The Laryngoscope

Examining multi-level immune response to determine prevalence of COVID-19 in pediatric tonsillectomy

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Objective: To determine the prevalence of COVID-19 in a cohort of children undergoing tonsillectomy through assessment of B cell immune responses to SARS-CoV-2 in both peripheral blood and tonsil tissue.

Methods: In this cohort study at a tertiary pediatric hospital (Children's National Hospital) in Washington, DC, we recruited 100 children undergoing tonsillectomy from late September 2020 to January 2021. Serum, peripheral blood cells, and tonsil tissue were collected and examined for immune reactivity to SARS-CoV-2. Parent-reported clinical histories were compared to antibody and B-cell responses.

Results: Among 100 children undergoing tonsillectomy, 19% had evidence of immune responses to SARS-CoV-2 (CoV2+), indicating prior COVID-19. In all seropositive participants, we detected SARS-CoV-2 specific B cells in both peripheral blood mononuclear cells and tonsils, providing evidence for tissue-specific immunity in these children. Of the 19, 63% reported no known history of COVID-19, and an additional 3 were asymptomatic or unaware of an acute infection when detected on pre-surgery screen. Hispanic children represented 74% of CoV2+ subjects compared to 37% of the full cohort. 100% of CoV2+ children lived in a zip code with poverty level >10%.

Conclusions: Nearly one-fifth of children undergoing tonsillectomy at an urban U.S. hospital had evidence of prior COVID-19 during the early pandemic, with the majority unaware of prior infection. Our results underscore the ethnic and socio-economic disparities of COVID-19. We found concordant evidence of humoral immune responses in children in both blood and tonsil tissue, providing evidence of local immune responses in the upper respiratory tract.

Key Words: COVID, SARS-CoV-2, pediatric, immune, tonsils, COVID disparity.

Level of Evidence: 3

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus, SARS-CoV-2, and includes a range of symptoms from mild upper respiratory symptoms to severe acute respiratory distress syndrome. It is known that children may be infected with no presenting symptoms.[1–3] As of April 2021, over 32 million individuals in the United States had confirmed COVID-19, with 2% reported to be in children aged 0–4 years and 10% in children aged 5–18 years.[4] The estimated SARS-CoV-2 seroprevalence up to October 2020 in children was 9.46% for the Washington Metropolitan area.[5]

In the early pandemic, prior to available vaccines and prior to widespread understanding of variants, much of the United States was in a lock-down of sorts with most schools offering only virtual options, many companies were shut-down with only essential workers presenting in person, and much of the public required masks when in a group setting. Given this unique time in our country; research leading to an improved understanding of vulnerable populations, patterns of spread, and immune responses to this novel virus seemed prudent. Acknowledging the susceptible pediatric population as well as the availability to safely obtain both blood and local immune tissue for the study of immune response in this population, we sought further understanding of SARS-CoV-2 in children undergoing adenotonsillectomy.

We hypothesized that the SARS-CoV-2 infection rate in children was underestimated due to the high asymptomatic rate in the pediatric population. We sought to determine whether participants had immune responses to SARS-CoV-2, indicating prior COVID-19, through

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identification of antibodies in serum as well as through examination of B cell responses in the tonsil, a local immune organ. We aimed to utilize this data to determine the percentage of patients who had evidence of prior COVID-19 and the characteristics of these patients.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board at Children's National Hospital (CNH) [Protocol 14454]. Patients scheduled to undergo tonsillectomy at CNH were invited to participate in the study between September 25, 2020 and January 15, 2021. We recruited 100 patients randomly over the study period with recruitment limited by resources available to process tissues on the same day as surgery. There were 251 patients undergoing adenotonsillectomy during this period. Participants were given information about the study prior to or on the date of surgery and were consented on the day of surgery by an investigator and/or research coordinator. There were no exclusions to involvement in the study, and study involvement was voluntary. The study was completed before vaccinations were available to children and prior to widespread circulation of variants such as delta or omicron. In addition, most Washington Metropolitan public schools were offering only virtual formats for learning during the time of this study. Importantly, we did not recruit based on known/unknown status of COVID-19 and did not exclude patients with known disease or PCR + result. Our goal was to reflect the entire cohort of children undergoing adenotonsillectomy during this period.

A parental questionnaire, (available in English and Spanish, see Supplement 1), querying personal history of COVID-19, results of prior testing for COVID-19, exposure to infected individuals, and symptoms that may reflect COVID-19 during the pandemic period was completed for all participants. The screening questionnaire included information on prior polymerase chain reaction (PCR) or antigen testing for SARS-CoV-2, illness in a household member along with information regarding household essential workers, number of household members, and attendance in daycare or in-person school. A provider questionnaire on patient-reported demographics, medical history, and surgical findings was obtained.

All patients were required to have a negative PCR testing for SARS-CoV-2 within 72 h before surgery. Patients with prior positive testing required surgical delay for a minimum of 21 days from positive PCR or date of the last symptom per Centers for Disease Control and Prevention (CDC) recommendations at that time.

Sample Collection

In the operating room, research blood was drawn from the intravenous line placed for anesthesia in serum separator tubes for serum collection and sodium heparin tubes for peripheral blood mononuclear cell (PBMC) extraction. Coded biologic samples were transported to and analyzed at the National Institutes of Health (NIH).

In the laboratory, serum separator tubes were spun at 1200 g for 10 minutes and serum was aliquoted and stored at −80°C. PBMCs were isolated by density gradient centrifugation (Lymphocyte Separation Medium, MP Biomedicals) the day after collection. Tonsillar tissue was stored in RPMI (Roswell Park Memorial Institute) media with 5% FBS (Fetal Bovine Serum), gentamicin 50 mg/mL, 1X antibiotic/antimycotic solution (Gibco) on ice immediately after collection. The following day, the tissue was mechanically dissociated to a single cell suspension, strained through a 100 μm cell strainer, lysed with ACK (Ammonium-Chloride-Potassium) buffer, and washed with PBS (Phosphate Buffered Saline) three times.

Serologic Assays

After thawing serum, IgG and IgM antibodies against the spike (S) protein and receptor-binding domain (RBD) of the S protein of SARS-CoV2 were analyzed using ELISA as previously described.[6,7] Positivity thresholds were based on mean optical density (absorbance) plus 3 standard deviations. The final criterion of S+ and RBD+ for any combination of positive IgG or IgM gave estimated sensitivity and specificity of 100% based on prior studies of this assay.

Flow Cytometry

Freshly isolated PBMCs and tonsillar cell suspensions were surface stained and analyzed by flow cytometry. 5 million cells per sample of PBMC or tonsillar cells were resuspended in PBS with 2% FBS and 2 mM EDTA. Fluorochrome-conjugated streptavidin was split into 5 aliquots and conjugated to biotinylated probes for Spike (S1) and RBD (BioLegend) at a molar ratio of 4:1 (probe: streptavidin) by vortexing at 4°C for 20 min with each aliquot. Cells were first stained with Live/Dead dye (ThermoFisher) for 15 min at room temperature, washed twice, and then incubated with True-Stain monocyte blocker (BioLegend) for 5 min. The antibody mix containing the rest of the surface antibodies, fluorochrome-conjugated S1 and RBD probes, and Brilliant Stain Buffer Plus (BD) was then added to the cells and incubated for 30 min at room temperature. Cells were washed three times and fixed in 1% paraformaldehyde for 20 min before acquiring on a spectral flow cytometer (Aurora, Cytek). The markers used to characterize SARS-CoV-2 antigen-specific B cells are detailed in supplement 2.

Statistical Analysis

The results of laboratory testing were returned to CNH investigators for the analysis of patient-specific risk factors for COVID-19. Baseline demographic characteristics in those with evidence of prior COVID-19 and those without were compared using the non-parametric Mann–Whitney U test for continuous variables and were summarized as medians with interquartile ranges (IQR). Binary and categorical variables were compared using the Chi-square test or Fisher's exact test (if any of the expected cell sizes was <5) and presented as frequencies with percentages.

Univariate (unadjusted) analyses were performed to compare various risk factors between two groups using Chi-square or Fisher's exact test and simple logistic regression. Multivariable (adjusted) analyses adjusted for age at surgery, gender, race/ethnicity, language, and surgical indication were performed using multivariate logistic regression. Odds ratios (OR) and adjusted odds ratios (aOR) with 95% confidence intervals were reported for the unadjusted and adjusted analyses respectively. Multicollinearity between the potential predictor variables in the multivariate regression model was assessed by checking the variance inflation factor (VIF). All statistical tests were two-sided and performed at the 0.05 level of significance. Statistical analysis was performed using R statistical software, version 4.0.10.[8]

RESULTS

Immune Findings

Of the 100 participants who were recruited (median age 5.4 years, range 1.7–18.6 years, with 44 <5 years old), 19 (19%) showed objective evidence of immune memory to SARS-CoV-2, either through serologic testing (presence of IgG/IgM to SARS-CoV-2) or presence of SARS-CoV-2-specific B cells identified in PBMC or tonsil, and were considered convalescent (CoV2+); the remaining 81 (81%)

were considered to be CoV2-(Fig. 1). Eighteen participants were seropositive, 78 were seronegative, 1 had inconclusive serologic testing, and 3 did not have serum drawn (Supplement 3). One patient, who did not have blood or serum drawn, was found to be CoV2+ based on identification of SARS-CoV-2 specific B cells in the tonsil alone; this individual also had a prior positive PCR test. The participant with indeterminate serology had no SARS-CoV-2 specific B cells in the peripheral blood and tonsil. All participants who were seropositive also had SARS-CoV-2 specific B cells in the tonsil and PBMC. Therefore, we were able to detect evidence of immune memory to SARS-CoV-2 in both blood and tissue.

History of COVID-19 PCR Testing

Of the 100 participants, 24 participants had undergone SARS-CoV-2 PCR testing prior to the preoperative screen (median age 5.7 years, range 2.8–16.4 years, with 10 <age 5 years). Most of these patients (55%) were tested because of symptoms concerning COVID-19 or exposure. Sixteen of these participants had negative PCR(s) (some with multiple tests); none of these were found to be CoV2+ in this study.

Eight participants had a prior positive PCR (33%, median age 4.4 years, range 2.9–16.4 years, with 5 that were <5 years). Of these eight, 7 were CoV2+ and showed evidence of a humoral immune response with a range of 25–201 days between the positive PCR and surgery. Five of the participants with prior positive PCR reported symptoms and all these symptomatic individuals were identified as CoV2+. The three asymptomatic participants who were PCR positive were identified through prior pre-operative screening at CNH (surgery was then delayed); none of these participants reported symptoms at the time of testing nor household members with disease. One participant who underwent surgery 21 days after their previous positive PCR had no evidence of immune responses to SARS-CoV-2 with no serum IgG or IgM detected to the virus as well as no SARS-CoV-2 specific B cells in the tonsil or PBMC (CoV2-). The other two participants were CoV2+.

An additional 12 patients were identified as CoV2+ with no known history of disease; 6 of these patients reported symptoms that may have been COVID-19 and 1 had a known exposure, although no PCR was obtained at those times. In total 19% of participants were CoV2+ in this study of which only 7 knew of prior infection.

Demographics

A comparison of the demographics of the CoV2+ and CoV2-groups is presented in Table I. Both groups were similar in terms of age at surgery, gender, indication for surgery, prior PCR testing, and reported COVID-19-related symptoms and tonsillitis during the pandemic period. However, patients who were CoV2+ were significantly more likely to identify as Hispanic (74% of CoV2+ participants versus 37% of the entire cohort, $p = 0.002$). If excluding those with a known diagnosis of COVID-19, 64% CoV2+ were Hispanic versus 33% of those without infection, $p = 0.012$.

Socio-Economic and Household-Related Factors

CoV2+ participants were also significantly more likely to reside in zip codes with a high poverty rate and with low education levels. All 19 CoV2+ patients (100%) lived in a zip code with a poverty level greater than 10% compared to only 8/81 (9.9%) CoV2-patients ($p < 0.001$). More CoV2+ patients were from areas with high school completion rate below 25% (CoV2+ 84% vs. CoV2–33%, $p < 0.001$) and college completion rates below 25% (CoV2+ 68% vs. CoV2–37%, $p = 0.013$). This significance was also seen if excluding those patients with prior PCR+ result. Parent-reported household size, number of essential workers in the household, and daycare/school attendance of the participant were not associated with CoV2+ (Table II). Thirteen participants had a known exposure to another individual with COVID-19. Of these, 7 were CoV2+ (6 had a history of + PCR and one was not previously tested). Six CoV2-participants had a known exposure, three of whom were within their households.

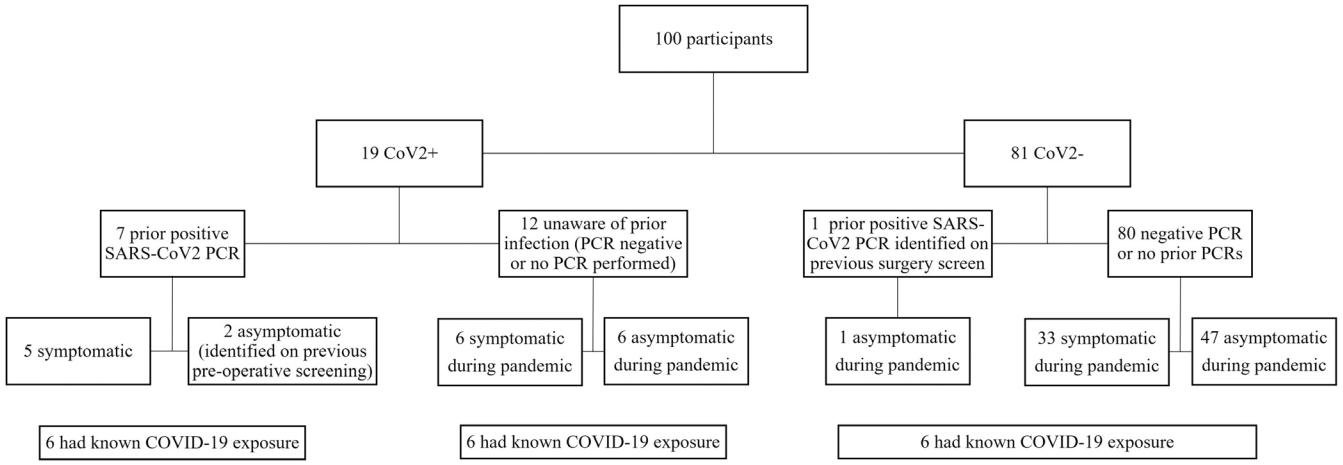

Fig. 1. Overall distribution of participants.

TABLE I.
Demographic Factors of All Participants.

Demographic factors	Total ($N = 100$)	CoV2 negative ($N = 81$)	CoV2 positive ($N = 19$)	p value*
Age at surgery (years), median (IQR)	5.3 (3.4, 9.0)	5.3 (3.3, 9.2)	6.0 (3.4, 8.0)	0.94
Female, *n* (%)	48 (48.0%)	38 (47%)	10 (53%)	0.65
Race/Ethnicity, *n* (%)				
White	24 (24%)	24 (30%)	0 (0%)	0.002
Black	26 (26%)	23 (29%)	3 (16%)	
Hispanic	37 (37%)	23 (29%)	14 (74%)	
Asian	2 (2%)	1 (1%)	1 (5%)	
Other	9 (9%)	8 (10%)	1 (5%)	
Indication, *n* (%)				
Obstruction (ATH/SDB)	55 (55.0%)	44 (54%)	11 (58%)	0.28
OSA	32 (32%)	23 (30%)	8 (42%)	
Recurrent/Chronic tonsillitis	6 (6.0%)	6 (7%)	0 (0%)	
PFAPA	7 (7.0%)	7 (9%)	0 (0%)	
History of PCR testing, *n* (%)	24 (24.0%)	16 (20%)	8 (42%)	0.040
PCR test reason†, *n* (%)				
Symptoms	12 (55%)	7 (47%)	5 (71%)	0.39
Screening	6 (27%)	4 (27%)	2 (29%)	
Exposure	4 (18%)	4 (27%)	0 (0%)	
History of PCR positive†, *n* (%)	9 (38%)	1 (6%)	8 (100%)	<0.001
Symptoms during pandemic, *n* (%)	44 (44.0%)	33 (41%)	11 (58%)	0.21
Tonsilitis during pandemic, *n* (%)	32 (32.0%)	25 (31%)	7 (37%)	0.62

*p values were obtained from Mann–Whitney U test for continuous data and Chi-square/Fisher's exact test for binary and categorical data.
†Among patients with history of PCR testing.
Abbreviations: ATH, adenotonsillar hypertrophy; IQR, interquartile range; OSA, Obstructive sleep apnea; PFAPA, Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis; SDB, sleep disordered breathing.

Symptoms and Diagnosis

Of the CoV2+ patients, the most common symptom reported was sore throat (37%) followed by cough (32%) and fever (21%). The most common symptom reported in those with prior PCR-confirmed COVID-19 was body aches (63%) followed by GI symptoms (50%) (Table III).

Presence of shortness of breath and presence of body aches were found to be associated with being CoV2+ in the univariate analysis (OR/aOR >1.00). However, these symptoms were not found to be statistically significant predictors in the multivariable analysis (Table IV).

DISCUSSION

Seroprevalence Rate and Immune Memory

We found that 19% of children in the Washington Metropolitan area undergoing tonsillectomy between September 2020 through January 2021 had been infected

TABLE II.
Socio-Economic Status and Parent-Reported Household Factors by CoV2 Status.

Patient characteristics	Overall ($N = 100$)	CoV2- ($N = 81$)	CoV2+ ($N = 19$)	p value
Socio-economic status				
Spanish language preferred, *n* (%)	19 (19.0)	11 (13.6)	8 (42.1)	0.004
Public Insurance, *n* (%)	69 (69.0)	51 (63.0)	18 (94.7)	0.007
Median income <$75000*, *n* (%)	30 (30.0)	25 (30.9)	5 (26.3)	0.697
Poverty level >10%*, *n* (%)	27 (27.0)	8 (9.9)	19 (100.0)	<0.001
High school completion rate <25%*, *n* (%)	43 (43.0)	27 (33.3)	16 (84.2)	<0.001
College completion rate <25%*, *n* (%)	43 (43.0)	30 (37.0)	13 (68.4)	0.013
Parent-reported factors				
Essential worker in family, *n* (%)	69 (72.6)	53 (68.8)	16 (88.9)	0.086
Avg. household size, mean (SD)	4.4 (1.5)	4 (1.5)	4.6 (1.6).	0.501
Daycare or school, *n* (%)	25 (25.0)	21 (25.9)	4 (21.1)	0.659

*Poverty level, Median income, High school and college completion data are zip code level data.

TABLE III.
Symptoms During the Pandemic Period.

Symptoms Full cohort ($N = 100$)	Stratified by CoV2 status	
	CoV2− ($N = 81$)	CoV2+ ($N = 19$)
Fever	13 (16%)	4 (21%)
Cough	11 (14%)	6 (32%)
Shortness of breath	2 (2%)	3 (16%)
Anosmia	0 (0%)	2 (11%)
Body aches	2 (2%)	3 (16%)
GI symptoms	7 (9%)	3 (16%)
Tonsilitis/sore throat	25 (31%)	7 (37%)
Any of the above	33 (41%)	11 (58%)
None of the above	48 (59%)	8 (42%)
SARS-CoV2 PCR + participants ($N = 8$)	CoV2− ($N = 1$)	CoV2+ ($N = 7$)
Fever	0 (0%)	2 (25%)
Cough	0 (0%)	2 (25%)
Shortness of breath	0 (0%)	2 (25%)
Anosmia	0 (0%)	2 (25%)
Body aches	0 (0%)	5 (63%)
GI symptoms	0 (0%)	4 (50%)
Any of the above	0 (0%)	5 (63%)
None of the above	1 (100%)	2 (25%)

with SARS-CoV-2 and had mounted a humoral immune response; the majority (74%) were asymptomatic or unaware of their acute infection. The expected seroprevalence in the Washington Metropolitan area around the time of sample collection was around 10% based on prior studies, including a seroprevalence study completed at CNH through October 2020.[6] Based on parental report and history of PCR positive testing (8%), we expected a similar infection rate in this study. However, a higher-than-expected CoV2+ rate of 19% was seen, as an additional 12 patients were determined to be CoV2+. As our study was done 6–9 months after the start of the pandemic, we believe the higher prevalence rate reflects the accumulation of cases during the Fall 2020 surge.

A more recent publication from CNH details the rise and fall of PCR positivity and the emergence of antibodies.[9] The median time to seropositivity in children was 18 days. Of note, the single patient with a prior positive PCR result who was seronegative in our study had their positive PCR test 20 days prior to surgery. Perhaps, though asymptomatic, this patient had not yet developed adequate antibody levels to show a serologic response; alternatively, this could also be a false positive PCR result, as we did not find SARS-CoV2-reactive B cells in either PBMCs or the tonsil. A recent study of SARS-CoV-2 challenged subjects in the UK also found a number who transiently tested positive by PCR (only one positive PCR) but failed to seroconvert.[10]

We found that the presence of serum antibodies to SARS-CoV-2, peripheral blood B cells specific to SARS-CoV-2, and tonsillar B cells specific to the virus were highly correlated indicating that children with systemic evidence of infection also have localized immunity in the upper respiratory tract. As SARS-CoV-2 is a respiratory virus, tissues in the upper respiratory tract are the first-line of immune defense, and mucosal antibodies and memory cells in these tissues likely play an important role in combating respiratory viral infections.[11] The tonsils and adenoids have been reported to be sites of SARS-CoV-2 infection and are known to have an important regional immune function within the nasopharynx and oropharynx.[12] There is, however, considerable redundancy of inductive lymphoid tissue in this area. Whether local immunity in the tonsils protects from repeated SARS-CoV-2 infection is unknown. A study from Italy reported that tonsillectomized adults had significantly higher risk of symptomatic COVID-19, though an increase in hospitalization was not seen.[13] In addition, though studies have shown decreased immune function in chronic tonsillitis, it is unclear if adenotonsillar hypertrophy leads to an effective change in immune function in these organs.[14] Further studies are needed to understand the differences in local immune function in those

TABLE IV.
Symptoms During Pandemic Period Associated with CoV2+.

Overall symptoms	Univariate (unadjusted)		Multivariable (adjusted)*	
	OR (95% CI)	p value	aOR (95% CI)	p value
Number of symptoms	1.4 (1.0, 1.9)	0.052	1.5 (1.0, 2.2)	0.077
Any symptoms	2.0 (0.7, 5.5)	0.180	2.2 (0.7, 7.1)	0.168
Specific symptoms				
Fever	1.4 (0.4, 4.9)	0.602	1.8 (0.4, 9.3)	0.471
Cough	2.9 (0.9, 9.3)	0.068	2.5 (0.7, 9.3)	0.178
Shortness of breath	7.4 (1.1, 48.0)	0.036	6.7 (0.6, 76.8)	0.125
Anosmia	NA†	NA†	NA†	NA†
Body aches	7.4 (1.1, 48.0)	0.036	NA†	NA†
GI symptoms	2.0 (0.5, 8.5)	0.357	2.6 (0.5, 14.4)	0.282

*Multivariable models were adjusted for age at surgery, gender, race or ethnicity, and surgery indication.
†There were no/enough patients with this symptom in the CoV2-group.
Abbreviations: CI, confidence interval; OR, odds ratio; aOR, adjusted odds ratio.

children with tonsillar disease states including adenotonsillar hypertrophy, OSA, and chronic tonsillitis in addition to whether humoral immunity at these mucosal sites is protective against future infection. In our study, the longest period from PCR-positive infection to serologic testing was 201 days and this individual still had circulating antibodies and SARS-CoV-2-specific B cells in the tonsil and peripheral blood. It is reassuring that these participants show continued presence of circulating antibody in addition to peripheral blood and tonsillar B cells that recognize the virus over 6 months after infection suggesting that they may have continued immunity, including localized immunity in the oropharynx.

Given that most participants in this cohort were asymptomatic, we can only speak to the duration of circulating antibodies and virus-specific B cells in those with prior PCR+ results and assume that was the time of a single infection leading to an immune response. Memory B cells may be maintained even in the absence of measurable levels of serum antibodies and are likely more important for long-term immunity.[15] T cell memory in the tonsil may also play an important role in viral defense and is currently under study. Further longitudinal analyses of patients with COVID-19 may help understand the durability of immune responses in children, as well as the long-term effects of SARS-CoV2 infection on the immune system. Our team is currently expanding the findings from this clinical cohort to more deeply examine the adaptive immune responses to SARS-CoV2 in the tissue and peripheral blood.

Demographics, Socio-Economic, and Parental Reported Home-Related Factors

Health disparities among different socioeconomic, racial, and ethnic groups have long been identified in the medical literature. Advocacy and public health policies have attempted to narrow these gaps; however, during a crisis such as the current COVID-19 pandemic, their existence resurfaces.[15] Our study highlights the effects of the early pandemic on existing disparities in children, like those observed in adult studies.

We found that Hispanic children were over-represented among those who were CoV2+. These observations are in congruence with prior studies demonstrating disproportionate infection among the Hispanic population,[16–19] particularly in the Washington metropolitan area at the time of the study.[19] Early in the pandemic, CNH reported racial/ethnic and socioeconomic disparities among children tested at a community testing site between March and April 2020. In that study, a large cohort of 1000 children (20% White, 23% Hispanic, and 30% Black) were tested for SARS-CoV-2 by PCR from nasopharyngeal swabs; 20.7% tested positive with higher rates of infection in minority children (7% White, 46% Hispanic, and 30% Black).[20] In the previous seroprevalence study of children at CNH, Hispanic ethnicity was also a significant risk factor for COVID-19 with 18.5% of Hispanic patients with SARS-CoV2 IgG as of October 2020.[5]

Cultural and socioeconomic factors related to how patients live, and work have also been found to influence COVID-19 risk. These factors can also influence infection rate and clinical course. A study from Denver, Colorado revealed that 86% of Hispanic patients with COVID-19 worked in an essential industry including construction, healthcare, food service, cleaning, and waste management, and many worked although ill due to lack of sick days.[16] In our study, having a parent who is an essential worker was not a statistically significant risk for CoV2+; however, all patients who reported an exposure and were symptomatic when PCR positive had an essential worker in the home, often with a household member noted as the source of exposure. No participants in this study reported exposure outside of the home such as in school or daycare; however, only 25% of the study cohort had attended daycare or school prior to study enrollment, likely due to the young age of participants and school closures at the time of this study.

Other social determinants of health that were significant risk factors for infection further highlight the disparities of COVID-19: all CoV2+ patients lived in a zip code with high levels of poverty, a finding seen in other pediatric studies,[20] and in zip codes with lower rates of high school completion. These and previous findings suggest that improving public health outreach and education to Spanish-speaking families, as well as to low-income families and families with lower education levels may be critical to controlling the COVID-19 pandemic.

Limitations

The primary limitation of this study is recall bias of families. Use of parental questionnaires to determine historical data is subject to recall bias that may introduce inaccuracy in the historical data. This is an inherent bias with our study design. Where possible, we corroborated historical recall with diagnostic information that might have been documented in the patient's chart, such as the date of testing or infection. We made every effort to overcome limitations due to language. Spanish-speaking participants made up 18% of the study population. These families were given questionnaires in the Spanish language and consented to using a live Spanish interpreter. No additional languages were represented in this study. All patients were given a chance to clarify survey questions with the investigators in their preferred language at the time of completion. In cases where answers were skipped or clarification was needed, the study team contacted the family or evaluated the medical record. Additional limitations may include a lack of willingness to share potentially sensitive information regarding history of COVID-19.

The Washington Metropolitan area (with patients in this study residing in Maryland, Virginia, District of Columbia, and West Virginia) is a culturally and racially diverse area, but the population that CNH serves is largely considered urban. This may somewhat limit the generalizability of our results. In addition, the results particularly related to race might not be easily translated to other locations where minorities do not represent a considerable proportion of patients undergoing care. It would be interesting to examine this study in a multi-institutional setting that includes diverse care centers to

see if a high seroprevalence is seen in other locations, and if there are other sociodemographic risk factors for COVID-19.

The timing of this study, ranging from late September 2020 to mid-January 2021, allowed us to have a glimpse into the early impacts of COVID-19 where precautions were high, variants were few, and vaccination was yet not available to children. As much has changed because of the start of the pandemic and factors including access to vaccination, re-opening of schools and community socialization, changes in mask mandates and acceptance, along with surges and SARS-CoV2 variants, it would be significant to look at changes in demographics, immune responses, and awareness of infections of the population over time.

CONCLUSION

In this study of 100 children undergoing tonsillectomy from late September 2020 to January 2021, we identified 19% of patients as having immunologic evidence of prior COVID-19 with only 7/19 patients having a known history of COVID-19 by prior positive PCR test. The use of seroprevalence in addition to PBMC and immune tissue to evaluate immune responses to SARS-CoV2 may more fully and accurately determine rates of infection and provide a window into local tissue immune memory to this novel virus. Ethnicity and socio-economic disparities of COVID-19 in the early pandemic are highlighted and continue to bring awareness of children as a vulnerable population in the COVID-19 pandemic.

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