Prevalence of IgG against SARS-CoV-2 and evaluation of a rapid MEDsan IgG test in children seeking medical care

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ABSTRACT

In a sample of 208 children seeking medical care, seropositivity rate of anti-SARS-CoV-2 IgG antibodies was 8.7%, suggesting a similar infection rate to that observed in adults, but >100-fold the incidence of RT-PCR-confirmed pediatric cases. Compared to the gold-standard combined ELISA+immunofluorescence, the MEDsan IgG rapid diagnostic test performed accurately.

Keywords: COVID-19; pediatrics; seroprevalence; rapid diagnostic test; antibody

INTRODUCTION

Children are underrepresented in terms of recognized acute COVID-19 cases, either because of a lower infection rate[1, 2] and/or because of more frequent asymptomatic or atypical presentations leading to underdiagnosis of the acute phase[3]. In this context, seroprevalence studies are helpful for identifying the proportion of children that has been infected by SARS-CoV-2, irrespective of clinical testing practices. The region of Geneva was severely affected, with an estimated RT-PCR confirmed 1000 cases/100'000 inhabitants and reaching a seroprevalence of around 10% in the general population[4]. The first aim of this work was to evaluate the prevalence of SARS-CoV-2 IgG in children who presented to our institution for non COVID-19-related blood testing in the month following the local epidemic peak of infections. The other aim of the study was to evaluate the SARS-CoV-2 IgG detection performance of the MEDsan COVID-19 IgG rapid diagnostic test in children (RDT), recently shown to display a satisfying diagnostic accuracy when compared to centralized tests in adults[5]. Multiple COVID-19 serology rapid tests are currently being developed and widely distributed, often without extensive or published performance assessments, especially in children.

METHODS

Study design. All patients <16 years-old (yo) with blood collected at Geneva University Hospitals between April 1st and 30th, and for which leftover EDTA plasma was available were eligible, with the exception of neonates. Patients who presented to the pediatric emergency room (ER; n=86), who were admitted (n=64) or coming to outpatient clinics (n=58) were enrolled. The study was approved by the local ethics committee (protocol #2020-0835). Assessment of seropositivity rate was performed using a commercial enzyme-linked immunosorbent assay (ELISA) with confirmation by immunofluorescence (rIFA) and used as a gold-standard against which the MEDsan COVID-19 IgG RDT (MEDsan GmbH, Hamburg, Germany) was challenged. Further details are available in the Supplementary Methods.

ELISA. ELISA was performed using the Euroimmun IgG assay targeting the S1 domain of the spike protein (Euroimmun AG, Lubeck, Germany)[6]. Quantitative results were interpreted following previously published cut-offs, with values <0.8 considered as negative, between 0.8 and 1.5 as indeterminate, and ≥1.5 as positive. Indeterminate and positive ELISA were confirmed by immunofluoresence (rIFA), as previously described[4, 7].

Spike-expressing recombinant immunofluorescence assay. rIFA was performed by determination of the IgG antibody response against the complete spike (S) protein (both S1 and S2 domains) of SARS-CoV-2, as previously validated for SARS-CoV-2[7]. Results were assessed by two independent readers with satisfactory interobserver kappa coefficients[7]

RDT. For the RDT IgG, 5 uL of plasma was applied to the cassette, and results read after 10 min but no later than 15 min as per the manufacturer's instruction.

RESULTS

SARS-CoV-2 IgG prevalence in children. Among the 316 children with blood collected in our institution during the study period, leftover plasma was available and used for this study in 208 patients (65.8%), with a median age of 9.0 years (Supplementary Table 1). Among those, 19 (9.1%) were tested as seropositive, 8 (3.8%) as indeterminate, and 181 (87.0%), as seronegative by ELISA. Sixteen and two of the respectively 19 positive and eight indeterminate were confirmed positive by rIFA, leading to a seropositivity rate of 8.7% (18/208) in the dataset. The positivity rate increased from 1.6% (1/61) between April 1-10th, to 10.8% (7/65) between April 11-20th, and to 12.2% (10/82) between April 21-30th (Figure 1). In comparison, the cumulative incidence of RT-PCR confirmed < 16 yo cases was <0.08% at the end of the study period (Figure 1), suggesting that only 1 out of 100 children infected by SARS-CoV-2 is clinically diagnosed. Median age did not significantly differ between IgG positive (10.6 [IQR 8.0-13.7]) and negative children (8.8 [3.5-13.0]; p=0.149). However, there was a trend towards a lower seropositivity rate in children <10 yo (5.4% [6/111]) when compared to older children (12.4% [12/97]; p=0.075). The seropositivity rate did not differ according to where the patient was sampled (Supplementary Table 1).

RDT performance. Using the MEDsan RDT, IgG were positive in 26 (12.5%) and negative in 182 (87.5%) of children. Performance of SARS-CoV-2 RDT IgG detection using RDT against ELISA+rIFA showed a sensitivity and specificity of 88.9% (16/18 [95% confidence interval {CI} 64%-98%]) and 94.7% (180/190 [95%CI 90%-97%]), respectively. The positive predictive value (PPV) and negative predictive value (NPV) were 61.5% (16/26 [95%CI 41%-79%]) and 98.9% (180/182 [95%CI 96%-100%]), respectively. The Kendall correlation coefficient between the RDT and ELISA+rIFA was 0.71 (p<0.001).

DISCUSSION

The key finding of the present study is that the seropositivity rate in children presenting to the hospital during this period is of the same magnitude than what observed in adults over the same period[4], suggesting that the SARS-CoV-2 infection rate is similar in children and in adults. In our dataset, the overall prevalence of IgG antibodies against SARS-CoV-2 during the month following the epidemic peak was 8.7% and increased from 1.6% to 12.2% over a short period. In comparison, seroprevalence increased from 4.8% to 10.8% during the five weeks following the peak in the large seroprevalence study conducted in our region[4]. Similarly, another large seroprevalence study in a region less heavily affected has shown a seroprevalence of 2.8% among children, in line with the adult seroprevalence[8]. In our study, despite a similar overall infection rate when compared to adults[4], children <10 yo were 2.3-fold less likely to be seropositive than those aged 10-16; even though these results were not significant, they suggest that either younger children were less exposed to SARS-CoV-2 during the first wave, or they are less susceptible to SARS-CoV-2 infection because of reduced ACE-2 receptor density in the upper airways, stronger innate immune system and/or cross-reactive immunity provided by recent exposure to human coronaviruses. Along the same lines as our data, seropositivity rates in our region's populational study, were respectively 0.8% and 9.6% in children 5-9 yo and 10-19 yo, over the five weeks following the epidemic peak[4], and respectively 4.3% and 7.9% over the twelve weeks following the peak (S. Stringhini, personal communication). Altogether, available data suggest that even though children have lower RT-PCR-confirmed secondary attack rates inside households[1, 2, 9], they might be infected as frequently as adults[4, 8]. Interestingly, the seropositivity rate in our dataset at the end of the study was >100-fold the cumulative incidence of RT-PCR confirmed cases <16 yo at the same period, suggesting that despite the absence of RT-PCR reagents shortage during the epidemic and wide RT-PCR pediatric testing criteria[10], only about 1% of the pediatric COVID-19 cases were diagnosed.

There are limitations to this pediatric seroprevalence study. First, our sample is possibly not representative of the general pediatric population because patients were enrolled in a clinical setting. Some of the enrolled patients might have sought medical attention for an illness of possible infectious origin with or without a SARS-CoV-2 RT-PCR test, thus conceivably increasing the pre-test probability of SARS-CoV-2 infection compared to the overall children population. Nevertheless, we believe this is a minor bias, because the prevalence of IgG antibodies was not lower in elective outpatients than in patients from the ER or those admitted in our hospital.

Second, the relatively small sample size and the lower number of preschool-aged children compared to older children do not allow to definitively conclude whether the first group is less susceptible to the virus. This pilot study provides the basis for larger population-based study enrolling preschool children to better evaluate the impact of public health policies on seroprevalence in younger age groups.

The study also shows that the performance of the MEDsan IgG RDT in children is acceptable, with a negative predictive value of 99%, in line with previously published NPVs of 97-100%[5, 11]. The sensitivity and specificity were respectively 89% and 95% in our dataset, which is in the range of previously reported sensitivities and specificities[5, 11, 12]. As expected, the PPV of 62% was similar than published PPV with a 10% seroprevalence[11], but lower than the PPV of 95% reported in the published method validation study[5], emphasizing the importance of the prevalence on PPV. In addition, in the previous validation study by Andrey et al., plasma samples were obtained from hospitalized patients, likely to have higher antibody levels than those with milder disease[5]. Finally, this RDT performance assessment was obtained against a validated ELISA method combined with immunofluorescence, unlike most SARS-CoV-2 seropositivity detection studies which lack reliable, validated, IgG detection reference methods as comparator. Altogether, these data show that in absence of centralized test availability, the MEDsan IgG RDT could be an acceptable option for rapid assessment of SARS-CoV-2 IgG seroprevalence in children. The rapid turnaround time without the need of a laboratory, and the possibility to use capillary blood makes it particularly convenient for children.

This RDT performance evaluation has one limitation, since the use of plasma might overestimate sensitivity and NPV when compared to whole blood which would be used in capillary tests. Nevertheless, at least in adults, plasma and whole blood samples showed similar results when tested with the MEDsan RDT[5].

In conclusion, our data show a 8.7% seropositivity rate of SARS-CoV-2 IgG in children, which is in line with adult data derived from the same region[4]. There was however a trend towards a lower rate of seropositivity among children <10 yo, suggesting a lower infection rate in younger children. Our serological data also suggest that only a small percentage of pediatric COVID-19 cases were diagnosed by RT-PCR. We also showed that the MEDsan IgG RDT performs accurately in the pediatric setting and could be -despite its limitations- considered for individual testing or even serosurveys in difficult-to-reach populations.

NOTES

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Conflicts of Interest:

KPB reports personal fees to their institution by MSD Advisory Board and Pfizer Advisory Board, outside the submitted work. The other authors have no conflict

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Figure Legends

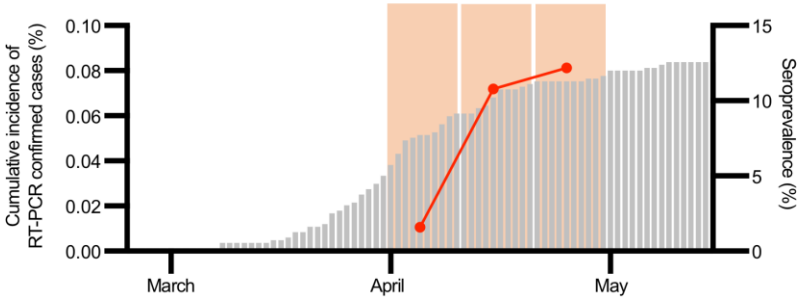
Figure 1. Evaluation of the seroprevalence of IgG against SARS-CoV-2 with time in patients < 16 yo in comparison to the cumulative incidence of RT-PCR confirmed cases <16 yo.

The grey bars represent the cumulative incidence of RT-PCR confirmed SARS-CoV-2 infections in <16 years old. The red dots represent the prevalence of igG against SARS-CoV-2 in our dataset of children <16 years old. The orange tan bars represent the three study periods (April 1-10th, 11-20th, and 21-30th).

Of note, serological tests were performed on children who had blood collected in our institution regardless of the indication for blood collection, whereas RT-PCR tests were performed on children who met the definition for a suspect COVID-19, which was the presence of any respiratory symptom or fever.

IgG: immunoglobulin G; RT-PCR: reverse-transcription polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Figure 1


Cumulative incidence of RT-PCR confirmed cases (%)
0.10
0.08
0.06
0.04
0.02
0.00
March
April
May
15
10
5
0
Seroprevalence (%)