Serology confirms SARS-CoV-2 infection in PCR-negative children with Paediatric 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 U.K., this is termed Paediatric Inflammatory Multisystem Syndrome- temporally associated with SARS-CoV-2 (PIMS-TS), and in the U.S, multi-system 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 anti-viral antibodies have not been useful to date in the immediate diagnosis of active COVID-19 infection, largely due to the 7-14 day lag between infection and antibody responses developing. In primary infections, IgM responses develop first, before eventually waning 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 8 patients of mixed ethnicity (5 male and median age 9 (range 7-14) years) admitted to hospital between 28th April-8th May 2020 with a case definition consistent with the criteria described by the Royal College of Paediatrics and Child Health (Table I). In all cases PCR tests for SARS-CoV-2 infection were negative. Seven patients had overlapping features of hyper-inflammation with either typical or atypical Kawasaki disease, and one patient had overlapping features of hyper-inflammation and toxic shock syndrome (patient details summarised in table II). All patients had fever and at least one gastrointestinal symptom (abdominal pain, vomiting and diarrhoea) and 75% had a

rash. 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 paediatric 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 S glycoprotein[5] were detected by a commercially available ELISA to detect IgG,IgA,IgM (The Binding Site, U.K.). This test was also modified to detect individual antibody isotypes. Nucleocapsid antibodies were detected using an in-house ELISA[6]. To both antigens, the adapted ELISA used HRP-labelled 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 8 children were tested against viral spike glycoprotein. For negative controls, we used sera obtained from adults before 2019 and as positive controls, we used plasma from adults hospitalised 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 spike glycoprotein (Fig. 1A). Since antibody isotypes can reflect recent infection (IgM), or more historic infections (IgG and IgA), we examined individual antibody isotypes. In children, IgM levels were similar to pre-2019 sera; in contrast, spike glycoprotein-specific IgM levels were higher in adult ITU COVID-19 patients (Fig. 1B). IgA and IgG were more similar in children and adult COVID-19 patients (Fig. 1C and 1D). Assessment of IgG isotypes, revealed IgG1 and IgG3 were the predominant isotypes present in these children and in adults (Fig. 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 nucleocapsid protein. IgG was readily detectable, and this was predominantly of IgG1 and IgG3 subclasses (Fig. 1B, 1E and 1F). Only minimal IgG2 and IgG4 responses were detected (data not shown). IgA responses were also detectable to nucleocapsid in PIMS-TS subjects (Fig. 1D). In contrast, anti-nucleocapsid IgM responses were minimal (Fig. 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[1,7]. These eight hospitalised children presenting with PIMS-TS had strong IgG and IgA responses to the viral spike glycoprotein and IgG to the nucleocapsid 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[8]. 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.

One interpretation of PIMS-TS presenting when the infection has resolved is that it could mean that host immune responses play an important part in disease pathogenesis. If so, then some overlap between the immunopathological mechanisms that drive severe disease in adults and children may exist[9]. 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[10], which is enhanced in adult patients[11]. Otherwise, antibodies may contribute to disease in other way, such as recognising self-antigens. Such considerations may be relevant as we get closer to the roll out 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. Lastly, this indicates the importance of serology as an appropriate diagnostic tool in select patient groups.

Marisol Perez-Toledo, PhD[1a]; Sian E. Faustini, PhD[1a]; Sian E. Jossi, MSc[1a]; Adrian M. Shields, MRCP, PhD[1]; Edith Marcial-Juarez, PhD[1]; Hari Krishnan Kanthimathinathan, MD[2,3]; Joel D. Allen, MBiochem[4]; Yasunori Watanabe, DPhil[5]; Margaret Goodall, PhD[1]; Benjamin E. Willcox, DPhil[1]; Carrie R. Willcox, PhD[1]; Mahboob Salim, PhD[1]; David C. Wraith, PhD[1]; Tonny V. Veenith, FFICM, PhD[6]; Eleni Syrimi, MD, MRCPCH[1]; Mark T. Drayson, PhD[1]; Deepthi Jyothish, MRCPCH[7]; Eslam Al-Abadi, MBBS, MRCPCH[2,8]; Ashish Chikermane, MRCP[9]; Steven B. Welch, FRCPCH[10]; Kavitha Masilamani, FRCPCH[7]; Scott Hackett, FRCPCH, MD[11]; Max Crispin, DPhil[4]; Barnaby R Scholefield, PhD[3,12,b]; Adam F. Cunningham, PhD[1b]; Alex G. Richter, MD[1b].

[a]These authors contributed equally

[b]Joint senior authors.

1. Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, B15 2TT, U.K.
2. Birmingham Clinical Trials Unit, University of Birmingham, Birmingham, B15 2TT, U.K.
3. Paediatric Intensive Care Unit, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, B4 6NH, U.K.
4. School of Biological Sciences, University of Southampton, Southampton SO17 1BJ, U.K.
5. Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.
6. Department of Critical Care Medicine, University Hospitals Birmingham NHS Trust, Birmingham, B15 2TH, UK
7. Department of General Paediatrics, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, B4 6NH, U.K.
8. Childhood Arthritis and Rheumatic Diseases Unit, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, B4 6NH, U.K.
9. Department of Cardiology, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, B4 6NH, U.K.
10. Department of Paediatrics, Birmingham Chest Clinic and Heartlands Hospital, University Hospitals Birmingham, Birmingham, B9 5SS, U.K.
11. West Midlands Immunodeficiency Centre, Heartlands Hospital, University Hospitals Birmingham, Birmingham, B9 5SS, U.K.
12. Institute of Inflammation and Ageing, University of Birmingham, Birmingham, B15 2TT, U.K.

Emails - a.g.richter@bham.ac.uk; a.f.cunningham@bham.ac.uk; barney.scholefield1@nhs.net

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Ethics statement. 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 was 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.

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Table I. Case definition for Paediatric Inflammatory Multisystem Syndrome temporally associated with SARS-CoV-2 pandemic†

Any child (<16 years) presenting with 1 and 2 and 3 below	
1. Presenting with	• Persistent fever • Inflammation (neutrophilia, elevated CRP and lymphopaenia) • Evidence of single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder) • Additional features such as coagulopathy. • This may include children fulfilling full or partial criteria for Kawasaki disease
2. Exclusion of any other microbial cause (waiting for results of these investigations should not delay seeking expert advice)	
3. SARS-CoV-2 pcr testing may be positive or negative[a]	

† Based on RCPCH guidance: https://www.rcpch.ac.uk/sites/default/files/2020-05/COVID-19-Paediatric-multisystem-%20inflammatory%20syndrome-20200501.pdf

Age (median, years)	Sex (%)	Fever	Rash	Lymph-adenopathy	Conjunctivitis non-exudated	Mucosal changes	Peripheral changes	Cardiac involvement
10 (7-15)	Female (37.5) Male (62.5)	100%	75%	25%	75%	37.5%	37.5%	100%

Table II. Summary of clinical features of the PIMS-TS population reported in this study

Figure legend

Figure 1. Detection of antibodies against S 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 or nucleocapsid 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) to F)** Optical Density for individual sera against spike glycoprotein and nucleocapsid from pre-2019 negative control donors (green), children with PIMS-TS (red), or plasma from adult ITU patients (yellow) serially diluted five-fold 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 line represents one sample.