, Chang SH, et al. Clinical Characteristics and Viral RNA Detection in Children With Coronavirus Disease 2019 in the Republic of Korea. *JAMA Pediatr*. 2021;175(1):73-80.
34. Yonker LM, Shen K, Kinane TB. Lessons unfolding from pediatric cases of COVID-19 disease caused by SARS-CoV-2 infection. *Pediatr Pulmonol*. 2020;55(5):1085-1086.
35. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465-469.
36. Huang Y, Chen S, Yang Z, et al. SARS-CoV-2 Viral Load in Clinical Samples from Critically Ill Patients. *Am J Respir Crit Care Med*. 2020;201(11):1435-1438.
37. Liu Y, Yan LM, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis*. 2020;20(6):656-657.
38. Luo Y, Trevathan E, Qian Z, et al. Asymptomatic SARS-CoV-2 Infection in Household Contacts of a Healthcare Provider, Wuhan, China. *Emerg Infect Dis*. 2020;26(8):1930-1933.
39. Zhang W, Cheng W, Luo L, et al. Secondary Transmission of Coronavirus Disease from Presymptomatic Persons, China. *Emerg Infect Dis*. 2020;26(8):1924-1926.
40. Teherani MF, Kao CM, Camacho-Gonzalez A, et al. Burden of Illness in Households With Severe Acute Respiratory Syndrome Coronavirus 2-Infected Children. *J Pediatric Infect Dis Soc*. 2020;9(5):613-616.
41. L'Huillier AG, Torriani G, Pigny F, Kaiser L, Eckerle I. Culture-Competent SARS-CoV-2 in Nasopharynx of Symptomatic Neonates, Children, and Adolescents. *Emerg Infect Dis*. 2020;26(10):2494-2497.
42. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med*. 2020;382(12):1177-1179.

43. Inciardi RM, Lupi L, Zaccone G, et al. Cardiac Involvement in a Patient With Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol*. 2020;5(7):819-824.
44. Puntmann VO, Carerj ML, Wieters I, et al. Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol*. 2020;5(11):1265-1273.
45. Lu Y, Li Y, Deng W, et al. Symptomatic Infection is Associated with Prolonged Duration of Viral Shedding in Mild Coronavirus Disease 2019: A Retrospective Study of 110 Children in Wuhan. *Pediatr Infect Dis J*. 2020;39(7):e95-e99.
46. Boulad F, Kamboj M, Bouvier N, Mauguen A, Kung AL. COVID-19 in Children With Cancer in New York City. *JAMA Oncol*. 2020;6(9):1459-1460.
47. Assaad S, Avrillon V, Fournier ML, et al. High mortality rate in cancer patients with symptoms of COVID-19 with or without detectable SARS-COV-2 on RT-PCR. *Eur J Cancer*. 2020;135:251-259.

Table 1: Demographic and Clinical Characteristics of Children with SARS-CoV-2 Infection

Characteristic	No. (%)
Mean Age	9.78 ± 6.56 years
Female	49/102 (48)
Race and Ethnicity	
Black Non-Hispanic	42/102 (41.1)
White Hispanic	26/102 (25.5)
White Non-Hispanic	33/102 (32.3)
Asian	1/102 (0.9)
Disease Severity	
Asymptomatic	45/102 (44)
Mild	45/102 (44)
Moderate-Severe	12/102 (11.7)
Non-invasive ventilation	9/93 (9.6)
Invasive ventilation	9/93 (9.6)
Co-morbidities	
None	48/102 (47)
1 co-morbid condition	36/102 (35.3)
> 1 co-morbid condition	18/102 (17.6)
Obesity	20/102 (19.6)
Endocrine (T1DM/T2DM)	14/102 (13.7%)
Hematology (HbSS, HbSC, Fanconi's)	8/102 (7.8)
Chemo/Immunomodulatory treatment	12/102 (11.7)
Pulmonary (asthma, chronic lung disease)	12/102 (11.7%)
Hypertension	4/102 (3.9)
COVID + contact	34/93 (36.5)
Symptoms at presentation	
Fever	41/57 (71.9)
Cough	23/57(40.3)
Median hospital stay	
Asymptomatic	1 day
Mild	3 days ($p<0.0001$)
Moderate-Severe	15 days
CXR obtained	36/102 (35.3)
Abnormal CXR	19/36 (52.7)

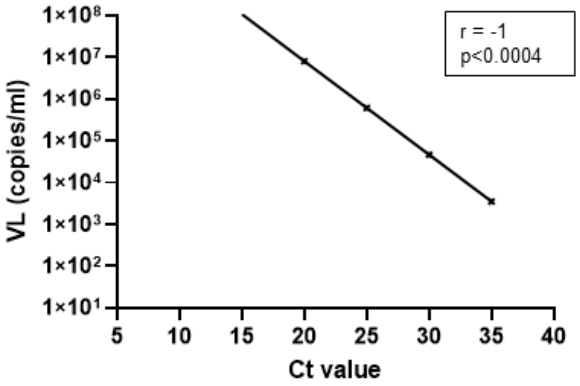
T1DM – Type-1 Diabetes Mellitus

T2DM – Type 2 Diabetes Mellitus

HbSS – Hemoglobin SS

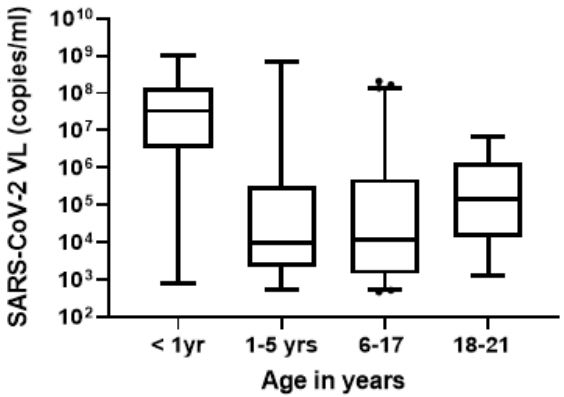
HbSC – Hemoglobin SC

CXR – Chest X ray

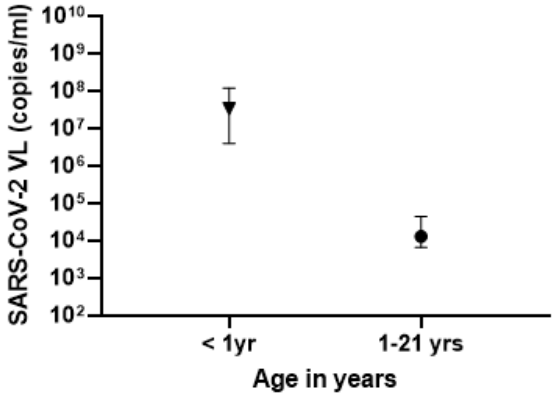


There was a strong inverse correlation between Ct value and Viral Load levels in samples obtained from children with COVID-19 (r = -1)

Figure 1: Correlation between Ct and Viral Load

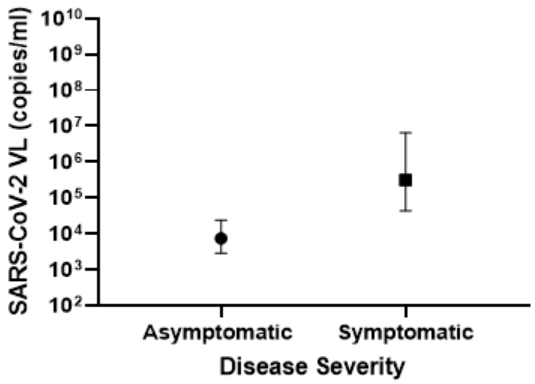


Panel A: Nasopharyngeal VL comparison in children based on age showed significantly higher VL levels in children ≤ 1 year compared to children 1-5 years (p = 0.01), 6-17 years (p=0.0002) or 18-21 years (p = 0.001). The midlines represent median and boxes represent interquartile ranges. Whiskers represent data points within 5-95th percentile for the group.

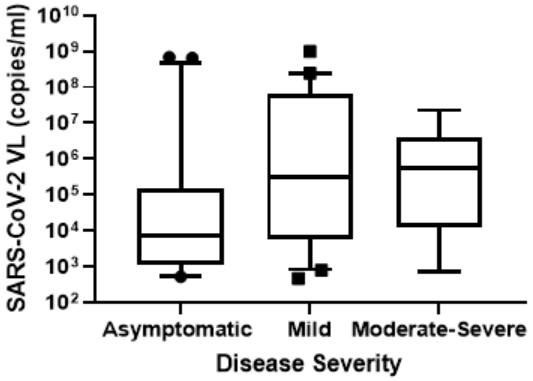


Panel B: Nasopharyngeal VL comparison between children < 1year and 1-21 years showed significantly higher VL levels in children <1 (p <0.0001). Symbol for each group represents median and whiskers represent 95% confidence interval.

Figure 2: Nasopharyngeal Viral Load comparison by age

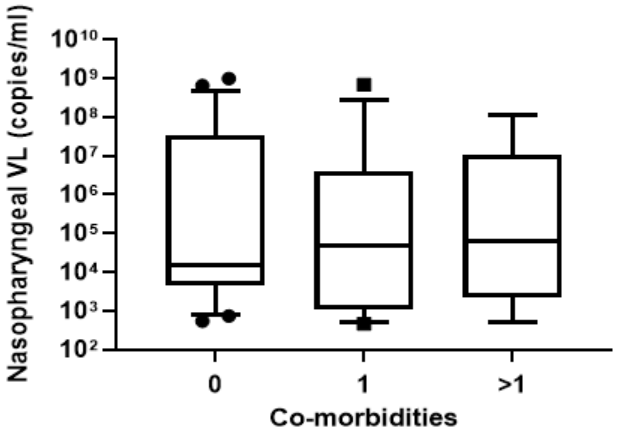


Panel A: Children with symptomatic infection had significantly higher VL than those with asymptomatic infection (p = 0.001). Symbol represents median and whiskers represent 95% confidence interval.



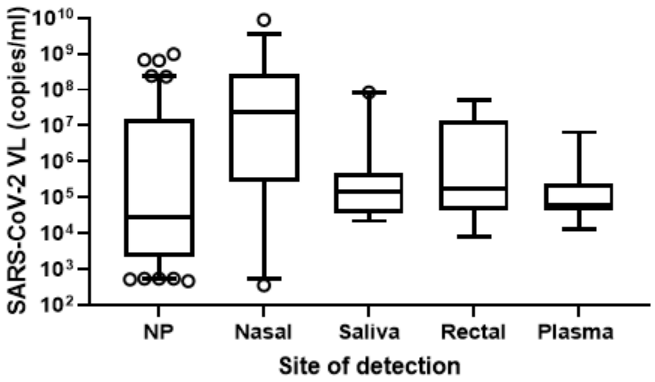
Panel B: Children with either mild or moderate-severe infection were noted to have higher VL levels compared to children with asymptomatic infection. There was no significant difference in VL levels between mild and moderate-severe disease (p = 0.9). Midlines represent median and boxes represent 95% CI. Whiskers represent 5-95th percentile for the group and outliers are represented by circles and squares.

Figure 3: Nasopharyngeal swab Viral Load comparison by Disease Severity



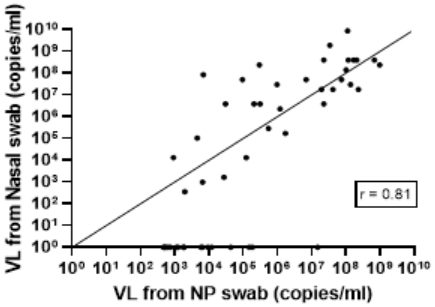
Nasopharyngeal VL comparison between groups with zero, one or > 1 underlying co-morbid conditions did not reveal a significant difference between groups (p = 0.8). Midlines represent median and boxes represent 95% CI. Whiskers represent 5-95th percentile for the group and outliers are represented by circles.

Figure 4: Nasopharyngeal Viral Load comparison by underlying co-morbidities

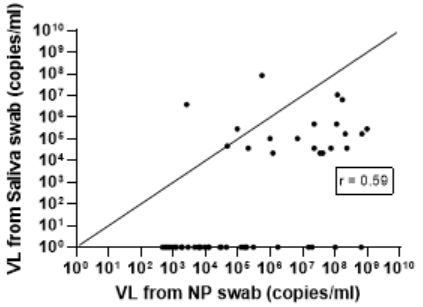


Comparison of VL at different sites did not reveal a significant difference between sites. Midlines represent median and boxes represent 95% CI. Whiskers represent 5-95th percentile for the group and outliers are represented by circles.

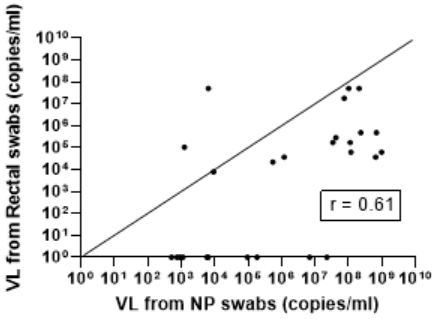
Figure 5: Viral Load comparison by site of detection



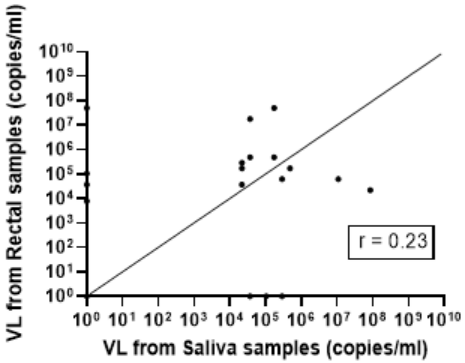
Panel A: VL comparison between paired NP and nasal swabs



Panel B: VL comparison between paired NP and saliva swabs



Panel C: VL comparison between paired NP and rectal swabs



Panel D: VL comparison between paired saliva and rectal swabs

Figure 6: Correlation between Respiratory and Gastrointestinal Samples

Supplement

Specimen Collection:

Swabs: Puritan swabs (hydraflock #25-3320-hemb 80mm) were used for NP swab collection. For collection of mid-turbinate nasal, saliva and rectal swabs, Fisherbrand polyester-tipped applicators were used (cat no. 23-400-111).

Methodology: NP specimens were obtained on the day of admission and swabs from other mucosal sites and blood were obtained within 3 days of admission to the hospital. CDC guidelines for the collection of respiratory specimens were followed for the collection of respiratory specimens as follows:

- Nasopharyngeal: The patient's head was tilted back to 70 degrees. The swab was gently through the nostril, parallel to the palate until resistance was encountered (distance equivalent to that from the ear to the nostril of the patient), indicating contact with the nasopharynx. The swab was left in place for several seconds to absorb secretions and slowly removed while rotating.

- Mid-turbinate Nasal: The patient's head was tilted back to 70 degrees. While gently rotating, the swab was inserted less than one inch (about 2 cm) into either nostril, parallel to the palate until resistance was met at the turbinates and rotated several times against the nasal wall.

- Saliva: The swab was placed in the floor of the mouth and allowed to saturate with secretions for collection of saliva swabs.

- Rectal: The swab was gently inserted 1-2 cm into the anal canal and rotated while removing the swab.

The swabs were placed in collection tubes with 3 ml of viral transport media (VTM) and processed within 24 hours (NP swabs) or stored at -80ºC. The composition of the VTM is - potassium phosphate dibasic 4.016g, potassium phosphate monobasic 2.016g, sucrose 136.9 g in 2000 ml of deionized water. The material is sterile filtered with a 0.22 micron filter and aliquoted into 500ml portions and stored at 4º C. Complete VTM is prepared by the addition of the following: 1 ml amphotericin B (250ug/ml), 1 ml vancomycin (100mg/ml in DMSO 0.2um), 5 ml fetal bovine serum and 1 ml Phenol red solution.

Blood: 2ml whole blood was collected in BD vacutainer tubes with EDTA (cat no: 368047) for separation of plasma and VL determination as follows.

Laboratory Analysis: RNA was extracted from samples using commercial spin column kits (QAmp viral RNA mini kit, Qiagen, Inc., Valencia, CA). Our laboratory developed a real-time RT-PCR assay for the detection of SARS-CoV-2 RNA is based on the CDC protocol (https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html) using reagents obtained from Integrated DNA technologies (Coralville, IA,https://www.idtdna.com/pages/landing/coronavirus-research- reagents/cdc-assays). The RT-PCR was set up using 5 µL of extracted RNA and the CDC N1 and N2 primers and probes targeting the N gene of SARS-CoV-2 using ABI 7500 real-

time PCR system (Applied Biosystems, Foster City, CA). The primer and probe nucleotide sequences include: N1 forward primer GAC CCC AAA ATC AGC GAA AT, N1 reverse primer TCT GGT TAC TGC CAG TTG AAT CTG, N1 probe FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1; N2 forward primer: TTA CAA ACA TTG GCC GCA AA, N2 reverse primer GCG CGA CAT TCC GAA GAA, and N2 probe FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1.

The RT-PCR assay was performed using the TaqPath 1-step-RT-qPCR Master Mix (Thermofisher, Waltham, MA) and 20 µL of the reaction mix contained 5 µL of Master Mix, 1.5 µL of primer/probe mix, and 5 µL of test specimen. The cycling parameters included 15 minutes at 50°C for reverse transcription, 2-minute inactivation at 95°C followed by 45 cycles of 3 seconds at 95°C and 30 seconds at 55°C. The samples were run in duplicate and each RT-PCR run also included a no-target control. In addition, a synthetic RNA standard incorporating target sequences of SARS-CoV-2 (SeraCare Life Sciences, Milford, MA) was included in each RT-PCR run as a positive control. A specimen was considered positive if one or more copies per reaction were detected in both wells by 40 PCR cycles. Viral load (VL) was quantitated by generating a standard curve based on dilutions of known SARS-CoV-2 genomic RNA obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA, Galveston, TX). The VL was expressed as copies/mL of VTM and as shown in figure S1, there was a strong inverse correlation between Ct and VL in samples. The detection limit of our RT-PCR assay was determined to be between 600 and 800 copies/mL.

EMR Data Extraction: For children who tested SARS-CoV-2 PCR positive and were included in this study, the following data was abstracted from the EMR:

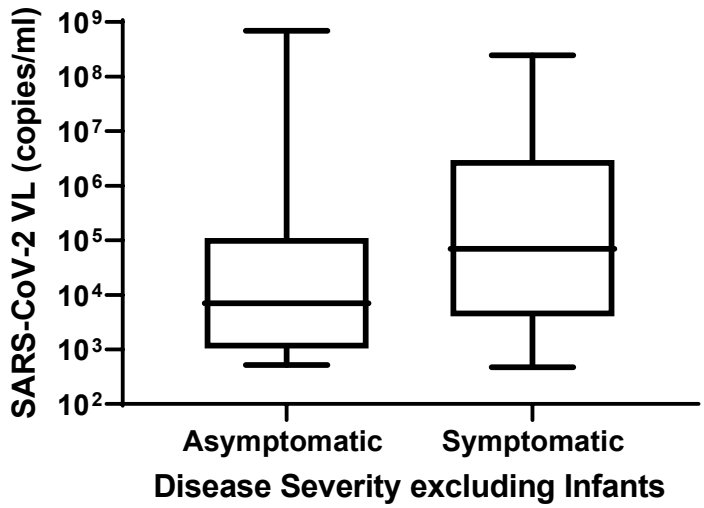
1) Demographic data: age, sex, race and ethnicity

2) Clinical data: presenting symptoms, exposure history, co-morbidities, ICU stay, oxygen requirement, requirement for invasive ventilation, duration of hospital stay, laboratory and radiological findings, admission diagnosis and co-morbid conditions was abstracted from the electronic medical records (EMR).

Co-morbidities: Co-morbid conditions that were considered significant for this study are: asthma or chronic lung disease, obesity, diabetes, hypertension, chronic kidney disease, congenital heart disease, underlying hematological (HbSS, HbSC, congenital neutropenias), children with malignancies receiving chemotherapy, hematopoietic or solid organ transplant recipients on immunosuppression, recipients of immunomodulatory therapy and children with medical complexity (children with multiple chronic conditions that affect many parts of the body who are often dependent on technology and other significant supports for daily life).

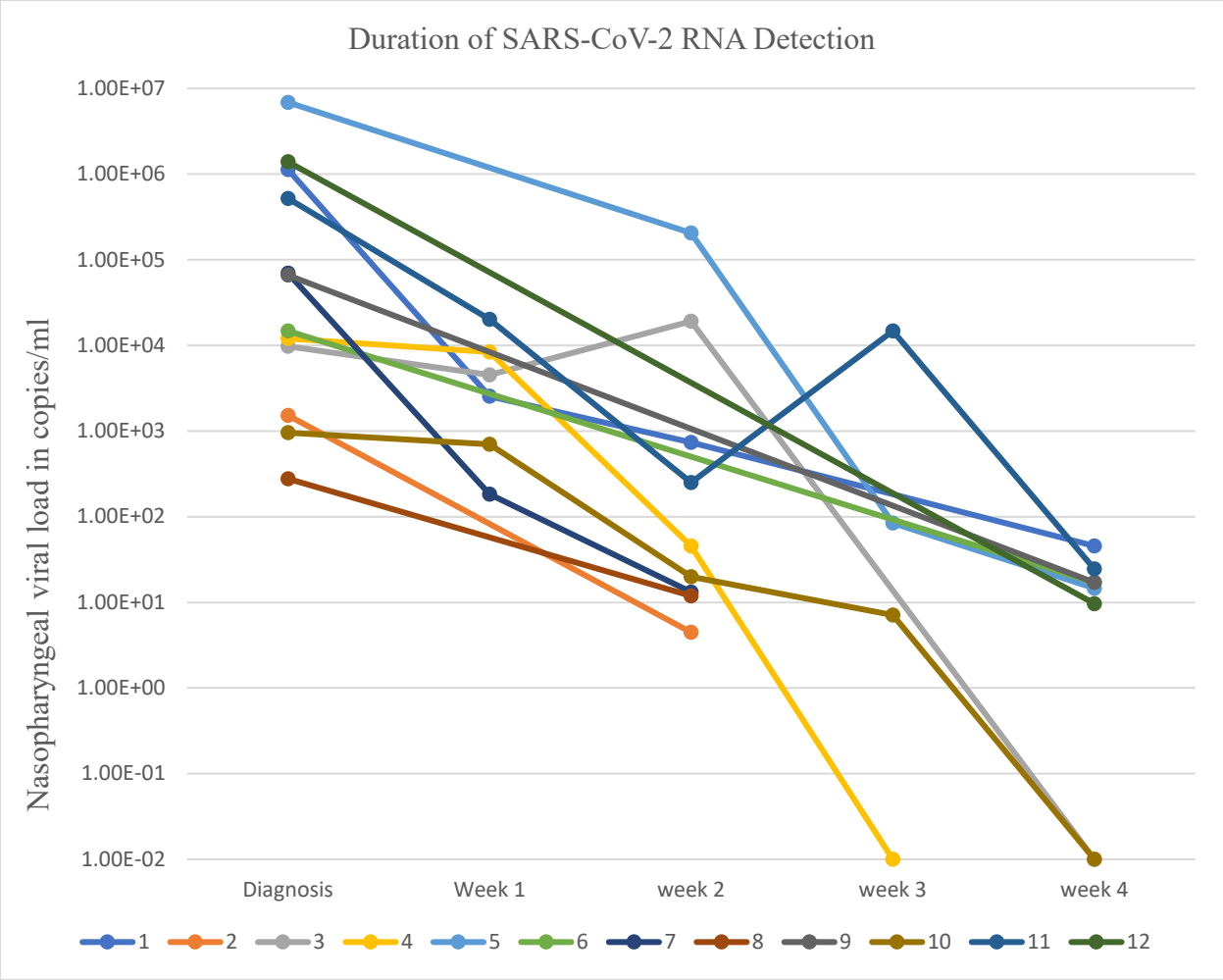
Results:

Figure S1: Nasopharyngeal swab Viral Load comparison by Disease Severity



Comparison of VL levels based on disease severity after excluding < 1year, a majority of whom presented with mild illness, showed a significant difference in VL between children with asymptomatic and symptomatic infection ($p = 0.02$). Midlines represent median and boxes represent 95% CI. Whiskers represent 5-95th percentile for the group.

Figure S2: Longitudinal detection of SARS-CoV-2 RNA



Most children (12/13) continued to test positive for SARS-CoV-2 RNA in NP swabs at 2 weeks after initial diagnosis and 6/13 (46%) were PCR positive beyond 4 weeks with decreasing VL from samples obtained serially

Table S1: Cycle threshold (Ct) values of the paired discordant samples; positive nasopharyngeal and negative mid-turbinate nasal swabs and the corresponding RNAse P PCR to ascertain sample quality. (Published in - Pinninti S, Trieu C, Pati SK, et al. Comparing Nasopharyngeal and Mid-Turbinate Nasal Swab Testing for the Identification of SARS-CoV-2. Clin Infect Dis. Jun 2020.)

Patient	Nasopharyngeal swab		Mid-turbinate Nasal (MT) swab	
	SARS-CoV-2	RNAse P	SARS-CoV-2	RNAse P
1	30	21	>40	20
2	32	18	>40	19
3	21	23	>40	20
4	28	19	>40	23
5	32	23	>40	25
6	35	17	>40	21
7	32	19	>40	23
8	33	22	>40	27
9	38	26	>40	21
10	30	21	>40	26
11	34	25	>40	20
12	32	22	>40	22
13	32	24	>40	22

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