Serum IgG levels in children 6 months after SARS-CoV-2 infection and comparison with adults

Silvia Bloise1 · Alessia Marcellino1 · Alessia Testa1 · Anna Diliillo1 · Saverio Mallardo1 · Sara Isoldi1 · Vanessa Martucci1 · Maria Teresa Sanseviero1 · Emanuela Del Giudice1 · Donatella Iorfida1 · Flavia Ventriglia1 · Riccardo Lubrano1

Abstract
Since the outbreak of SARS-CoV-2 among the population has occurred quite recently, there is a lack of evidence on the long-term duration of antibody response, especially in children. It is therefore crucial to clarify this aspect, considering its implications in the development of successful surveillance strategies, therapies, and vaccinations. The aim of this study was to assess the antibody response in a children group after SARS-CoV-2 infection, and to compare it with that of their parents affected by SARS-CoV-2 infection. We enrolled 12 children and their parents, both groups being affected by COVID-19 in April 2020. In the children’s group, we collected real-time RT-PCR cycle threshold (Ct) values and gene characterization of first nasal-throat swab at the time of diagnosis (T0); 30 days after the diagnosis (T30), we performed blood tests to detect anti-SARS-CoV-2 IgM and IgG. Finally, 180 days after the diagnosis (T180), we measured anti-SARS-CoV-2 IgG in both children and parents. In children, antibody levels declined significantly at 180 days (T180) after first measurement (T30). There were no significant differences in IgG level related to age, sex, and clinical manifestations. We found a significant correlation between IgG titers at T30 and Ct value of gene N. Children showed a lower level of antibodies against SARS-CoV-2 at T180 compared to their parents.

Conclusion: Antibody responses in children waned 180 days after SARS-CoV-2 infection, and at the same time, their parents showed a different antibody response to the virus. These results highlight that serological tests should be used with caution in surveillance strategies among the general population.
What is known:
• Currently is not known how long antibody response will be maintained or if it protects from reinfection.
• Recent reports in adults suggest that antibodies to SARS-CoV-2 declined several months after infection, but data are missing in pediatric age.

What is new:
• We showed that antibody responses to SARS-CoV-2 wane several months after infection also in children with quantitative differences in antibody levels between children and adults.
• In this context, serological tests should be used with caution in surveillance strategies.

Keywords  Antibody response  •  Children  •  COVID-19  •  Immunity

Abbreviations
COVID-19  Coronavirus disease 2019
E  Envelope protein
RT-PCR  Real-time reverse transcription polymerase chain reaction
Ct  Real-time-PCR cycle threshold
SARS-CoV-2  Severe acute respiratory syndrome coronavirus 2
capable of controlling the infection, and to provide further information for the development of successful surveillance strategies, therapies and vaccines.

For these reasons, we conducted a study to evaluate the antibody response in a children group after SARS-CoV-2 infection, also comparing the antibody response with that of their parents, also affected by SARS-CoV-2 infection.

Materials and methods
This was a prospective cohort study conducted at the Pediatric Unit of Santa Maria Goretti Hospital, in Latina—Sapienza University of Rome (Polo Pontino) between May 2020 and October 2020.

Recruitment was conducted within the geographical area of the province of Latina by general pediatricians, who were engaged via email invitations to participate in the study. All children who resulted positive for SARS-CoV-2 infection at nasopharyngeal swab test in April 2020 were offered to participate in the study if at least one of their parents was infected as well; also, in parents the infection was detected by nasal-throat swab test for SARS-CoV-2 nucleic acid by Real-time reverse transcription PCR (rRT-PCR). All those who consented to the study protocol, which included serology testing in children 1 month after the initial infection, and a serology testing both in children and their parents 6 months after the initial infection, were consecutively enrolled.

All participants were interviewed on symptoms and signs of infection and on the possible existence of other family members infected with SARS-CoV-2. We investigated the presence of comorbidities; risk factors, as obesity (defined by BMI $\geq$ 30 kg/m$^2$) and smoking (smoker was defined by having smoked at least 100 cigarettes in his or her lifetime, being a smoker at the time of the interview, or having quit smoking for less than 6 months).

Furthermore, we investigated the use of medications influencing antibody response.

In the children’s group, we collected real-time-PCR cycle threshold (Ct) values and gene characterization of first nasal-throat swab at the time of diagnosis (T0); 30 days after the diagnosis (T30), we performed another nasal-throat swab and blood tests to detect anti-SARS-CoV-2 IgM and IgG. Finally,
180 days after the diagnosis (T180), we measured anti-SARS-CoV-2 IgM and IgG both in children and parents.

The primary aim of our study was to describe the dynamic changes of serum antibody levels against SARS-CoV-2 30 days and 180 days after the infection in children.

Secondary aims were to evaluate possible demographic, clinical, and laboratory factors influencing the antibody response in our pediatric cohort and to compare it with that of their parents, 180 days after the infection.

Laboratory test

The infection was detected by nasal-throat swab test for SARS-CoV-2 nucleic acid by real-time reverse transcription PCR (rRT-PCR) targeting three genes: envelope protein (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid protein (N).

The STARMag 96 × 4 Universal Cartridge Kit (Seegene Inc.) was used to extract total RNA, and gene fragments were detected by Alplex TM 2019 n-CoV assay (Seegene Inc.).

The cycle threshold (Ct) values of rRT-PCR represent the number of replication cycles required to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads.

According to the manufacturer’s instructions, samples with a Ct value < 40 were regarded as SARS-CoV-2 detected, and a Ct value < 40 for only one of the three targets was considered positive.

The serum antibodies against SARS-CoV-2 were detected by chemiluminescent immunoassay (CLIA), using the iFlash Immunoassay Analyzer (YHLO Biotech Co., Ltd, Shenzhen, China).

The iFlash-SARS-CoV-2 IgM and IgG assay used present a sensitivity of 97.3% and specificity 96.3% [14]. These antibodies are against nucleocapsid and spike protein.

The antibody levels were expressed as arbitrary unit per ml (AU/ml). The results ≥10 AU/ml were considered positive, while the results < 10 AU/ml negative.

Statistical analysis

The statistical analysis was performed with JMP® 15.2.0 program for Mac (SAS Institute Inc.). For all variables the approximation of population distribution to normality was tested by Kolmogorov-Smirnov One-Sample Test and statistics for kurtosis and symmetry. Because the results were asymmetrical distributed all data were expressed as median, 25° and 75° quartiles and consequently presented as box and whisker plot. For the analysis of the differences between the group of the study the non-parametric Wilcoxon tests was used. For the correlation of continuous variables Pearson’s or Spearman’s coefficient was calculated according to the distribution.

A descriptive analysis using percentage values was performed for qualitative variables.

A p value < 0.05 was considered significant.

Results

Demographic, clinical, and virological characteristics of the patients

We enrolled 12 children and 12 parents. In the children group, 7 subjects were male and 5 were females; the median age was 13.37 (9.6–14.3) years.

In the children group two patients were asymptomatic (17%), while 10 (83%) suffered from a mild clinical condition, showing fever (80%), acute upper respiratory symptoms (25%), gastrointestinal symptoms (25%), and other symptoms, as myalgia, ageusia, anosmia and headache (60%). Children comorbidities were allergic rhinitis (one patient) and coeliac disease (one patient).

In the parent group, 7 subjects were males and 5 were females; the median age was 47 (40.5–51.2) years.

All parents were symptomatic, showing fever (100%), upper respiratory symptoms (83%), gastrointestinal symptoms (25 %), and other symptoms, as myalgia, ageusia, anosmia, and headache (83%). Three parents showed cough and dyspnea and were hospitalized with diagnosis of pneumonia.

Parent’s comorbidities were allergic rhinitis (one parent) and hypertension (two parents).

Furthermore, four parents were smokers.

No patients enrolled were taking medications influencing antibody response.

Patients’ demographic and clinical characteristics are summarized in Table 1.

The genomic characterization and RT-PCR cycle threshold (Ct) values of nasal-throat swabs of children at T0 are summarized in Table 2.

Levels of serum antibodies at T30 and at T180 in children

As shown in Fig. 1, the IgM and the IgG levels declined significantly (IgM: p < 0.0262; IgG: p < 0.0001) at 180 days (T180) compared to previous measurement (T30).

At T30, the median IgM level was 1.29 (1.02–1.47) AU/ml; at T180, it was 0.74 (0.64–1.01) AU/ml.

At T30, the median IgG level was 90.61 (71.5–101) AU/ml; at T180, it was 16.53 (9.1–24.1) AU/ml.

At T30, no child had detectable IgM levels, while all children (100%) showed positive IgG levels.
At T180, no child had detectable IgM levels, while 9 children (75%) showed positive IgG levels.

**Relationship between antibody response and demographic, clinical, and virological characteristics in children**

There were no significant difference in IgG levels related to age ($r^2 =0.27$, $p=0.07$), sex ($p=0.56$), presence of symptoms (fever, $p=0.87$; respiratory symptoms, $p=0.40$; gastrointestinal symptoms, $p=0.87$; others symptoms, $p=0.63$) and duration of symptoms ($r^2 =0.10$, $p=0.63$). Instead, analyzing the correlation between IgG level and virological characteristics, we observed that children positive for E gene have significantly higher titers of IgG levels (100.51 (116.59–92.36) AU/ml vs 72.16 (57.54–80.66) AU/ml, $p < 0.02$), while the IgM levels did not show significant variations (1.29 (1.01–1.42) AU/ml vs 1.26 (0.91–1.76) AU/ml, $p=0.77$). Moreover, we found a significant correlation between IgG titers at T30 and Ct value of gene N ($r^2 =0.38$, $p<0.033$) (Fig. 2), while correlations between IgG titers at T30 and Ct value of gene E and gene RdP were not significant ($r^2 =0.026$, $p=0.61$; $r^2 =0.026$, $p=0.095$).

**Comparison of children’s antibody levels, IgG, and IgM with their parents at T180**

At T180, children showed lower level of IgG antibodies against SARS-CoV-2 compared to their parents ($p <0.0001$), while IgM levels were similar ($p=0.93$) (Fig. 3).

In children, the median IgM level was 0.74 (0.64–1.01) AU/ml; in parents, the median IgM level was 0.83 (0.53–1.19) AU/ml.

In children, the median IgG level was 16.5 (9.1–24.1) AU/ml; in parents, the median IgG level was 92.7 (44.1–163.3) AU/ml.

At T180, no child or parent had detectable IgM levels, while all parents (100%) and 9 children (75%) showed positive IgG levels.

**Discussion**

There is a lack of evidence regarding the long-term duration of antibody response against SARS-CoV-2, especially in children.
In adult patients, recent reports suggest that antibody response to SARS-CoV-2 declined significantly in the 3 months following SARS-CoV-2 infection [15–19].

In our study, we observed a significant decay of antibody response also in pediatric age 6 months after SARS-CoV-2 infection. Nevertheless, we think that these data should not be considered alarming, since the absence of specific antibodies does not mean absence of immune memory. In fact, our immune system has several strategies to ensure an immune memory, as T cell.

Currently, different studies showed that T cells are also implied during SARS-CoV-2 infection and polyfunctional T cells (PFC) with a stem-like memory phenotype were detectable both in convalescent patients and in blood samples from people who had not been exposed to the virus [20–22].

Differently from adults, whose antibody levels seem to correlate with the severity of the disease [23–25], in our pediatric cohort we found no correlation in antibody levels related to presence of symptoms; This may be in line with what has been reported in the literature, considering that all our symptomatic children suffered from a mild clinical condition and nobody developed severe complications of disease.

Instead, analyzing the virological characteristics of our patients, we observed that children positive for E gene had significantly higher titers of IgG level at T30. We hypothesized that the highest levels of antibodies in this group of children were due to a higher viral load. In fact, the majority of children with E gene was also positive for the other two genes researched; furthermore, we found a significant correlation with the Ct of N gene. This aspect sustained our hypothesis, considering the relation between Ct value and viral copy number value [26, 27].

Finally, our results showed quantitative difference in the anti-SARS-CoV-2 specific antibody response between children compared with their parents, supporting the results of a recent study of Weisberg et al. [28], where the authors demonstrated a reduced protective serological response in children compared to adults. These data support the hypothesis of a distinct immune response in pediatric age that could be at the base of lower susceptibility to SARS-CoV-2 in children. This could be related to a more robust innate immune response resulting in an efficacious viral clearance and consequent reduced acquired immune response [29]. Since the outbreak occurred many authors sustained this hypothesis [30, 31] and recently the research in this area has allowed to identify biological and cellular characteristics at the base of age-related different host immune responses. Perce et al. [32] demonstrated that pediatric patients had higher serum concentrations of interleukin-17A and interferon-γ shortly after infection, contributing to immune protection, particularly against lung disease. Instead, other authors focused on early and more effective polyclonal innate or IgM memory B cell response [33], or increased number of naïve T cells [28] that make children capable of a more rapid reaction to new pathogens.
Our study presents several limitations: the small sample size that was composed by children with mild infection, not requiring hospitalization; the lack of discrimination between antibodies against N and S protein of SARS-CoV-2; the lack of the data related to antibodies level of adults at 30 days after the infection.

However, given that parents and their children are often infected in the same household, we arguably recorded the immune response against the same strain of virus.

Conclusion

Our study showed that antibody responses wane after SARS-CoV-2 infection also in pediatric age.

However, pending new evidences about the duration of immunity, we think that preventive measures, as universal facial masking, should be implemented also in children to limit the spread of infection [34].

Furthermore, the reduced antibody response in children compared with their parents confirmed a distinct infection course between the two age groups and corroborated the hypothesis of an age-related immune response, with a more robust innate immune response in the pediatric population.

Finally, analogously to adults, the rapid decline of antibody levels in children should prompt to cautiously consider serological tests for surveillance strategies among the general population.

Authors’ contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Dr Silvia Bloise, Dr Alessia Marcellino, Dr Alessia Testa, Dr Vanessa Martucci, Dr Sara Isoldi, Dr Donatella Iorlida, Dr Anna Dilillo, Dr Emanuela Del Giudice, Dr Maria Teresa SanSeviero, Dr Saverio Mallardo, and Dr Flavia Ventriglia.

The first draft of the manuscript was written by Dr Silvia Bloise and Dr Alessia Marcellino and all authors commented on previous versions of the manuscript.

Prof. Riccardo Lubrano conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

All authors read and approved the final manuscript.

Funding Open access funding provided by Università degli Studi di Roma La Sapienza within the CRUI-CARE Agreement.

Data availability All data and materials support published claims and complied with field standards

Code availability N/A

Declarations

Ethics approval and consent to participate N/A. Informed consent was obtained from all individual participants included in the study.

Consent for publication Patients signed informed consent regarding publishing their data

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. World Health Organization Press Conference. The World Health Organization (WHO) has officially named the disease caused by the novel coronavirus as COVID-19. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
26. Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, Gao G, Wang S, Ma C, Xie R, Wang F, Tan C, Zhu L, Guo Y, Zhang F (2020) Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis 71(15):793–798. https://doi.org/10.1093/cid/ciaa345

27. Sethuraman N, Jeremiah SS, Ryo A (2020) Interpreting diagnostic tests for SARS-CoV-2. JAMA. 323(22):2249–2251. https://doi.org/10.1001/jama.2020.8259

28. Weisberg SP, Connors T, Zhu Y, Baldwin M, Lin WH, Wontakal S, Szabo PA, Wells SB, Dogra P, Gray JI, Idzikowski E, Bovier F, Davis-Porada J, Matsumoto R, Li Poon MM, Chait MP, Mathieu C, Horvat B, Decimo D, Bitan ZC, La Carpia F, Ferrara SA, Mace E, Milner J, Moscona A, Hod EA, Porotto M, Farber DL (2020) Antibody responses to SARS-CoV2 are distinct in children with MIS-C compared to adults with COVID-19. medRxiv; 2020.07.12.20151068. https://doi.org/10.1101/2020.07.12.20151068.

29. Kikkert M (2020) Innate immune evasion by human respiratory RNA viruses. J Innate Immun 12(1):4–20. https://doi.org/10.1159/000503030

30. Cristiani L, Mancino E, Matera L, Nenna R, Pierangeli A, Scagnolari C, Midulla F (2020) Will children reveal their secret? The coronavirus dilemma. Eur Respir J 55(4):2000749. https://doi.org/10.1183/13993003.00749-2020

31. De Luca CD, Esposito E, Cristiani L, Mancino E, Nenna R, Cortis E, Midulla F (2020) Covid-19 in children: a brief overview after three months experience. Paediatr Respir Rev 35:9–14. https://doi.org/10.1016/j.prrv.2020.05.006

32. Pierce CA, Preston-Hurlburt P, Dai Y, Aschner CB, Cheshenko N, Galen B, Garforth SJ, Herrera NG, Jangra RK, Morano NC, Orner E, Sy S, Chandran K, Dziura J, Almo SC, Ring A, Keller MJ, Herold KC, Herold BC (2020) Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. Sci Transl Med 12(564):eabd5487. https://doi.org/10.1126/scitranslmed.eabd5487

33. Carsetti R, Quintarelli C, Quinti I, Piano Mortari E, Zumla A, Ippolito G, Locatelli F (2020) The immune system of children: the key to understanding SARS-CoV-2 susceptibility. Lancet Child Adolesc Health 4(6):414–416. https://doi.org/10.1016/S2352-4642(20)30135-8

34. Lubrano R, Bloise S, Testa A, Marcellino A, Dilillo A, Mallardo S, Isoldi S, Martucci V, Sanseverio M, Del Giudice E, Malvasi C, Iorfida D, Ventriglia F (2021) Assessment of respiratory function in infants and young children wearing face masks during the COVID-19 pandemic. JAMA Netw Open 4(3):e210414. https://doi.org/10.1001/jamanetworkopen.2021.0414

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.