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SARS-CoV-2-specific IgG1/IgG3 but not IgM in children with Pediatric Inflammatory Multi-System Syndrome

To the Editor,

There is a low rate of symptomatology associated with SARS-CoV-2 infection in children and a substantially lower risk of death than in adults.[1] Nevertheless, in rare cases, some children present with features of a multisystem inflammatory syndrome with overlapping features of Kawasaki disease and toxic shock syndrome.[2,3] In the United Kingdom, this is termed pediatric inflammatory multisystem syndrome—temporally associated with SARS-CoV-2 (PIMS-TS), and in the United States, multisystem inflammatory system in children (MIS-C). Since children with PIMS-TS can be PCR-negative for SARS-CoV-2, understanding the antibody response to SARS-CoV-2 in these children may help develop diagnostic strategies and help understand the nature of the immune response.

Two antigens have been included in most serology tests—the surface-exposed spike (S) glycoprotein and the non-exposed nucleocapsid (N) protein.[4] Serological tests for antiviral antibodies have not been useful to date in the immediate diagnosis of active COVID-19 infection, largely due to the 7- to 14-day lag between infection and antibody responses developing. In primary infections, IgM responses develop first, before eventually declining and IgG responses dominate thereafter. High levels of IgG without IgM are typically suggestive of infection weeks previously. This may be relevant in diseases such as PIMS-TS, since if it follows the pattern of Kawasaki disease, then the causative agent is often not determinable at the time of clinical presentation.

We examined antibody responses in sera from eight patients of mixed ethnicity (five male and median age 9 [range 7-14] years) admitted to hospital between April 28 and May 8, 2020, with a case definition consistent with the criteria described by the Royal College of Paediatrics and Child Health.[5] In all cases, PCR tests for SARS-CoV-2 infection were negative. Seven patients had overlapping features of hyperinflammation with either typical or atypical Kawasaki disease, and one patient had overlapping features of hyperinflammation and toxic shock syndrome. All patients had fever and at least one gastrointestinal symptom (abdominal pain, vomiting, and diarrhea), whereas six patients had a rash and non-exudative conjunctivitis. Four children showed mucosal and peripheral changes and only two children presented with lymphadenopathy.

Hyperinflammation was supported by presence of fever and the median (IQR) CRP was 188 (136-255) mg/L and ferritin was 1325 (819-2121) µg/L in this cohort of children. 63% of patients had impaired myocardial function on echocardiography. 75% required admission to pediatric intensive care predominantly for cardiovascular support due to hypotension. All patients improved with supportive therapy that included immunomodulation with immunoglobulins and/or steroids and were discharged from PICU, remaining hospital inpatients.

Antibodies to the trimeric viral spike glycoprotein (S)[6] were detected by a commercially available ELISA to detect combined IgG, IgA, and IgM (The Binding Site). This test was also modified to detect individual antibody isotypes. Nucleocapsid (N) antibodies were detected using an in-house ELISA.[7] To both antigens, the adapted ELISA used HRP-labeled mouse monoclonal anti-human IgG, IgA IgM, IgG_{1-4} secondary antibodies, generated at the University of Birmingham (available from Abingdon Health Ltd).

Sera from these eight children were tested against viral S glycoprotein. For negative controls, we used sera obtained from adults before 2019, and as positive controls, we used plasma from adults hospitalized with PCR-confirmed severe COVID-19. Screening of sera, diluted 1:40, to detect IgG, IgA, and IgM demonstrated that all children had antibodies against the SARS-CoV-2 S glycoprotein (Figure 1A). Since antibody isotypes can reflect recent infection (IgM), or more historic infections (IgG and IgA), we examined individual antibody isotypes and presented these results as area under the curve (AUC). In children, IgM levels were similar to pre-2019 sera; in contrast, both S glycoprotein and N-specific IgM levels were higher in adult ITU COVID-19 patients (Figure 1B). Although anti-S IgA and IgG were more similar in children and adult
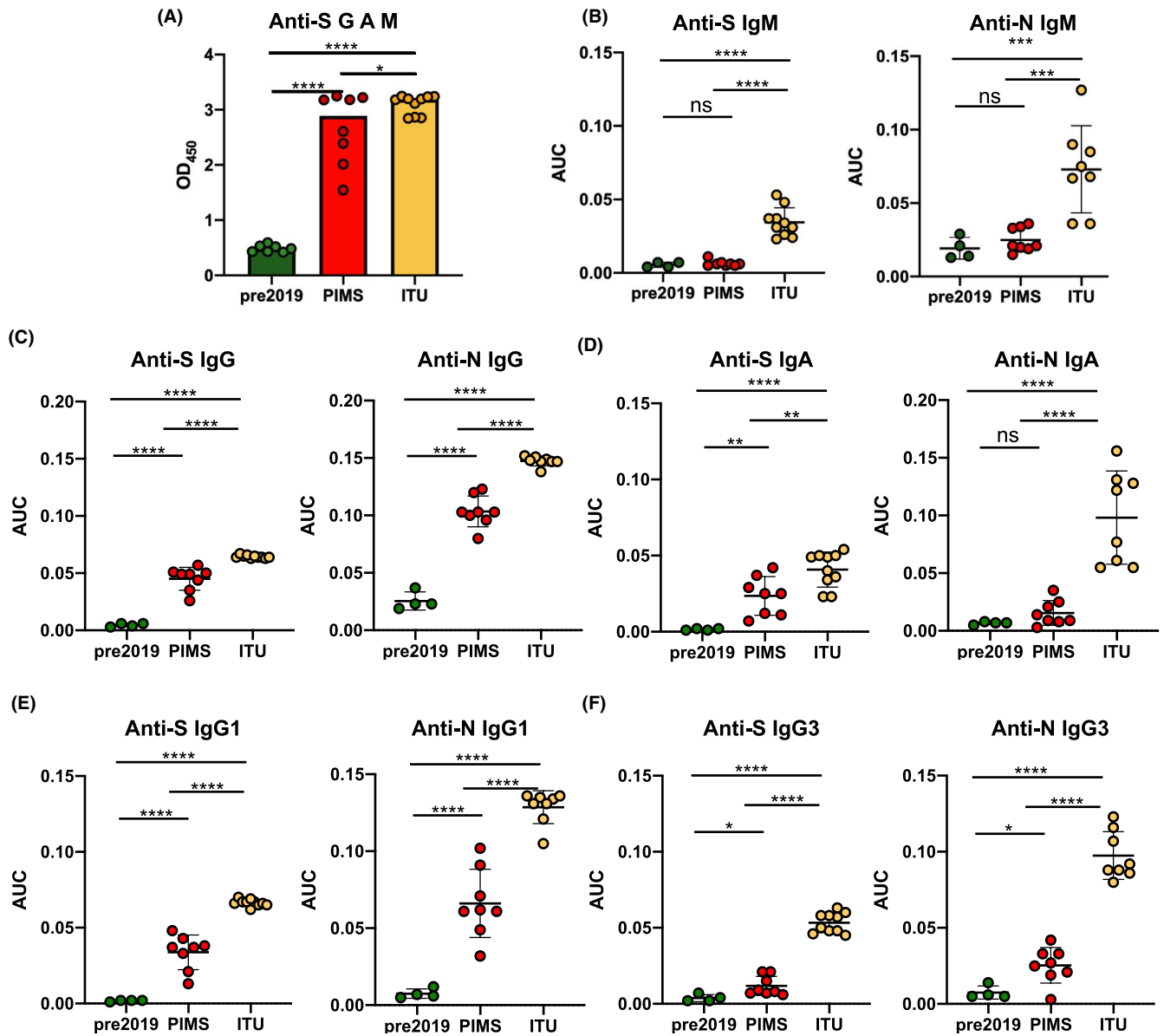
FIGURE 1 Detection of antibodies against spike glycoprotein and nucleocapsid from SARS-CoV-2 in children with PIMS-TS. Serological responses were detected against purified near-full-length trimeric SARS-CoV-2 viral spike glycoprotein (S) or nucleocapsid (N) by ELISA. A, Optical density at 450 nm (OD_{450}) of individual sera at a single dilution (1:40) from pre-2019 healthy adult donors (green), sera from children with PIMS-TS (red), or plasma from adult patients in intensive therapy unit (ITU, yellow) detected using combined anti-IgG, IgA, and IgM. One symbol represents results for a single serum, and the bar shows the median values for each group. B-F, Area under the curve (AUC) for individual sera against S glycoprotein and N from pre-2019 negative control donors (green), children with PIMS-TS (red), or plasma from adult ITU patients (yellow) serially diluted fivefold from 1:20 or 1:50; primary antibodies were detected with (B) anti-IgM, (C) anti-IgG, (D) anti-IgA, (E) anti-IgG1, or (F) anti-IgG3. Each point represents one individual; horizontal line represents the mean $\pm$ SD. Two-tailed one-way ANOVA with Holm-Sidak's test *post hoc* was applied to calculate statistical differences. *$P <.05$, **$P <.005$, ***$P <.001$, ****$P <.0001$, ns, non-significant [Colour figure can be viewed at wileyonlinelibrary.com]

COVID-19 patients, anti-N IgA and IgG antibodies were higher in ITU patients (Figure 1C,D). Assessment of IgG isotypes revealed IgG1 and IgG3 were the predominant isotypes present in these children and in adults (Figure 1E), with IgG2 and IgG4 similar to negative controls in all but one child, who had a weak IgG4 response (data not shown). We then tested the same sera against the N protein; this response was predominantly of IgG1 and IgG3 subclasses (Figure 1B,E,F). Only minimal IgG2 and IgG4 responses

were detected (data not shown). IgA responses were also detectable to N in PIMS-TS subjects (Figure 1D). In contrast, anti-N IgM responses were minimal (Figure 1B). Therefore, children with Kawasaki-like inflammatory syndrome, negative by PCR, can have IgG1, IgG3, and IgA antibody levels to SARS-CoV-2 in the absence of maintained IgM responses.

Recent anecdotal reports of a Kawasaki-like inflammatory syndrome, often without detection of SARS-CoV-2 virus, appear at odds

with the relatively mild or asymptomatic presentation of SARS-CoV-2 infection in the vast majority of children.[1,8] These eight hospitalized children presenting with PIMS-TS had strong IgG and IgA responses to the viral S glycoprotein and IgG to the N antigen, despite being PCR-negative. Although PCR detection of infection is an imperfect technique, it is the nearest to a gold standard for determining active infection.[9] It is likely that the virus had been cleared by these children, a conclusion supported by the high levels of SARS-CoV-2-specific IgG detected without concomitant IgM. Indeed, it is possible that infection may have resolved weeks or even months previously.

The antibody levels detected in the children with PIMS-TS were lower than those in adults with severe COVID-19 infections. The reasons for this are unclear but may reflect differences in age and the presence of comorbidities in adults. Higher antibody levels in adults could reflect higher levels of virus, and therefore, more antigen, particularly as the children, did not present with notable symptomatology recent to the development of PIMS-TS. Such a hypothesis would be consistent with the concept that PIMS-TS is likely to be host-driven rather than due to direct cytopathic effects of the virus on the host. Such an interpretation of PIMS-TS presenting when the infection has resolved may mean that host immune responses play an important part in disease pathogenesis. If so, then some overlap between the immunopathologic mechanisms that drive severe disease in adults and children may exist.[10] Associated with this is the detection of IgG1 and IgG3 in these children to both S and N proteins, and these isotypes are associated with complement activation,[11] which is enhanced in adult patients.[12] Otherwise, antibodies may contribute to disease in other way, such as recognizing self-antigens. Such considerations may be relevant as we get closer to the rollout of vaccines against SARS-CoV-2.

Strong IgG antibody responses can be detected in PCR-negative children with PIMS-TS. The low IgM response in these patients is consistent with infection having occurred weeks previously and that the syndrome onset occurs after the control of SARS-CoV-2 viral load. This implies that the disease is largely immune-mediated. This indicates the importance of serology as an appropriate diagnostic tool in select patient groups. The major limitation of this study is the small number of subjects. However, the results from this work will provide a valuable addition to the literature base and emphasize the need to study the presence and importance of IgG and, equally, the absence of IgM.

AUTHOR CONTRIBUTION

Marisol Perez-Toledo: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Writing-review & editing (equal). **Sian E. Faustini:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Writing-review & editing (equal). **Sian E. Jossi:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Writing-review & editing (equal). **Adrian M. Shields:** Data curation (supporting); Writing-review & editing (supporting). **Edith Marcial-Juarez:** Investigation (equal); Writing-review & editing (supporting). **Hari Krishnan Kanthimathinathan:** Resources (supporting); Writing-review & editing (supporting). **Joel D. Allen:** Resources (supporting); Writing-review & editing (supporting). **Yasunori Watanabe:** Resources (supporting); Writing-review & editing (supporting). **Margaret Goodall:** Investigation (supporting); Resources (equal). **Benjamin E. Willcox:** Resources (lead); Writing-review & editing (supporting). **Carrie R. Willcox:** Resources (lead);

ETHICAL APPROVAL

The patients' samples were either tested as part of routine diagnostics on in-house COVID-19 antibody ELISAs run by the UKAS accredited Clinical Immunology Service at the University of Birmingham or used for assay development. The ethical approval for this work and the use of these samples were provided by the awarding bodies of the University of Birmingham Research Ethics Committee, the South Birmingham Research Ethics Committee, and the National Research Ethics Service Committee West Midlands. All approvals are overseen by the United Kingdom National Health Service and this is therefore a NHS Health Research Authority-approved study. All patients and/or their parents/legal guardians provided signed informed consent to inclusion of de-identified data in this report.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pai.13504.

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