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COVID-19 seroprevalence and clinical picture in Swedish pediatric oncology and hematology patients

Emil Sundberg[1,2] | **Tove Hoffman**[3] | **Anna Nilsson**[4] | **Simon Pahnke**[5] | **Gunilla Enblad**[6] | **Linda Kolstad**[3] | **Bengt Rönnberg**[3,7] | **Åke Lundkvist**[3] | **Milla Torkki**[1] | **Otto Zhou**[1] | **Jenna Anderson**[2] | **Arja Harila-Saari**[1,2] | **Josefine Palle**[1,2]

[1]Department of Children's Oncology and Hematology, Uppsala University Hospital, Uppsala, Sweden

[2]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

[3]Department of Medical Biochemistry and Microbiology, Zoonosis Science Center (ZSC), Uppsala University, Uppsala, Sweden

[4]Department of Women's and Children's Health, Pediatric Oncology, Karolinska Institutet, Stockholm, Sweden

[5]Unit of Oncology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden

[6]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

[7]Laboratory of Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden

Correspondence
Arja Harila-Saari, Department of Women's and Children's Health, Uppsala University, Uppsala 751 85, Sweden.
Email: arja.harila-saari@kbh.uu.se

Abstract

Background: Children develop symptomatic coronavirus disease 2019 (COVID-19) more rarely than adults upon infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Pediatric oncology and hematology patients may be at increased risk of severe COVID-19 due to their underlying disease or treatment. We investigated COVID-19 and seroprevalence of anti-SARS-CoV-2 antibodies, respectively, in a Swedish cohort of pediatric oncology and hematology patients.

Procedure: Patients ($n = 136$) were recruited between June 2020 and September 2021 at Uppsala University Children's Hospital, Sweden. Up to six consecutive blood samples per patient were analyzed for wild-type anti-S1 IgM and IgG antibodies (including after vaccination, $n = 4$). Clinical data on COVID-19 (including polymerase chain reaction [PCR] test results) were collected from electronic medical records. A questionnaire was completed at recruitment.

Results: A cumulative seroprevalence (IgM and IgG) of 33% (45/136 patients, 95% confidence interval: 25%–41%) was observed in this patient cohort, of whom 66% (90/136 patients) were under severe immunosuppressive treatment during the study period. Increasing patient age ($p = .037$) and PCR test results ($p < .002$) were associated with seropositivity in nonvaccinated cases. Most seropositive, nonvaccinated cases (32/43, 74%) were never PCR-verified for SARS-CoV-2 infection. Of the 13 patients with PCR-verified infection, nine (69%) reported mild disease. A majority (63%) reported continued school attendance during the pandemic.

Conclusions: Swedish pediatric oncology and hematology patients developed antibodies against SARS-CoV-2, despite their diagnosis and/or treatment, and the observed

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplant; CI, confidence interval; COVID-19, coronavirus disease 2019; HSCT, hematopoietic stem cell transplant; IQR, interquartile range; N, nucleocapsid (protein); OR, odds ratio; PCR, polymerase chain reaction; PHA, Swedish Public Health Agency; S, spike (protein); SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

seroprevalence was similar to that in national pediatric outpatients. PCR-verified cases underestimate the true incidence of COVID-19 in this patient cohort.

KEYWORDS
COVID-19, hematology, immunology, oncology, pediatrics

1 | INTRODUCTION

Children develop symptomatic coronavirus disease 2019 (COVID-19) more rarely than adults upon infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and generally present with mild and nonspecific symptoms.[1–4] Pediatric oncology and hematology patients, however, may suffer from an increased risk of symptomatic COVID-19, and the pandemic's effects on patients' medical care remains a concern. An American study on pediatric cancer patients with COVID-19 observed low morbidity and mortality, albeit higher than the general pediatric population,[5] whereas a recent multinational study reported severe or critical illness in 19.9% of cases and a higher mortality than previously reported for the pediatric population, with increased risk of severe outcomes in low-income countries.[6] A multinational study further found that while most pediatric cancer patients experienced asymptomatic or mild disease, 13% of patients suffered from severe disease and 3% died from COVID-19.[7] While some data suggest that younger children are less susceptible to SARS-CoV-2 than adults,[8] other data indicate a similar risk of infection[9,10] and of seropositivity[11,12] in children and adults.

Antibodies to SARS-CoV-2 typically develop against all virus structural proteins to varying degrees. Anti-SARS-CoV-2 antibody analyses frequently target the spike (S) protein, needed for viral binding to cellular receptor ACE-2 and/or the nucleocapsid (N) protein, necessary for viral replication. An American study found that in children, anti-SARS-CoV-2 antibodies were produced predominantly against the S-protein.[13] Immune cells are reduced by many treatment modalities targeting oncological and hematological diseases or by the disease itself; for example, CD19 and CD20 antibodies or CAR-T cells used for hematological malignancies may lead to complete B-cell depletion. Treated children may thus suffer an impaired ability to clear an ongoing infection or to form immunological memory post infection. However, early cross-sectional studies on COVID-19 in immunocompromised children, including oncology patients, reported detectable antibodies targeting SARS-CoV-2.[14,15] The main objective of this study was to evaluate the seroprevalence of SARS-CoV-2 antibodies in a cohort of Swedish pediatric oncology and hematology patients (including after vaccination where possible), and to collect self-reported information of the patients' and their families' physical school/work attendance during the pandemic. Second, we aimed to assess the disease severity of COVID-19 in this cohort.

2 | METHODS

2.1 | Study design and patient recruitment

This study was conducted as a prospective, single-center longitudinal cohort study. Study participants were recruited and sampled to September 7, 2021. Patients treated or on follow-up at the Department of Children's Oncology and Hematology, Uppsala University Children's Hospital (Sweden), were invited to participate; a clinic with approximately 65 new patients diagnosed annually and 110 patients diagnosed during the study period. Patients with an oncological or hematological diagnosis or receiving allogeneic hematopoietic stem cell transplant (allo-HSCT) were included from June 23, 2020, while patients with benign hematological disorders were included from November 23, 2020 after ethical amendment approval. Inclusion was independent of previously known COVID-19. This study was approved by the Swedish Ethical Review Authority (reference 2020-02154, approved May 22, 2020, with amendment 2020-04672 approved November 23, 2020).

2.2 | Blood sample collection

Venous blood was collected during already-scheduled clinical samplings (CAT Serum Clot Activator tube, VACUETTE, Greiner Bio-One Gmbh, Austria). Samples were collected up to six times per patient: once at inclusion, three collected after an interval of ≥6 weeks per sample, and finally two samples collected after an interval of ≥3 months per sample. After 2–24 hours storage at +8°C, samples were centrifuged at 2000 × *g* for 10 minutes, aliquoted, and stored at −74°C before transport on dry ice to Uppsala University (Sweden) for analysis.

2.3 | Patient data collection

Patient data were collected via electronic medical records and a brief questionnaire. Insufficient data were completed via patient or caregiver anamnesis, when possible, or by contacting the patients' local hospitals. Patients were categorized into age groups, representing preschool children (≤5 years old), school children (6–12 years old), and teenagers (13–18 years old). Results from polymerase chain reaction (PCR) tests performed for clinical purposes were obtained from patient medical journals; no PCR tests were performed for the study

TABLE 1 COVID-19 disease severity classification[5,16]

Disease severity	Definition
Asymptomatic disease	No reported symptoms of COVID-19
Mild disease	Symptoms of COVID-19 that did not require hospitalization (e.g., runny nose, fever, cough, sore throat, stomachache), including long-lasting symptoms. If the patient was hospitalized it was mainly for other indications than management of COVID-19
Moderate disease	Symptoms of COVID-19 requiring hospitalization, but not ICU-care
Severe disease	Symptoms of COVID-19 requiring ICU-care

Abbreviation: ICU, intensive care unit.

alone. COVID-19 disease severity in PCR-verified patients was classified as asymptomatic, mild, moderate, or severe disease (Table 1).[5,16] Patient treatments were classified into four degrees of immunosuppression, defined as the maximum degree of immunosuppressive treatment experienced during the pandemic (i.e., since March 2020 in Sweden; Table 2).

The questionnaire was completed at recruitment to approximate family constellation and physical school/work attendance since the start of the pandemic in Sweden (March 2020). Questions included whether the child had been physically attending school and at what mean frequency; number and age category of family members living with the patient; and number of family members physically attending work, school, or daycare.

2.4 | Laboratory analyses

Sera were analyzed for wild-type SARS-CoV-2 S1-specific IgG and IgM antibodies using a COVID-19 suspension immunoassay.[17] Briefly, S1 protein was coupled to carboxylated differentially color-marked magnetic beads (MagPlex microspheres, Luminex Corp., TX, USA). For IgM determination, sera were pre-incubated with GullSORB (Meridian Life Science, TN, USA) to remove IgG, mixed with PBS-T (0.05% Tween-20) and microsphere mixture to a final serum dilution of 1:50, then incubated with biotinylated anti-human IgM (Sigma Aldrich, Merck, Darmstedt, Germany) or biotinylated protein G (Pierce Biotechnology, ThermoFisher Scientific, MA, USA), followed by addition of streptavidin–phycoerythrin (Invitrogen, ThermoFisher Scientific). Analyses were performed in a Luminex MagPix instrument. Serum samples were classified as seronegative (Median Fluorescence Intensity, MFI <300) or seropositive (MFI >800). Samples falling between these cutoffs were re-analyzed and classified as seropositive if subsequent MFI >300.

2.5 | Statistical analysis

Standard descriptive statistics were performed. Mann–Whitney *U* tests were performed to compare the mean number of study samples collected between the seropositive and seronegative groups. Logistic regressions, adjusting for number of samples collected, were performed for univariable and multivariable subgroup analysis. For the continuous independent variable "age in years," median and interquartile range (IQR) were calculated, and for other variables, frequency and percentage were calculated. Subgroup analyses both including and excluding postvaccination samples were performed. All tests adopted a value of $p \leq .05$ for statistical significance. A confidence interval (CI) of 95% was used when applicable. Analyses were conducted using SPSS (version 27.0).

3 | RESULTS

3.1 | Patient demographics

Of patients offered to participate, 80% consented, resulting in a total of 139 patients recruited between June 2020 and September 2021. Three patients were excluded due to not meeting inclusion criteria ($n = 2$) and never being sampled ($n = 1$), for a final study population of 136 children. Patients were sampled from one to six times (median two times), with a mean frequency of 73 days between samplings for those with multiple samplings (33–301 days, median 56 days). A difference ($p = .012$) was observed in the mean number of study samples collected between the seropositive (mean 2.9 times) and seronegative groups (mean 2.3 times), both when including and excluding postvaccination samples. Mean follow-up time from first sampling to last potential sampling in September 2021 was 250 days. Patient demographics are presented in Table 3 (and in Table S1 for subgroup analyses excluding postvaccination samples). Patient ages, defined as age in years at inclusion, ranged from 0 to 18 years (median 8 years, IQR 10 years). Patient diagnoses included leukemia ($n = 44$), lymphoma ($n = 11$), solid tumor ($n = 40$), brain tumor ($n = 26$), and hematological disorder post-transplant ($n = 15$). One patient had a history of multiple cancers, but diagnosis was classified as "solid tumor" due to this being the most recent tumor and the one under active treatment. The category "hematological disorder or post-transplant" consisted of 13 patients with benign hematological disorders and two patients who received allo-HSCT due to chronic mucocutaneous candidiasis and multiple sclerosis, respectively. Six patients died during the study, none of whom were suspected to have died from COVID-19, but rather from their underlying disease. Ninety patients (66%) experienced severely immunosuppressive treatment during the study period.

3.2 | Vaccination

To our knowledge, four teenaged patients received a COVID-19 vaccine during the study, two of whom were seropositive at subsequent samplings. These patients' postvaccination blood samples are included in the results unless otherwise stated. One seroposi-

TABLE 2 Maximum degree of immunosuppressive treatment experienced during the pandemic

Degree of immunosuppression	Definition
No immunosuppression	Not immunosuppressive treatment or only on follow-up
Mild immunosuppression	Oral low-intensive chemotherapy (e.g., maintenance therapy in leukemia), radiotherapy alone, immuno-affecting treatment of benign hematological disease, more than 1 year post allo-HSCT without immunosuppressive treatment, or targeted agents
Moderate immunosuppression	Some lower intensity intravenous solid tumor protocols, interim maintenance chemotherapy for ALL, or oral chemotherapy in combination with radiotherapy, more than 1 year post allo-HSCT with immunosuppressive treatment
Severe immunosuppression	Intensive intravenous chemotherapy (e.g., induction, consolidation, and delayed intensification) for ALL, all AML chemotherapy courses, induction regimens for other malignancies, within 1 year post allo-HSCT

Abbreviations: ALL, acute lymphoblastic leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplant; AML, acute myeloid leukemia.

tive patient had received the first dose (Comirnaty, Pfizer/BioNTech, Germany) 3 weeks prior to seropositive sampling, and the other patient the second dose (Comirnaty) 3 months prior to seropositive sampling. One seronegative patient had received the second dose (Comirnaty) 1 week prior to seronegative sampling and had received the second of two allo-HSCTs to treat pre-B-ALL at 3 months prior to the first dose. The other seronegative patient received the first dose (Spikevax, Moderna, Spain) at 1 month prior to seronegative sampling, and was treated with sirolimus during the period for vaccination.

3.3 | Seroprevalence

Forty-five patients (33%; 95% CI: 25%–41%), including two vaccinated patients, had detectable anti-SARS-CoV-2 antibodies at one or more visits during the study, of whom 43/136 patients (30%) were IgG-positive (with or without IgM). When excluding postvaccination samples, 43/135 patients were seropositive (32; 95% CI: 24%–40%). Two patients (1%) were only IgM-positive at sampling; while one of these patients was sampled only once, the other patient was IgG-negative at two consecutive visits, 2 months apart, indicating an absence of seroconversion. Eight previously IgG-positive, unvaccinated patients became seronegative during the study, of whom only one were ever positive when PCR tested (see below, *PCR testing*); none of the seven other patients had a previous positive PCR test or seronegative blood sample, so time of infection for these patients remains unknown and antibody durability cannot be assessed.

When performing subgroup analyses and adjusting for number of samples collected (Table 3 and Table S1), only PCR test results were associated with seropositivity ($p = .002$ and .003 when excluding and including postvaccination samples, respectively). No associations were found between seropositivity and degree of immunosuppression, or any of the other variables analyzed. In a multivariable regression model, excluding postvaccination samples (adjusted for number of samples, sex, diagnosis category, and degree of immunosuppression), increasing patient age was associated with an increased likelihood of being seropositive, with an odds ratio (OR) of 1.086 (95% CI: 1.005–1.173, $p = .037$) per year. The corresponding OR when including postvaccination samples was 1.096 (95% CI: 1.015–1.184, $p = .019$) per year.

3.4 | PCR testing

Ninety-seven (71%) patients had been PCR tested for active SARS-CoV-2 infection, of whom only 13 patients (13% and 10% of PCR tested and total study participants, respectively) were ever PCR-positive. Most seropositive, unvaccinated cases, 32/43 (74%), were never PCR-positive for SARS-CoV-2 infection. Two seronegative patients received inconclusive test results at some point, but were classified as PCR-negative due to subsequent negative PCR tests. Nine of the 13 patients (69%) with PCR-verified SARS-CoV-2 infection became seropositive post infection, and of whom eight were still seropositive at last available follow-up sampling, after a mean of 250 days (range 118–487 days) after their positive PCR test. Additional data on antibody durability beyond this follow-up timeframe is yet unavailable, but data collection is ongoing and aimed to be published in the future. The only PCR-positive, seropositive patient who became seronegative during the study was PCR-positive in January 2021, sampled seropositive in March, April, and June 2021, and seronegative in August 2021. Two of the 13 patients (15%) were both seronegative at subsequent sampling approximately 4 months post infection; one of these two patients suffered from multiple cancers and the other had a primary hemophagocytic lymphohistiocytosis and had undergone allo-HSCT in 2016. Additionally, while PCR test results from the time of their initial infection was missing in both cases, two previously seropositive patients experienced PCR-verified reinfection. One patient was seronegative when sampled 6 months prior to the reinfection and had received an allo-HSCT in the period between the first seropositive sample and the PCR-verified reinfection. The other patient was seropositive when sampled only 2 weeks prior to the PCR-verified reinfection.

3.5 | Disease severity

Of the 13 patients with PCR-verified SARS-CoV-2 infection, nine (69%) experienced mild disease, whereas moderate disease occurred in three

TABLE 3 Patient demographics and characteristics, grouped by distribution of seronegative and seropositive, respectively, presented as count (% within category)

Study participants ($n = 136$)	Seronegative ($n = 91$)	Seropositive ($n = 45$)	p-Value
Age in years, median (IQR)	6.0 (9.0)	10.0 (9.5)	
Age group			.106
Preschool children, ≤5 years ($n = 55$)	41 (75%)	14 (26%)	
School children, 6–12 years ($n = 46$)	32 (70%)	14 (30%)	
Teenagers, 13–18 years ($n = 35$)	18 (51%)	17 (49%)	
Sex			.510
Males ($n = 66$)	45 (68%)	21 (32%)	
Females ($n = 70$)	46 (66%)	24 (34%)	
Diagnosis			.571
Leukemia ($n = 44$)	30 (68%)	14 (32%)	
Lymphoma ($n = 11$)	7 (64%)	4 (36%)	
Solid tumor ($n = 40$)	26 (65%)	14 (35%)	
Brain tumor ($n = 26$)	20 (77%)	6 (23%)	
Hematological disorder or post-transplant ($n = 15$)	8 (53%)	7 (47%)	
Degree of immunosuppression			.489
No immunosuppression ($n = 11$)	6 (55%)	5 (46%)	
Mild immunosuppression ($n = 13$)	10 (77%)	3 (23%)	
Moderate immunosuppression ($n = 22$)	15 (68%)	7 (32%)	
Severe immunosuppression ($n = 90$)	60 (67%)	30 (33%)	
Have received allo-HSCT			.940
Yes ($n = 17$)	10 (59%)	7 (41%)	
No ($n = 119$)	81 (68%)	38 (32%)	
PCR tested for COVID-19			.003
PCR-positive ($n = 13$)	2 (15%)	11 (85%)	
PCR-negative ($n = 84$)	57 (68%)	27 (32%)	
Never tested/unknown ($n = 39$)	32 (82%)	7 (18%)	
Patient attending school[a]			.820
Yes, ≥3 days/week ($n = 77$)	51 (66%)	26 (34%)	
Yes, 1–2 days/week ($n = 3$)	1 (33%)	2 (67%)	
Yes, more rarely than every week ($n = 5$)	5 (100%)	0 (0%)	
No ($n = 51$)	34 (67%)	17 (33%)	
Number of family members (patient excluded)[a]			.247
1–2 ($n = 17$)	9 (53%)	8 (47%)	
3–4 ($n = 93$)	66 (71%)	27 (29%)	
≥5 ($n = 26$)	16 (62%)	10 (39%)	
Number of family members ≥18 years of age attending work or studies[a]			.776
0 ($n = 38$)	26 (68%)	12 (32%)	
1 ($n = 35$)	22 (63%)	13 (37%)	
2 ($n = 54$)	36 (67%)	18 (33%)	
3–4 ($n = 9$)	7 (78%)	2 (22%)	
Number of family members <18 years of age attending school or preschool[a]			.333
0 ($n = 30$)	19 (63%)	11 (37%)	
1–2 ($n = 69$)	46 (67)	23 (33%)	

(Continues)

TABLE 3 (Continued)

Study participants ($n = 136$)	Seronegative ($n = 91$)	Seropositive ($n = 45$)	*p*-Value
3–4 ($n = 28$)	22 (79%)	6 (21%)	
≥5 ($n = 9$)	4 (44%)	5 (56%)	

Note: Postvaccination samples ($n = 4$) are included; corresponding table excluding postvaccination samples is presented in Table S1. Logistic regression, adjusting for number of samples collected, was performed to analyze the individual variables between the seropositive and seronegative groups.
Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplant; IQR, interquartile range; PCR, polymerase chain reaction.
[a]Questionnaire questions, completed at the time of recruitment to the study.

(23%). One patient classified as having moderate disease was hospitalized for neutropenic fever, but with no focal symptoms, and the primary cause of the neutropenic fever was not verified. The only patient with severe disease suffered from a massive thoracic tumor and was persistently PCR-positive for approximately 6 weeks. In total, 9% (4/43) of seropositive, unvaccinated patients suffered from moderate or severe PCR-verified COVID-19.

3.6 | School attendance

While 51 (38%) patients reported that they did not physically attend school, the majority, 85 patients (63%), reported physical school attendance during the pandemic. In total, 77 patients (57%) attended school ≥3 days/week, three (2%) for 1–2 days/week, and five (4%) attended less than every week. Of the 51 patients who reported not attending school, most were recruited early in the study; 36/51 (71%) through October 2020 compared to 15/51 (29%) from November 2020 to September 2021.

4 | DISCUSSION

As of September 2021, 45 patients (33%) were found to be or have been seropositive against wild-type SARS-CoV-2, including two vaccinated, seropositive patients. The pediatric oncology and hematology patients in this cohort were thus able to develop antibodies against SARS-CoV-2, despite their diagnosis and/or treatment. Notably, no subgroup differences in seropositivity between diagnoses or the degrees of maximum immunosuppressive treatment experienced during the study period was observed; only positive PCR test results and increasing patient age were significantly associated with a higher chance of seropositivity. This observed association is consistent with previous data reporting lower risk of transmission in younger children than older children and/or adults.[1,18]

The cumulative seroprevalence of 33% in our cohort, including vaccinated cases, is comparable with the seroprevalence in Swedish pediatric outpatients, as reported by the Swedish Public Health Agency (PHA).[19] The PHA has repeatedly performed seroprevalence analyses in leftover blood sample material, collected nationally and for clinical reasons other than COVID-19 and including vaccinated cases. The SARS-CoV-2 seroprevalence in patients aged 0–19 years reported for the three sampling periods in 2021 (as of November 2021) are compiled in Table 4, with the two most recent estimates being 29.7% (95% CI: 27.41–32.07) in samples collected from May 24, 2021 to June 4, 2021 and 42.1% (95% CI: 39.31–44.94) in samples collected from September 20, 2021 to October 3, 2021. In all 2021 sampling periods, SARS-CoV-2 seroprevalence was lowest in children aged 0–11 years and highest in adolescents aged 16–19 years. This, too, is in line with our results, showing increasing seroprevalence with age. Vaccinations for Swedish adolescents aged 18–19 were initiated in summer 2021, and for adolescents aged 16–17 in August 2021.

Only 13 patients in this cohort tested PCR-positive to SARS-CoV-2 at least once, while 43 unvaccinated patients were seropositive and evidently previously infected. Detecting ongoing SARS-CoV-2 infection is challenging in children, reflected in a disparity between reported PCR-verified cases and seropositive cases, as observed here and previously. An Italian study observed a SARS-CoV-2 PCR-verified:seropositive ratio of 1:7 in children in May 2020,[20] while a Swiss study reported a ratio of 1:3.5 in March–April 2021,[21] similar to the observed ratio of 1:3.3 herein (1:3.5 when including vaccinated patients).

However, when interpreting this discrepancy, several biases must be considered. Children are PCR tested for SARS-CoV-2 infection less frequently than adults. Patients in this cohort were primarily PCR tested if symptomatic or if COVID-19 was suspected, although they were sometimes tested as screening, for example, prior to anesthesia. With children often presenting with mild or asymptomatic COVID-19, which also applies to this cohort, some patients were likely not PCR tested due to this testing strategy despite experiencing an active infection. Nasopharyngeal PCR testing for SARS-CoV-2 relies on testing during the acute phase of infection with sufficient viral loads; timing is thus crucial and hampered by children often being asymptomatic. Testing children could also have a lower methodological sensitivity, due to caution when sampling in consideration of the child's comfort. In summary, PCR-verified cases underestimate the true incidence of COVID-19 in this patient cohort.

Pediatric oncology or hematology patients are reported to suffer from an increased risk of symptomatic COVID-19 compared to the general pediatric population,[5] whom in general are mildly affected. However, most patients herein with PCR-verified SARS-CoV-2 infection or seropositive results experienced mild or asymptomatic disease.

The mild symptoms generally observed in children may be partly explained by a pre-activated and/or cross-reactive immune response, due to children frequently experiencing upper airway infections by other seasonal coronaviruses.[22] Data on the presence of cross-reactive

TABLE 4 The proportion of seropositive blood samples in national pediatric outpatients per age category (years) and time period of sampling (year/month/day), respectively, as reported by the Swedish Public Health Agency[19]

Sample period	Total (ages 0–19) % (95% CI)	Ages 0–11 % (95% CI)	Ages 12–15 % (95% CI)	Ages 16–19 % (95% CI)
2021/03/01 to 2021/03/12	23.4% (21.36–25.49)	20.2% (17.53–23.15)	24.7% (20.84–28.77)	29.5% (24.60–34.68)
2021/05/24 to 2021/06/04	29.7% (27.41–32.07)	23.3% (20.29–26.45)	34.9% (30.56–39.34)	36.3% (30.59–42.23)
2021/09/20 to 2021/10/03	42.1% (39.31–44.94)	28.4% (24.78–32.19)	39.5% (34.41–44.80)	81.1% (75.46–86.03)

Abbreviation: CI, confidence interval.

antibodies, predominantly IgG, in some individuals reveal that these were more prevalent in children and adolescents than in adults, indicating a pre-existing heterologous immunological memory.[13,23] Other studies have also observed cross-reactive $CD4^+$ and/or $CD8^+$ T cells in unexposed individuals.[24–26] Furthermore, epithelial and immune cells of the upper airways in children are primed for virus sensing, thereby facilitating an efficient early production of interferon to which SARS-CoV-2 is highly susceptible. This provides a strong innate antiviral response, which in turn may lead to faster viral clearance and milder disease.[27]

Swedish schools have remained open throughout the pandemic, excluding high schools, which switched to distance education in March 2020 but reopened after the summer holidays in August 2020. Most patients reported at recruitment that they continued to physically attend school during the pandemic, with over half attending ≥3 days/week. A distributional skew was observed regarding physical school attendance, with most recruited early in the pandemic. Early uncertainty and caution regarding COVID-19 may have resulted in an increased tendency to stay home from school, and some patients reporting physical absence at recruitment may have physically attended school later during the pandemic.

In Sweden, most pediatric oncology and hematology patients (excluding transplanted patients) are encouraged to continue physical school attendance after diagnosis. This has continued during the pandemic and patients have been advised to follow general recommendations by the PHA. In fact, to avoid negative implications on children's education and well-being, physical school closures have been recommended only as a last resort during the pandemic.[1,28] A 2013 Swedish study reported that school participation (compared to school absence) did not increase the risk of infection requiring antimicrobial treatment for patients undergoing oncological treatment.[29] The present study indicates that despite exposure to SARS-CoV-2, most patients in this cohort were not clinically diagnosed with symptomatic COVID-19, suggesting that recommendations for continued school attendance were adequate for most pediatric oncology and hematology patients.

We aimed for a low risk of sampling bias by including patients independent of previous COVID-19 and diagnosis. We included samples from vaccinated patients in analyses to assess the patients' ability to produce antibodies independent of virus versus vaccine exposure, to limit any biases from excluding known vaccinated individuals, and to increase comparability to the seroprevalence observed in Swedish pediatric outpatients.

Study participants were recruited continuously from June 2020 and the number of possible follow-up samples has varied depending on time since inclusion. The individual time of recruitment, questionnaire completion and of sampling adds temporal confounders that limit interpretability and comparability of the results. Samples were only collected in connection to already scheduled clinical visits, and no PCR tests were performed for study purposes only, further limiting interpretability.

Whether some patients were sampled when their antibody titers had decreased below detectable limits, or had been exposed to SARS-CoV-2 but unable to produce antibodies, remains unknown. As the analysis antigen used was wild-type S1-protein, any antibodies not reactive to wild-type SARS-CoV-2 but to other variants have not been detected. Furthermore, to better describe immunity against SARS-CoV-2, analyses of antibody titers and dynamics, cellular immunity, and immunological memory should be performed. Finally, the results herein should be interpreted in the context of the COVID-19 pandemic until September 2021 in Sweden, and before the spread of the Omicron variant, which seems to possibly affect children differently than previous variants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author (on group-level). The data are not publicly available due to privacy or ethical restrictions.

ORCID

Emil Sundberg https://orcid.org/0000-0001-5087-1400
Anna Nilsson https://orcid.org/0000-0001-5885-7101
Arja Harila-Saari https://orcid.org/0000-0003-2767-5828

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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