



Case report

Robust humoral immune response after boosting in children with Multisystem Inflammatory Syndrome in Children

Arthur J. Chang, Sarah Baron, Mark D. Hicar^{*}

Department of Pediatrics, University at Buffalo, Buffalo, NY, USA

ARTICLE INFO

Keywords:

Multisystem Inflammatory Syndrome in Children
Post-vaccination myocarditis
Auricular chondritis
Coxsackievirus

ABSTRACT

The exact pathogenesis of Multisystem Inflammatory Syndrome in Children (MIS-C) is unknown. Reports on response to vaccination in children who had MIS-C are lacking. Using prospectively enrolled children, we report on humoral immune responses prior to and after SARS-CoV-2 immune rechallenge. Recurrent auricular chondritis was also noted in one child.

Introduction

Multisystem Inflammatory Syndrome in Children (MIS-C), is marked by high inflammatory markers, organ dysfunction, and symptomatic overlap with inflammatory conditions such as Kawasaki disease [1]. Early in the pandemic it was unclear if vaccination should be encouraged for those who recovered from MIS-C. In May of 2021, statements from CDC were open to the choice of vaccination in children who had MIS-C, but no official recommendation has been put forth by the ACIP or CDC as of December of 2021. Recent studies suggest vaccination will prevent cases of MIS-C [2,3]. However, passive surveillance data suggests that in rare cases MIS-C may occur secondary to vaccination [4]. As there are no reports of SARS-CoV-2 rechallenge in patients formally diagnosed with MIS-C, herein we highlight three cases of MIS-C who were vaccinated or reinfected and compare their antibody response to acute COVID-19 cases.

Methods

Clinical subjects

Patients under consideration for acute COVID-19 (PCR positive with respiratory complaints and no alternative diagnosis) were recruited and used as controls for the assays under University at Buffalo (UB) IRB approved STUDY00004340 as previously described [5]. During peripheral blood mononuclear cell (PBMC) isolation, plasma was withdrawn and saved codified aliquots in a -80°C freezer and associated

codified clinical information was retained. Epidemiologic case data for New York and Erie county are freely available at <https://coronavirus.health.ny.gov/covid-19-data-new-york>.

Proteins utilized

Human expressed proteins of Receptor Binding Domain (RBD; Synthetic construct SARS_CoV_2RBD_his gene; GenBank: MT380724.1) and Spike (Synthetic trimerized construct SARS_CoV_2_ectoCSPP gene; GenBank: MT380725.1) based on Wuhan-Hu-1 were expressed utilizing expression vectors from Florian Krammer's laboratory in 293F cells following published protocol [6].

ELISA

96-well ELISA plates were coated with recombinant proteins at 10 ng/well. The ELISA protocol used followed a recently published SARS-CoV-2 related protocol [7] with minor adjustments (use of Goat Anti-Human Ig-HRP (Southern Biotech) secondary) and TMB Ultra (Thermo Fisher) as developer as previously published [5]. Optical density results were read at 450 nm. Titers were calculated with four times background as positive cutoff for IGG and two times background for IGA and IGM.

^{*} Correspondence to: University at Buffalo, 6072 UB CTRC, 875 Ellicott Street, Buffalo, NY 14203, USA.

E-mail addresses: markhica@buffalo.edu, markhicar@gmail.com (M.D. Hicar).

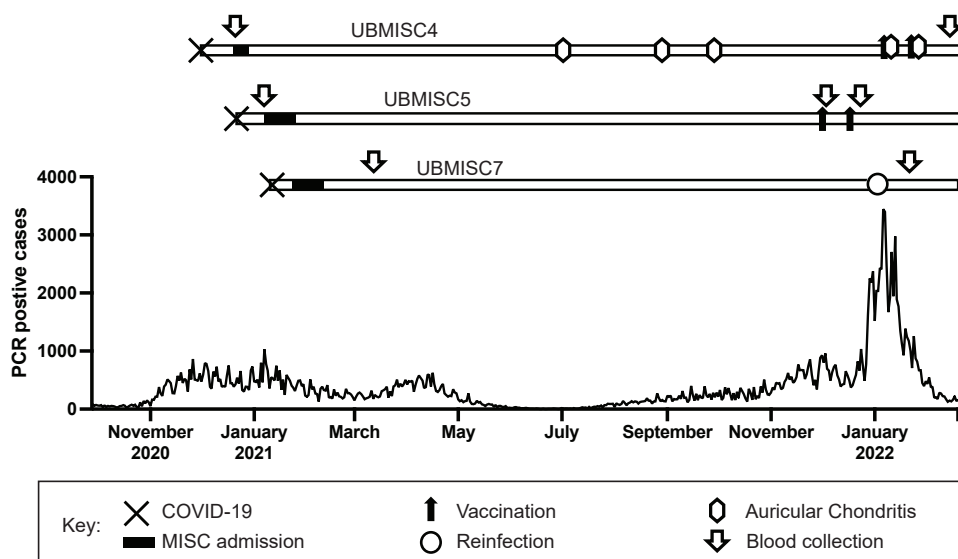


Fig. 1. Immunologically boosted MIS-C cases in relation to local case epidemiology. This illustration highlights study interventions and timing of presentation and immune challenge of subjects UBMISC4, UBMISC5, and UBMISC7. Age-appropriate Pfizer-BioNTech COVID-19 vaccine was used. Local county PCR confirmed cases are graphed by date for comparison. Case findings are not perfectly to scale.



Fig. 2. Auricular chondritis after MIS-C. Photograph of child’s normal ear (on left), taken between events, compared to the first event of recurrent auricular inflammation (on right) in Subject MISC4.

Results

Case summaries

All three children highlighted in this report were originally infected in our “January 2021 peak” cluster of cases (Fig. 1). All were treated with IVIG, methylprednisolone and anakinra, received aspirin, and completed steroid tapers following our published protocol [1]. All of the cases of MIS-C in this study met the MIS-C CDC definition and were reported to the New York State Public Health Department.

Subject UBMISC4 is a 6-year-old twin with eosinophilia, allergic rhinitis and asthma who had initial URI of 3 days with known PCR-positive confirmed family members one month prior to admission for MIS-C. MIS-C presented with 4 days of high fever and significant abdominal pain prompting transfer to our facility for appendicitis concern. He was hypotensive on presentation, but echocardiography only showed mild tricuspid and mitral regurgitation. He was PCR positive for COVID-19 at the time, but had a clear chest CT, no respiratory

symptoms, and his SARS nucleocapsid IgG were 5.4 (ref. < 1.4). Other notable labs showed ALC of 1.2×10^9 cells/L, platelets of 90×10^9 /L, D-Dimer 5.6 mcg/mL, Troponin-I 0.14 mcg/L, BNP 359 ng/L, CRP 79 mg/L, procalcitonin 13.4 mcg/L, and ferritin 1803 mcg/L. Symptoms resolved quickly, hospital stay was 4 days, and he was well by two-week follow-up.

Months later, after several insect bites, his mom noted his ears swelled and became tender and red bilaterally, consistent with auricular chondritis (Fig. 2). No other symptoms of relapsing polycondritis were present (no fever, joint complaints, chest pain) [8] and his eosinophilia did not correlate. This resolved slowly over a week, but auricular chondritis recurred twice in association with non-covid viral syndromes. Each event was less severe than prior event. He was vaccinated 11 months after MIS-C resolved. Auricular chondritis returned with both doses, each event less severe than the last following the same established pattern, but no other complaints were noted. His twin sibling did not display auricular inflammation and notably never developed concern for MIS-C. On three-week follow-up, labs showed normal CBC, CRP < 0.20 mg/L, and anti-Spike antibodies > 25,000 (ref. < 50 AU). He was doing well without chest pain or other MIS-C symptoms. Due to the recurrent auricular chondritis, rheumatoid factor and autoantibodies were assessed (anti-nuclear, myeloperoxidase, neutrophil cytoplasmic, serine protease, PR3 IgG) and confirmed negative.

Subject UBMISC5 is a 6-year-old previously healthy girl who had PCR-positive mild COVID-19. She was admitted one month later, after four days of high fever which progressed to chills, diaphoresis, abdominal pain, rash, vomiting, and headache. Her notable labs were ALC of 0.4×10^9 cells/L, platelets of 103×10^9 /L, D-dimer of 1.49 mcg/mL, troponin-I < 0.01 mcg/L, BNP < 10 ng/L, CRP of 26.22 mg/L, procalcitonin of 0.71 ng/mL, and ferritin of 208 mcg/L. Her SARS-Cov-2 nucleocapsid IgG was 6.6 (ref. < 1.4); she had negative microbiologic workup and negative nasopharyngeal PCR studies (inclusive of enterovirus and SARS-CoV-2). Notably, coxsackie A titers were consistent with past infection, and coxsackie B complement fixation titers were low positive at 1:8 for B-1 and 1:16 for B-5. She became afebrile and was discharged in good condition after 6 days. Her admission and follow-up echocardiograms were normal. 9 months past full recovery, they chose to be vaccinated. Study blood was collected three days after the first dose and five days after the second dose. Vaccination was well tolerated.

Subject UBMISC7 is a healthy 7-year-old male who presented with high fevers one month after PCR-confirmed positive short-course URI

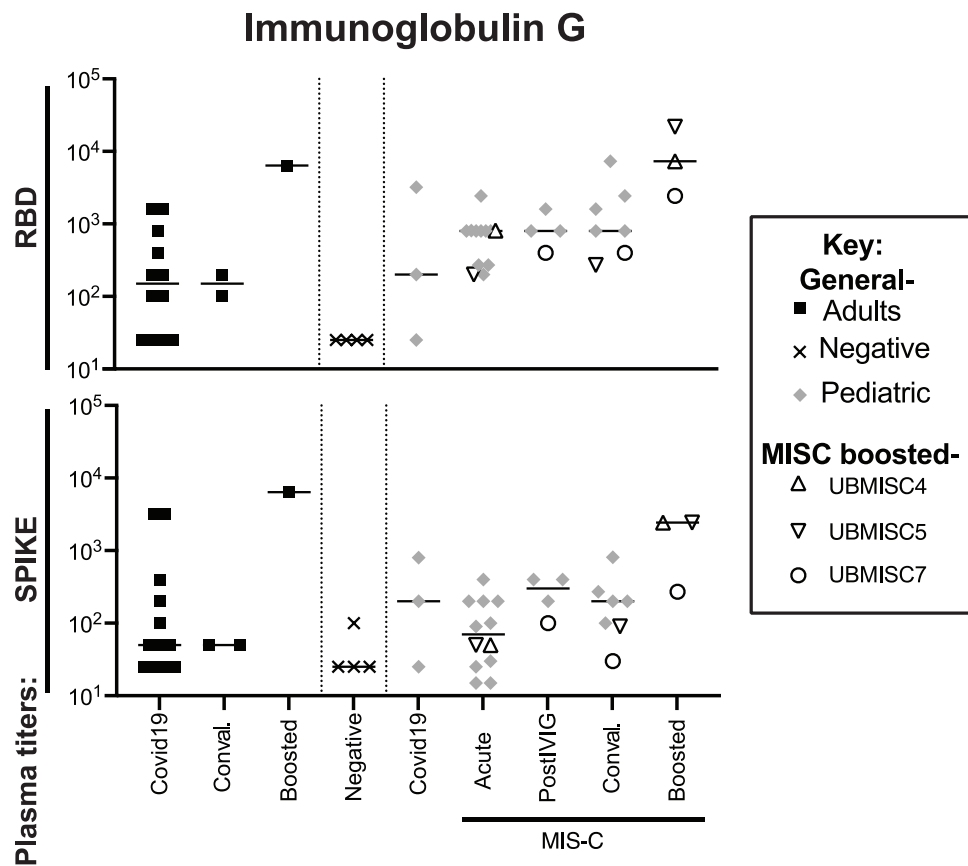


Fig. 3. Immunoglobulin G titers against SARS-CoV-2 RBD and trimerized spike. Data is from ELISA results using recombinant proteins expressed in human cell expression system. Symbols are noted in legend (black squares–adult cases, grey diamond–pediatric cases, open symbols–rechallenged pediatric cases).

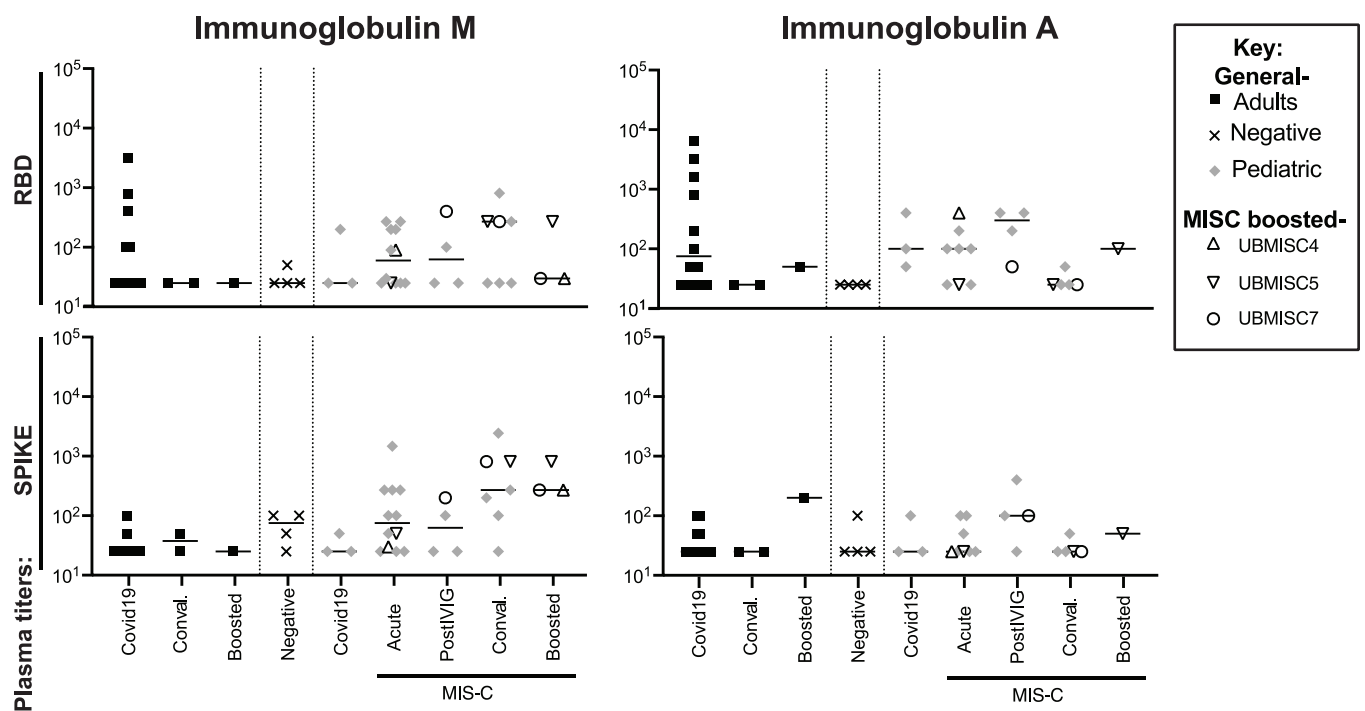


Fig. 4. Immunoglobulin M and A titers against SARS-CoV-2 RBD and trimerized spike. Data is from ELISA results using recombinant proteins expressed in human cell expression system. Symbols are noted in legend (black squares–adult cases, grey diamond–pediatric cases, open symbols–rechallenged pediatric cases).

illness. He also presented with diarrhea, nausea, vomiting, rash, and hypotension requiring epinephrine and a brief PICU admission. Notable laboratory values included ALC of 0.5×10^9 cells/L, platelets of 142×10^9 /L, D-dimer 2.96 mcg/mL, Troponin-I 0.02 mcg/L, BNP 322 ng/L, CRP 95 mg/L, procalcitonin 6.75 mcg/L, and ferritin 997 mcg/L.

Echocardiography only showed mild tricuspid regurgitation. His symptoms resolved over the 10-day hospitalization. On two-month follow-up, he was back to baseline. 10 months after the MIS-C admission, everyone in his family caught COVID-19; his symptoms were mild and lasted a few days. At the time, Omicron was the predominant strain, with NYS data showing 90.3 % omicron within days of his test. He was well on one-month follow-up and denied any symptoms similar to prior MIS-C or consistent with myocarditis. Lab evaluation showed ferritin 56 mcg/L, CRP 0.8 mg/L, normal CBC, and SARS-CoV-2 Spike antibodies were highly positive (34.84; ref. < 1.00).

MIS-C compared to acute cases

We recruited adults and children who were PCR-confirmed or had concerns for MIS-C throughout the pandemic. Titers, resolved by ELISA testing on Spike trimer and RBD, showed subjects with MIS-C had higher titers against RBD, consistent with many cases having resolved their acute infection (Fig. 3). Variable seroconversion on acute adult and pediatric samples is consistent with known literature and likely reflects timing of sampling, as most cases are seropositive if collected after 10 days of symptoms [5,6,9]. The IGG titers against the RBD were stable in MIS-C pre-IVIG, post-IVIG and convalescence. The Spike reactivity was more variable with slightly higher values post-IVIG, consistent with slight Spike cross-reactivity in IVIG we have previously shown [5]. As other studies have shown, IGA and IGM responses are more variable than IGG responses (Fig. 4) [6,10,11].

The three reported cases (UBMISC4, 5, 7) had clinical follow-up six-weeks post-vaccination or reinfection. All were doing well with no return of MIS-C-like symptoms. Their immune response against RBD and Spike protein showed significant boosting compared to acute and/or prior sampling (Fig. 3, open symbols as noted in figure legend). This was comparable to a post-vaccination adult sample shown.

Discussion

All of our immune rechallenge MIS-C cases are clinically well, and these cases are the first children with MIS-C to have post-immune challenge immunologic data described. The paucity of reports of MIS-C or MIS-A post vaccination and our prior data argues against a pure association with immunity to the Spike protein [5]. The recent reports of MIS-C cases directly from vaccination are rare (1 in 3 million) and subject to bias due to the methods of capture (surveillance survey) [4]. Analysis of MIS-A cases diagnosed post-vaccination support that natural infection was the impetus rather than vaccination [12]. Studies from a French cohort and the United States support that full vaccination is actually protective against developing MIS-C [2,3]. A genetic predisposition may play a role in MIS-C, so it is interesting that the twin brother of UBMISC4 who was infected concurrently did not show any concern for MIS-C. The low positivity of coxsackie B titers in UBMISC5 are also of interest, as a recent or prior cardiotropic viral infection may play a role in development of MIS-C. Further studies in our larger cohort are being pursued.

The focus on this report was to show that children with MIS-C who undergo vaccination have a robust immune response and in our limited experience do very well. This is consistent with the general lack of reporting of any cases of recurrent MIS-C. This small series also highlights a case of recurrent auricular chondritis after MIS-C which has not been previously described.

CRedit authorship contribution statement

MDH conceived of and designed the study, recruited subjects, analyzed and interpreted data, and co-authored the first draft. AJC recruited subjects, analyzed and interpreted data, and co-authored the first draft; and SB acquired, analyzed, and interpreted data. All authors revised the article and approved the final submission.

Ethical approval

This study was approved under University at Buffalo (UB) IRB #STUDY00004340.

Consent

Study was carried out under IRB approval (University at Buffalo (UB) STUDY00004340). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Funding

This work was supported by SUNY Research Seed Grant Program 2019–2020 and by the Wildermuth Memorial Foundation through Variety, the Children's Charity of Buffalo & Western New York. The authors have no financial relationships relevant to this article to disclose.

Conflicts of interest

None.

Acknowledgements

Dr. Patrick Kenney and Dr. Michael Croix were instrumental in recruiting subjects.

References

- [1] Hennon TR, Yu KOA, Penque MD, Abdul-Aziz R, Chang AC, McGreevy MB, et al. COVID-19 associated Multisystem Inflammatory Syndrome in Children (MIS-C) guidelines; revisiting the Western New York approach as the pandemic evolves. *Prog Pediatr Cardiol* 2021;62:101407.
- [2] Levy M, Recher M, Hubert H, Javouhey E, Flechelles O, Leteurtre S, et al. Multisystem inflammatory syndrome in children by COVID-19 vaccination status of adolescents in France. *JAMA* 2022;327(3):281–3.
- [3] Zambrano LD, Newhams MM, Olson SM, Halasa NB, Price AM, Boom JA, et al. Effectiveness of BNT162b2 (Pfizer-BioNTech) mRNA vaccination against multisystem inflammatory syndrome in children among persons aged 12–18 years – United States, July–December 2021. *MMWR Morb Mortal Wkly Rep* 2022;71(2): 52–8.
- [4] Yousaf AR, Cortese MM, Taylor AW, Broder KR, Oster ME, Wong JM, et al. Reported cases of multisystem inflammatory syndrome in children aged 12–20 years in the USA who received a COVID-19 vaccine, December, 2020–August, 2021: a surveillance investigation. *Lancet Child Adolesc Health* 2022.
- [5] Chang AJ, Croix M, Kenney P, Baron S, Hicar MD. Serum responses of children with Kawasaki disease against severe acute respiratory syndrome coronavirus 2 proteins. *Pediatr Infect Dis J* 2020;39(11):e366–7.
- [6] Amanat F, Stadlbauer D, Strohmaier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med* 2020;26(7):1033–6.
- [7] Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmaier S, Arunkumar GA, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. *Curr Protoc Microbiol* 2020;57(1):e100.
- [8] Emmungil H, Aydin SZ. Relapsing polyarthralgia. *Eur J Rheumatol* 2015;2(4): 155–9.
- [9] Figueiredo-Campos P, Blankenhaus B, Mota C, Gomes A, Serrano M, Ariotti S, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. *Eur J Immunol* 2020;50(12): 2025–40.

- [10] Kevadiya BD, Machhi J, Herskovitz J, Oleynikov MD, Blomberg WR, Bajwa N, et al. Diagnostics for SARS-CoV-2 infections. *Nat Mater* 2021;20(5):593–605.
- [11] Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claer L, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci Transl Med* 2021;13(577).
- [12] Belay ED, Godfred Cato S, Rao AK, Abrams J, Wilson WW, Lim S, et al. Multisystem inflammatory syndrome in adults after SARS-CoV-2 infection and COVID-19 vaccination. *Clin Infect Dis* 2021.