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ORIGINAL ARTICLE

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

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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.

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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

Background

At the end of 2019, a novel coronavirus was identified as responsible for a cluster pneumonia in Wuhan, Hubei Province, China. The evaluation with real-time polymerase chain reaction (RT-PCR) of bronchoalveolar lavage samples from a patient led to identify the etiologic cause: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded RNA virus, belonging to the b-coronavirus genus. In February 2020, the World Health Organization named the disease associated to its infection as the 2019 novel coronavirus disease (COVID-19), and in March, the outbreak was officially declared as a public health emergency of international concern [1].

Most of the published reports have primarily focused on the disease features; however, more recently, the attention and research shifted to the investigation of the host immune response to the virus.

Similar to other viral infections, SARS-CoV-2 stimulates an innate immune response and a subsequent adaptive immune response with development of neutralizing antiviral T cell and antibody [2].

Neutralizing antibodies are crucial for the establishment of a protective immunity [3, 4], but is not yet currently known how long antibody responses will be maintained or if humoral immune response protects from reinfection. In fact, the dynamics of humoral immune responses in pediatric age still remain unknown.

There is a growing need to clarify these issues, considering the lowest susceptibility to COVID-19 of children compared with adult population [5–13]; therefore, study their immune response could be important to understand the mechanism 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 Allplex 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 asymmetrically 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.

Table 1 Patients' demographic and clinical characteristics

	Children	Parents
Gender (males/females)	7/5	7/5
Age (median (25–75°))	13.37	47
Children (years)	9.6–14.3	40.5–51.2
Parents (years)		
Caucasians (*N* (%))	12 (100%)	12 (100%)
Comorbidities (*N* (%))	2 (17%) Allergic rhinitis, coeliac disease	3 (25%) Allergic rhinitis, hypertension
Risk factors (*N* (%))	0 (0%)	0 (0%)
- Obesity (BMI≥30)	0 (0%)	4 (33%)
- Smoking		
Symptoms	10 (80%)	12 (100%)
- Fever (TC ≥ 37.5 C°) (*N* (%))	3 (25%)	10 (83%)
- Upper respiratory symptoms (*N* (%))	3 (25%)	3 (25%)
- Gastrointestinal symptoms (*N* (%))	7 (60%)	10 (83%)
- Others (myalgia, ageusia, anosmia, and headache) (*N* (%))	0 (0%)	3 (25%)
- Cough and dyspnea (*N* (%))		
Hospitalized (*N* (%))	0 (0%)	3 (25%)

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).

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

Children	E gene-Ct	Gene RdRp-Ct	Gene N-Ct
1	37.05	40	40
2	21.64	21.88	24.38
3	40	40	36.63
4	40	40	38.70
5	40	40	35.88
6	40	33.85	35.55
7	17.24	19.34	20.07
8	40	33.40	34.22
9	25.86	26.93	29.27
10	30.44	31.45	33.50
11	30.54	34.78	33.77
12	40	18.17	40

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).

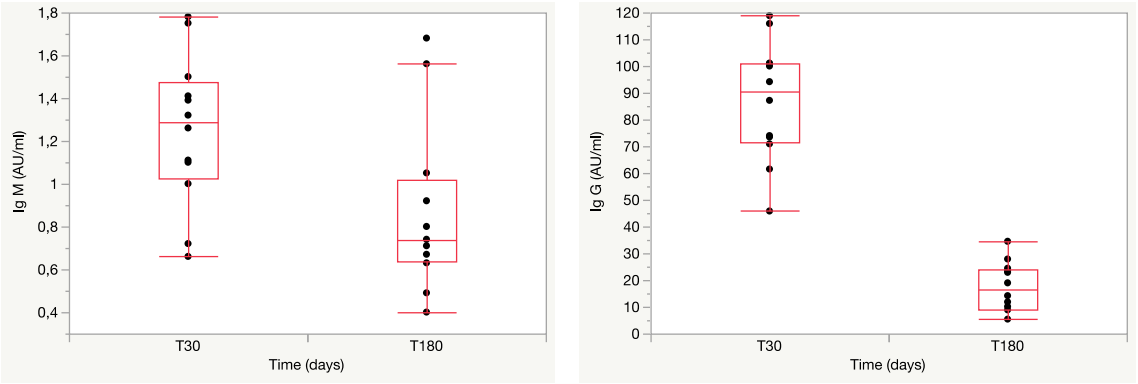
In children, the median IgM level was 0.74 (0.64–1.01) AU/ml; in parents, the median IgM level was 0.83 (0.53–1.19) AU/ml.

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.



Ig M Ig G

Fig. 1 Decline of IgM and IgG antibody levels in children 180 days after SARS-CoV-2 infection. The boxes include value of median, 25° and 75° quartiles; the whiskers include 10° and 90° quartiles

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 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.

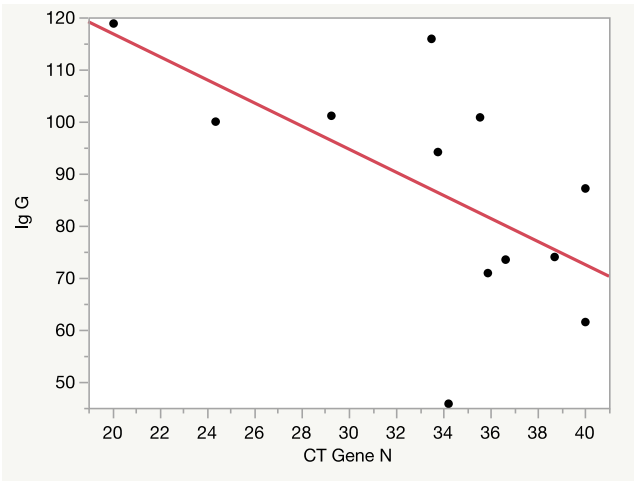


Fig. 2 Correlation between anti-SARS-CoV-2 IgG titers at T30 and Ct of gene N in children

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.

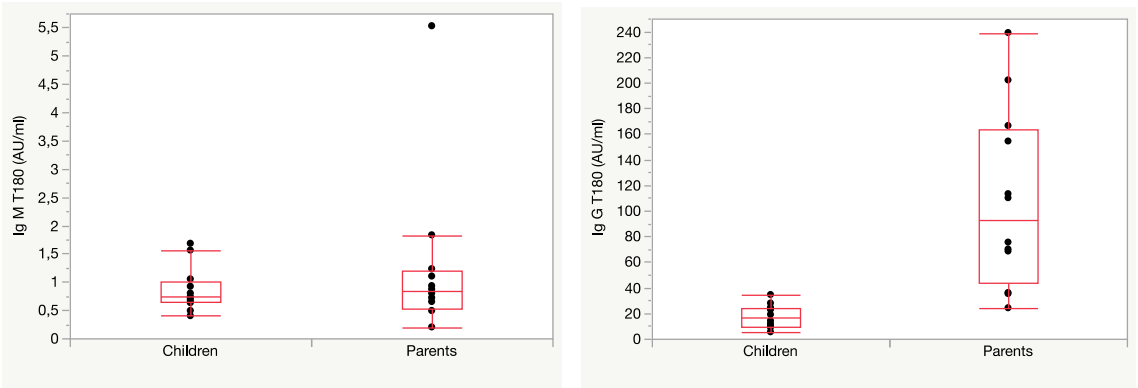


Fig. 3 Comparison of children's IgM and IgG antibody levels with their parents at T180. The boxes include value of median, 25° and 75° quartiles; the whiskers include 10° and 90° quartiles

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 Iorfida, 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.

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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.

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.

2. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen MC, O'Mahony L, Gao Y, Nadeau K, Akdis CA (2020) Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy 75(7):1564–1581. https://doi.org/10.1111/all.14364
3. Lanzavecchia A, Frühwirth A, Perez L, Corti D (2016) Antibody-guided vaccine design: identification of protective epitopes. Curr Opin Immunol 41:62–67. https://doi.org/10.1016/j.coi.2016.06.001
4. Corti D, Lanzavecchia A (2013) Broadly neutralizing antiviral antibodies. Annu Rev Immunol 31:705–742. https://doi.org/10.1146/annurev-immunol-032712-095916
5. Ludvigsson JF (2020) Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr 109(6):1088–1095. https://doi.org/10.1111/apa.15270
6. Liu YJ, Chen P, Liu ZS, Li Y, Du H, Xu JL (2020) Clinical features of asymptomatic or subclinical COVID-19 in children. Zhongguo Dang Dai Er Ke Za Zhi 22(6):578–582. https://doi.org/10.7499/j.issn.1008-8830.2004088
7. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, Tong S (2020) Epidemiology of COVID-19 among children in China. Pediatrics. 145(6):e20200702. https://doi.org/10.1542/peds.2020-0702
8. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, Zhang W, Wang Y, Bao S, Li Y, Wu C, Liu H, Liu D, Shao J, Peng X, Yang Y, Liu Z, Xiang Y, Zhang F, Silva RM, Pinkerton KE, Shen K, Xiao H, Xu S, Wong GWK (2020) SARS-CoV-2 infection in children. N Engl J Med 382:1663–1665. https://doi.org/10.1056/NEJMc2005073
9. Zimmermann P, Curtis N (2020) Coronavirus infections in children including COVID-19 an overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. Pediatr Infect Dis J 39(5):355–368. https://doi.org/10.1097/INF.0000000000002660
10. Sun D, Li H, Lu XX, Xiao H, Ren J, Zhang FR, Liu ZS (2020) Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: a single center's observational study. World J Pediatr 16(3):251–259. https://doi.org/10.1007/s12519-020-00354-4
11. Zheng F, Liao C, Fan QH, Chen HB, Zhao XG, Xie ZG, Li XL, Chen CX, Lu XX, Liu ZS, Lu W, Chen CB, Jiao R, Zhang AM, Wang JT, Ding XW, Zeng YG, Cheng LP, Huang QF, Wu J, Luo XC, Wang ZJ, Zhong YY, Bai Y, Wu XY, Jin RM (2020) Clinical characteristics of children with coronavirus disease 2019 in Hubei. China Curr Med Sci 40(2):1–6. https://doi.org/10.1007/s11596-020-2172-6
12. Wang D, Ju XL, Xie F, Lu Y, Li FY, Huang HH, Fang XL, Li YJ, Wang JY, Yi B, Yue JX, Wang J, Wang LX, Li B, Wang Y, Qiu BP, Zhou ZY, Li KL, Sun JH, Liu XG, Li GD, Wang YJ, Cao AH, Chen YN Clinical analysis of 31 cases of 2019 novel coronavirus infection in children from six provinces (autonomous region) of northern China. Chinese J Pediatr 58(4). https://doi.org/10.3760/cma.j.cn112140-20200225-00138
13. Isoldi S, Mallardo S, Marcellino A, Bloise S, Dilillo A, Iorfida D, Testa A, Del Giudice E, Martucci V, Sanseviero M, Barberi A, Raponi M, Ventriglia F, Lubrano R (2021) The comprehensive clinic, laboratory, and instrumental evaluation of children with COVID-19: a 6-months prospective study. J Med Virol 93(5):3122–3132. https://doi.org/10.1002/jmv.26871
14. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, Lan Z, Law S, MacLean E, Trajman A, Menzies D, Benedetti A, Ahmad KF (2020) Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 370:m2516. https://doi.org/10.1136/bmj.m2516
15. Murchu OE, Byrne P, Walsh KA, Carty PG, Connolly M, De Gascun C, Jordan K, Keoghan M, O'Brien KK, O'Neill M, Smith SM, Teljeur C, Ryan M, Harrington P (2020) Immune response following infection with SARS-CoV-2 and other coronaviruses: a rapid review. Rev Med Virol 23:e2162. https://doi.org/10.1002/rmv.2162
16. Zhou W, Xu X, Chang Z, Wang H, Zhong X, Tong X, Liu T, Li Y (2020) The dynamic changes of serum IgM and IgG against SARS-CoV-2 in patients with COVID-19. J Med Virol 93:924–933. https://doi.org/10.1002/jmv.26353
17. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, Hu JL, Xu W, Zhang Y, Lv FJ, Su K, Zhang F, Gong J, Wu B, Liu XM, Li JJ, Qiu JF, Chen J, Huang AL (2020) Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 26(8):1200–1204. https://doi.org/10.1038/s41591-020-0965-6
18. Wang X, Guo X, Xin Q, Pan Y, Hu Y, Li J, Chu Y, Feng Y, Wang Q (2020) Neutralizing antibodies responses to SARS-CoV-2 in COVID-19 inpatients and convalescent patients. Clin Infect Dis 4:ciaa721. https://doi.org/10.1093/cid/ciaa721
19. Seow J, Graham C, Merrick B, Acors S, Pickering S, KJA S, Hemmings O, O'Byrne A, Kouphou N, Galao RP, Betancor G, Wilson HD, Signell AW, Winstone H, Kerridge C, Huettner I, Jimenez-Guardeño JM, Lista MJ, Temperton N, Snell LB, Bisnauthsing K, Moore A, Green A, Martinez L, Stokes B, Honey J, Izquierdo-Barras A, Arbane G, Patel A, Tan MKI, O'Connell L, O'Hara G, MacMahon E, Douthwaite S, Nebbia G, Batra R, Martinez-Nunez R, Shankar-Hari M, Edgeworth JD, SJD N, Malim MH, Doores KJ (2020) Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol 5(12):1598–1607. https://doi.org/10.1038/s41564-020-00813-8
20. Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, Okba NMA, Endeman H, van den Akker JPC, Molenkamp R, Koopmans MPG, van Gorp ECM, Haagmans BL, de Swart RL, Sette A, de Vries RD (2020) Phenotype and kinetics of SARS-CoV-2-specifc T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol 5(48):eabd2071. https://doi.org/10.1126/sciimmunol.abd2071
21. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, Rawlings SA, Sutherland A, Premkumar L, Jadi RS, Marrama D, de Silva AM, Frazier A, Carlin AF, Greenbaum JA, Peters B, Krammer F, Smith DM, Crotty S, Sette A (2020) Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 181(7):1489–1501.e15. https://doi.org/10.1016/j.cell.2020.05.015
22. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, Llewellyn-Lacey S, Kamal H, Bogdanovic G, Muschiol S, Wullimann DJ, Kammann T, Emgård J, Parrot T, Folkesson E, Karolinska COVID-19 Study Group, Rooyackers O, Eriksson LI, Henter JI, Sönnerborg A, Allander T, Albert J, Nielsen M, Klingström J, Gredmark-Russ S, Björkström NK, Sandberg JK, Price DA, Ljunggren HG, Aleman S, Buggert M (2020) Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 183(1):158–168.e14. https://doi.org/10.1016/j.cell.2020.08.017
23. Zhang B, Zhou X, Zhu C, Song Y, Feng F, Qiu Y, Feng J, Jia Q, Song Q, Zhu B, Wang J (2020) Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. Front Mol Biosci 7:157. https://doi.org/10.3389/fmolb.2020.00157
24. Lee N, Chan PK, Ip M, Wong E, Ho J, Ho C, Cockram CS, Hui DS (2006) Anti-SARS-CoV IgG response in relation to disease severity of severe acute respiratory syndrome. J Clin Virol 35:179–184. https://doi.org/10.1016/j.jcv.2005.07.005
25. Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE, Ba L, Li W, Farzan M, Chen Z, Yuen KY, Ho D (2006) Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals. J Med Virol 78:1–8. https://doi.org/10.1002/jmv.20499

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.abd5487
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, Sanseviero M, Del Giudice E, Malvaso 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