Coronavirus antibody screening identifies children with mild to moderate courses of PMIS

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Abstract

Background: Children are affected rather mildly by the acute phase of COVID-19, but predominantly in children and youths, the potentially severe and life threatening pediatric multiorgan immune syndrome (PMIS) occurs later on. To identify children at risk early on, we searched for antibodies against SARS-CoV-2 and searched for early and mild symptoms of PMIS in those with high levels of antibodies. Methods: In a cross-sectional design, children aged 1-17 were recruited through primary care pediatricians for the study (a), if they had an appointment for a regular health check-up or (b), or if parents and children volunteered to participate in the study. Two antibody tests were performed in parallel and children with antibody levels >97th percentile (in the commercially available test) were screened for signs and symptoms of PMIS and SARS-CoV-2 neutralization tests were performed. Results: We identified antibodies against SARS-CoV-2 in 162 of 2832 eligible children (5.7%) between June and July 2020 in three, in part strongly affected regions of Bavaria. Approximately 60% of antibody positive children showed high levels of antibodies. In those who participated in the follow up screening, 30% showed some mild and minor symptoms similar to Kawasaki disease and in three children, cardiac and neuropsychological symptoms were identified. Symptoms correlated with high levels of non-neutralizing and concomitantly low levels of neutralizing antibodies and lower neutralizing capacity. Conclusions: Children exposed to SARS-CoV-2 should be screened for antibodies and those children with positive antibody responses should undergo a stepwise assessment for late COVID-19 effects.

Introduction

Early in the COVID-19 pandemic, children and adolescents were thought to be important transmitters of the disease but were also believed to be only mildly affected.[1] Later, evidence increased that children are not major spreaders.[2,3,4] However, reports of a multiorgan immune syndrome in children and youths surfaced from Italy, the UK and the US,[5] occurring weeks to months after the SARS-CoV-2 wave. Studies in clinical settings[6] and assessments of registry data from different countries followed,[7] and suggested the association of the multiorgan immune syndrome and SARS-CoV-2 infections in children and youths.

The syndrome was named pediatric multiorgan immune syndrome (PMIS), pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) or multiorgan immune syndrome in children (MIS-C). It features many characteristics of the Kawasaki disease but it also showed some peculiar differences. PMIS ranges from 3 to 17 years while Kawasaki disease usually presents before the age of 5 years. Cardiac dysfunction and shock as well as gastrointestinal and neurologic symptoms are more frequent in PMIS.[8] Interestingly, an association of Kawasaki disease with coronavirus infections has been discussed even before the COVID-19 pandemic.[9]

Recent studies linked PMIS to the presence of antibodies to SARS-CoV-2 and some authors suggested that high levels of antibodies against SARS-CoV-2 may in fact contribute to the occurrence of the syndrome.[10] However, these observations were based on*post hoc* measurements of antibodies in children who had already acquired PMIS and had been hospitalized for severe symptoms.

We speculated that if strong antibody responses occur in children after SARS-CoV-2 infection, which could be causal for the onset of PMIS, they should be present before PMIS manifests or while the disease is still mild and thus, could be used to detect those children at risk to develop severe PMIS. Thus, we to screen a large number of children in rather severely affected areas of Bavaria (Southern Germany) for neutralizing and overall antibody levels against SARS-CoV-2 in the first pandemic wave. Children with significantly elevated antibody responses were evaluated clinically for signs of PMIS using a three-stage work-up protocol.

Methods

Study Design and population

This study has a cross-sectional design. We established a network of pediatricians who volunteered to take part in the study and focused on three areas/counties within Bavaria with very high, moderate and average infection rates as indicated by positive PCR tests per 100,000 inhabitants according to the Robert Koch Institute, the German center for disease prevention (figure 1). In areas where the number of willful study participants exceeded the capacity of local pediatricians, a study team supported sample collections. The sample collection took place in the three study areas (Tirschenreuth; Regensburg city and county; Oberbayern/ alpine region from May 22nd to July 22nd 2020 in a sliding window approach. Invitation to participate for children age 1 to 14 years was based on two approaches: (1) All children of that age group who were scheduled for a prevention program visit in 2020 with the respective pediatrician were invited to participate and (2) all children whose parents and the children themselves wanted to participate voluntarily were also tested. In those cases, also siblings older than 14 years were included into the study. The study was approved by the Ethics Committee of the University of Regensburg (file-number: 20-1865-101).

Data collection and management

All data were collected in an online survey using self-administered parental questionnaires. All acquired data was fully anonymized and only accessible at an individual level to the participant using an individual code on the Qnome platform (www.qnome.eu) as previously described in detail.[11] Clinical follow-up data on PMIS symptoms acquired by their pediatricians were entered in the dataset when participants gave permission for data-linking by providing their individual code for that purpose. That way, anonymization of data on the level of the dataset and study center persisted while individual therapy for the attending pediatrician was still possible.

SARS-CoV-2 antibody tests

Specific antibody response to SARS-CoV-2 was evaluated by the use of two different test kits: the commercially available, licensed qualitative Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, Rotkreuz, Switzerland; https://diagnostics.roche.com) and a validated and published in-house ELISA.[12] The Elecsys Anti-SARS-CoV-2 assay does not discriminate between the antibody type(s) present and can detect IgA, IgM, and IgG. The test is based on a recombinant nucleocapsid (N) antigen and has a cutoff value of 1.0 (S/Co). The in-house ELISA is based on SARS-CoV-2 S-protein's receptor-binding domain, quantifies total IgG and has a cutoff value of 1.0 (S/Co). The detected reactivity correlates with the SARS-CoV-2 neutralization titer as

described previously.[12] All samples with S/Co <1.0 were considered negative. In this experimental setting, strong antibody responses of S/Co [?]100 in the Elecsys Anti-SARS-CoV-2 test were considered for clinical follow-up for PMIS.

SARS-CoV-2 Neutralization Test

Neutralizing antibodies were evaluated by titration of sera against SARS-CoV-2 pseudotyped Vesicular Stomatitis Virus (VSV). The test is based on VSV-ΔG*FLuc pseudotyped with SARS-CoV-2-Spike-ΔER which correlates with SARS-CoV-2 neutralization as described previously.[13,14] Pseudoviral titers were determined by limited dilution and fluorescence microscopy. For all samples, a fixed inoculum of 25,000 ffu was neutralized for 1 h and luciferase activity was determined 20 h post infection of HEK293T-ACE2^{+}-cells. IC50 values were fitted using the algorithm: 'log (inhibitor) vs. normalized response' and further analyzed in GraphPad Prism 8 software (GraphPad Software, San Diego, USA).

PMIS evaluation protocol

We used a three-stage evaluation protocol that was developed based on information available on PMIS at the end of May 2020 and deduced from Kawasaki's syndrome. Children with strong antibody responses were asked to contact their caring pediatrician or, in cases when this was not possible, to approach study doctors being pediatric cardiologists and Kawasaki experts. The first stage of evaluation included clinical parameters: fever for three days or more, conjunctivitis, lymphadenopathy, exanthema, enanthema, skin pealing, oedema/ erythema of extremities, gastrointestinal problems, and arterial hypotension. The second stage comprised laboratory investigations such as urine examination for protein, leukocytes and erythrocytes and inflammatory markers in blood. At the third stage, screening for blood coagulopathies and cardiac effects (by echocardiography) were performed. The protocol used for this evaluation is accessible online (www.cokiba.we-care.de).

Statistical analyses

Descriptive statistics were calculated using frequencies (percentages) for categorical data and median (interquartile range) for metric data. Participants' characteristics and symptoms are presented stratified by antibody response. Differences between groups were analyzed using χ^2 tests for categorical variables and t-test for independent groups, respectively. All analyses were performed using SPSS.23.

Results

Overall, 2934 children participated in the study of whom 2906 were tested successfully with at least one of the two applied antibody tests and 2832 (96.5%) had also entered necessary study data in the online tool. Demographic data of the children participating in the study are given in table 1 and locations of test-centers across counties are depicted in figure 1.

Both tests consistently identified the same children with antibody responses with some exceptions (figure 2). A positive result in at least one of the two tests defined a positive case. Overall, 5.7% of successfully tested children were positive for SARS-CoV-2 antibodies: 138 were positive in both tests, one only in the Elecsys test and 23 by the in-house ELISA (figure 2).

Strong regional differences were observed in the prevalence of SARS-CoV-2 antibodies in children (figure 1). Overall, children in the heavily affected county of Tirschenreuth (with 1638 positive PCR tests/100.000 inhabitants) had positive antibody response 3-4 times more often than in the two other test regions. When only those children randomly selected (approach 1) and only one child (youngest) per family were included in the analysis, 7.2% of tested children where positive in Tirschenreuth, 3.1% in Regensburg and 1.8% in Oberbayern/ Alpine region. In those who volunteered for testing, e.g. due to symptoms that may have been related to COVID-19 or suspected contact to a COVID-19 patient (approach 2), 15.9% were found positive in Tirschenreuth, 2.3% in Regensburg and 7.8% in Oberbayern/ Alpine region, again taking only one child per family into account.

The older the children, the more positive SARS-CoV-2 tests were found, with 4.9% positive in the 0-6 year-olds (n=1299), 5.7% in the 7-10 year-olds (n=849) and 7.3% positive in the 11-17 year-olds (n=684). Children with chronic diseases tended to be slightly less often positive (4.3% of 344) than those without chronic diseases. Within the study population, 263 children had already received a SARS-CoV-2 PCR test previously and 21 had a positive test result. Of these, 15 individuals showed elevated antibody responses (71.4%) while in 6 subjects no response in any of the two tests could be found. 238 children lived in a household with a positively tested family member and of these 32.4% developed antibodies against SARS-CoV-2. Thus, living with a SARS-CoV-2 positive family member is the single most prominent association with a SARS-CoV-2 infection in children in our study population. We assessed symptoms potentially related with SARS-CoV-2 infections in our study population but found very few specific features that would allow to discriminate COVID-19 from common viral infections in children (supplementary table 1).

Next, we invited children with highly positive antibody responses to participate in a clinical follow-up examination (figure 3). For this study, in the absence of any better criteria for selection at the time point of designing the study, children with S/Co [?] 100 in the Elecsys Anti-SARS-CoV-2 were considered to have a strong overall antibody response, even though the test is licensed for a qualitative interpretation of results only. Values of that kind were observed in 3.4% of all children tested and in 69.8% of the positive cases. As testing was performed in an anonymous way (in which only the parents/ participants could link test results to the proband), children were invited through the study app to participate in a clinical follow-up with their pediatrician. Thus, positive and highly positive children could not be approached directly by the study team or their pediatrician and only those children who responded (52 out of 97, 53.6%) could be screened for signs and symptoms of PMIS similar to Kawasaki disease in a three-stage approach. Of these, 18 (34.6% of the responders and 18.6% of all those with highly elevated measurements in the Elecsys test) showed at least one clinical symptom related to PMIS; while 8 (15.4%/8.2%) showed two or more clinical signs. One child showed additional changes in the laboratory parameters (elevated lactate dehydrogenase (LDH)) while two presented with cardiac findings and one with excessive chronic fatigue.

As highlighted in figure 2, N-protein specific antibody titers (Elecsys) did not correlate with our in-house S-protein ELISA, which has been demonstrated earlier to nicely correlate with neutralization. In a*post hoc* analysis, we determined the levels of neutralizing antibody titers within this subgroup of 52 children, selected based on their high N-specific antibody signal with follow up data available. While the neutralizing titers correlated with the S-protein ELISA (Spearman's p=0.0002, R=0.495) no correlation could be found with the N-protein based Elecsys test (figure 4a, b). Analysing the distribution of neutralizing antibody titers (figure 4c), 48% (n=15) of children with neutralization capacity below mean turned out to be symptomatic, while only 15% (n=3) of children with high neutralizing activity showed symptoms (supplementary table 2). This effect was even more pronounced when IC50 values were split into more defined groups (figure 4d). This effect was also seen (to a lesser degree) for the S-protein based ELISA, confirming the observation that S-protein specific antibody titers correlate with neutralizing capacity of the tested sera. In sum, this subgroup analysis revealed significantly lower neutralization titers in symptomatic patients as compared to children (p=0.034), who did not develop signs and symptoms suggestive for PMIS.

Discussion

In our study, performed in regions of Germany with a relatively high incidence of COVID-19 in adults in the first phase of the pandemic, approximately 6% of children tested positive for SARS-CoV-2 antibodies in two tests directed against the N and S proteins of the virus. Of those children with high N-protein specific antibody levels, 18 showed signs and symptoms suggestive for mild to moderate forms of PMIS weeks to months after the suspected infection with SARS-CoV-2. Within this subgroup, sera of children with symptoms showed significantly lower neutralizing capacity as compared to sera from children without PMIS or PMIS-like symptoms.

This study was not primarily aimed to investigate representative prevalence rates of SARS-CoV-2 infections in children in Germany in the first wave,[15] but to answer the question if screening of children for SARS-CoV-2 antibodies may help to detect those at increased risk for PMIS. Despite the closing of schools, kindergartens

and nurseries very early on in the pandemic in Bavaria, a surprisingly high number of children showed antibodies in our study. One possible explanation for that could be that many parents who participated in the study suspected a coronavirus infection in their children due to symptoms or outbreaks in the community. Indeed, children were explicitly not tested in the beginning of the pandemic when test capacities were limited. Thus, the study may have addressed an unmet need of parents to get their children tested, which was further supported by the observation that participation in the study was overwhelming.

We used values beyond S/Co of 100 in the Elecsys test as a proxy for strong antibody responses, as this value is approximately the 97th percentile of all previous available test values (provided by Roche, personal communication). We are aware that the assay is a qualitative test but nevertheless, in the absence of a better measure to determine strong antibody responses at the early point in the pandemic, we applied it in the context of a study in this first screening approach. Surprisingly, almost 70% of seropositive children with a mild to asymptomatic course of the initial SARS-CoV-2 infection, had such values compared to only 21% of seropositive adults with mild disease in one of our previous studies.[16] These data may suggest that children mount stronger antibody responses to SARS-CoV-2 than adults on a regular basis. It can also be speculated that this may contribute to milder acute course of the initial infection as observed in children. In contrast, high levels of S-specific antibodies seem to worsen the outcome in severe courses of COVID-19 in adults.[17] This hypothesis should be further l investigated in longitudinal studies.

Many children show values of S/Co beyond 100 in the Elecsys test, and a similar observation was made for the S protein based in-house test, where also high values were observed in more than half of the positively tested individuals. As we included only children with strong N-specific antibody responses in our follow-up, we may have missed some children that developed PMIS. Due to the anonymous design of our study, we cannot go back to study participants and extend the follow-up to those with lower antibody values. However, this would be needed to investigate possible cutoff points in antibody responses, which could predict PMIS with greater specificity and/or sensitivity. Therefore, it seems a good strategy at this point to evaluate all children who show antibody responses to SARS-CoV-2 for PMIS symptoms.

Our study indicates that very few symptoms are specific for COVID-19 in children. On the other hand, only 23% of the 162 children with detectable SARS-CoV-2 antibodies were free of symptoms in the weeks before the antibody test. Interestingly, even children as young as 6 years of age were able to indicate loss of smell and taste - the only specific symptom for COVID-19 we could identify in children. Thus, screening for SARS-CoV-2 infections in children by symptoms does not seem to be useful.

A large number of children acquired antibodies against SARS-CoV-2 when family members had developed COVID-19. Therefore, we suggest that children confronted with COVID-19 in the household should systematically be screened for SARS-CoV-2 antibody responses, e.g. 4 weeks after the diagnosis in the index case, thereby not missing out on potential childhood SARS-CoV-2 infections despite of mild or absent symptoms in children.

In our study population, at least 2 children showed PMIS with cardiac affection (A-V block grade I; thoracic pain and left axis deviation), even though the children were not recognized to be sick enough to be referred to the hospital. Of note, our study also identified 2 siblings of study participants (tested positive for SARS-CoV-2 antibodies outside the study) with cardiac PMIS symptoms. Furthermore, it became clear in the course of the study that initially mildly affected children suffer from long-term COVID-19 effects. As reported in adults ,[18] we identified a case of chronic fatigue syndrome timely associated with SARS-CoV-2 infection. Thus it seems necessary to follow up children after a COVID-19 infection for neuropsychological effects of COVID-19.

We found a significant correlation between PMIS / PMIS-like symptoms and low neutralization titers within a subgroup of children with high titers of N-specific antibodies. Lower neutralization titers were also found in severe PMIS compared to active COVID-19.[19] Our observation that lower neutralization capacities are associated even with mild PMIS symptoms suggests that this may at least contribute if not cause the development of PMIS. Further studies need to confirm the predictive value of high N-specific antibodies

and clarify whether or not high N-specific antibody titers and low neutralization capacity are independent predictors for the development of PMIS and PMIS-like symptoms.

Based on our results we propose to screen children from households with COVID-19 cases on a regular basis for SARS-CoV-2 antibodies as well as children from areas with high prevalence of COVID-19, if any symptoms suggestive for COVID-19 occur. Those that are found to have antibodies, no matter if S protein or N protein directed, should be offered a clinical follow-up for PMIS. Based on the experience with Kawasaki disease,[20] early intervention seems to have the potential to avoid long-term or chronic illness also in PMIS.

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Tables and Figures

Table 1:

Characteristics of study participants stratified for antibody (AB) test result

GENERAL CHARACTERISTICS	Negative AB test (N=2670)	Positive AB test (N=162)	p
Study participation due to			
... regular health check-up, % (N)	66.0 (1763)	32.1 (52)	
... COVID-19 symptoms/contacts, % (N)	34.0 (907)	67.9 (110)	<.001*
Sex (male), % (N)	51.7 (1380)	50.6 (82)	.792
Age (years) (Md, IQR)	7 (4.0-10.0) (range 0-17)	8 (4.7-11.0) (range 0-16)	.070
Any chronic disease, % (N)	12.3 (329)	9.3 (15)	.247
Does your child usually attend...			
Nursery, % (N)	6.1 (163)	4.9 (8)	
Kindergarten, % (N)	27.5 (733)	23.5 (38)	
Elementary school, % (N)	30.3 (809)	29.0 (47)	
Secondary school (Mittelschule), % (N)	4.9 (130)	9.9 (16)	
Secondary school (Realschule), % (N)	8.5 (227)	11.1 (18)	
Grammar school, % (N)	11.0 (295)	9.9 (16)	
School for special needs, % (N)	0.6 (17)	1.2 (2)	
None of them, % (N)	11.1 (296)	10.5 (17)	.138
SARS-CoV-2 PCR testing, % (N)	8.8 (234)	17.9 (29)	<.001*
Positive SARS-CoV-2 PCR test, % (N)	0.2 (6)	9.3 (15)	<.001*
Hospitalization due to COVID-19, % (N)	0.2 (6)	1.2 (2)	.019*
Household member COVID-19, % (N)	6.0 (161)	47.5 (77)	<.001*
Any symptom, % (N)	70.1 (1871)	76.5 (124)	.080

Notes: *: p <.05; chi^2 test, t-test for independent groups IQR - interquartile range, Md - median

Figure 1:

Map of Bavaria with location of centers contributing to the survey (red dots) and COVID-19 prevalence until July 2020 (color coded by county). Number for overall, negatively and positively tested children are given in the circle chart

Figure 2:

Comparison between the N protein directed Elecsys Anti-SARS-CoV-2 assay (total Ig) and the S protein directed in-house SARS-CoV-2 assay detecting IgG (IgG) in the total study population (N=2832). Strong

dotted lines represent the assay cutoff values, ±10% borderline intervals (gray areas). Signal-to-cutoff (S/Co) ratios are given for both assays.

Figure 3:

Study flowchart (upper panel) and findings from the follow-up of SARS-CoV-2 seropositive children for PMIS symptoms in the CoKiBa study (lower panel)

Legend: * only those 52 individuals who actively contacted their pediatrician could be included into the follow up. Due to the anonymous design of the study no reminder was possible. ** Individuals could report more than one symptom. AB is antibody, LDH is lactate dehydrogenase

Figure 4:

Neutralizing and S-protein specific binding antibody titer analysis of children from follow up with N-specific signal S/Co>100

Legend: **A.** Correlation of N-specific antibody signal (Elecsys, S/Co) with neutralizing antibody titers. **B.** Correlation of S-protein ELISA binding antibody titers (S/Co) with neutralizing antibody titers. **C.** Dotplot representation of the neutralizing antibody titers of all children examined in the follow up study, visualizing symptomatic children by dot colour and shape. **D** . Pie chart distribution of symptomatic children grouped according to neutralizing antibody titer. **E.** Comparison of the neutralization capacity of symptomatic and asymptomatic children.