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False-positive SARS-CoV-2 serology in 3 children with Kawasaki disease

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ABSTRACT

Background: Kawasaki disease (KD) is an acute febrile and eruptive disease with systemic vasculitis predominantly affecting young East Asian children. Recent reports showed that children with KD-like disease from KD low prevalence regions had positive SARS-CoV-2 serology despite a negative SARS-CoV-2 polymerase chain reaction (PCR) in respiratory samples.
Objectives: To describe 3 pediatric Kawasaki Disease patients with false positive SARS-CoV-2 serology.
Study design: We retrospectively recruited children with KD diagnosed during the COVID-19 outbreak in Hong Kong. Clinical characteristics and laboratory test results including SARS-CoV-2 PCR results were retrieved. We performed a microparticle-based immunoassay for the detection of IgG against nucleoprotein (NP) and spike protein receptor binding domain (RBD), and a microneutralization assay for the detection of neutralizing antibodies.
Results: Three Chinese children with typical KD were identified. They had no epidemiological links with COVID-19 patients and tested negative for SARS-CoV-2 NPA PCR. They were treated with IVIG and aspirin, and were discharged without complications. Subsequently 2 of them were tested positive against anti-RBD and anti-NP antibodies and 1 was tested positive against anti- RBD antibodies. However, microneutralization assay showed that neutralizing antibodies were absent, suggesting a false-positive IgG result.
Conclusion: Detection of neutralizing antibodies is recommended to confirm previous SARS-CoV-2 infection in IgG-positive but PCR-negative patients.

1. Background

Kawasaki disease (KD) is an acute systemic vasculitis complicated by coronary aneurysms that predominantly occurs in young East Asian children. Typical symptoms include fever for more than 5 days, exanthema, lymphadenopathy, conjunctival injection, altered oropharyngeal mucosa, and extremity changes. The etiology of KD remains uncertain and cases remain rare (McCrindle et al., 2017). Nevertheless, an upsurge of KD cases in Europe was observed during the Coronavirus Disease 2019 (COVID-19) pandemic. Out of the 10 KD cases reported in children from Bergamo, Italy, 2 tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by PCR, whereas 8 tested positive for SARS-CoV-2 antibodies (Viner and Whittaker, n.d.). A similar cluster of KD cases in children was reported in France during the outbreak, in which 14 children tested positive for SARS-CoV-2 IgG, but only 7 children tested positive for SARS-CoV-2 by PCR. As KD is known to be more prevalent in Hong Kong than in Europe (Uehara and Belay, 2012; Ng et al., 2005) and Hong Kong was close to the epicenter of the initial COVID-19 outbreak, concerns have been raised about new cases of KD in Hong Kong children during the outbreak and whether they were associated with SARS-CoV-2 infection. Therefore,

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serological testing for SARS-CoV-2 was offered to Hong Kong children diagnosed with KD between January and April 2020.

2. Objective

We aim to describe 3 pediatric Kawasaki Disease patients diagnosed during the COVID-19 outbreak with false positive SARS-CoV-2 serology.

3. Methods

Children diagnosed with KD between January and April 2020 were identified. Their clinical and laboratory data were reviewed. Serological testing was performed to determine possible exposures to SARS-CoV-2. This study was approved by the University of Hong Kong/Hospital Authority Hong Kong West Cluster Institutional Review Board (Reference number: UW 20-292). Written consent was obtained from parents prior to testing.

3.1. Real-time reverse transcription polymerase chain reaction (RT-PCR) assays for SARS-CoV-2 RNA testing of respiratory specimens

Nasaopharyngeal swabs (NPS) obtained during admission were tested by RT-PCR using the LightMix® Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol, Berlin, Germany) on a LightCycler Multiplex RNA Virus Master (Roche, Penzberg, Germany) according to the manufacturer's instructions.

3.2. Detection of IgG against SARS-CoV-2 nucleoprotein and spike protein receptor binding domain

Blood (5 mL) was collected from each patient and serum was obtained for the detection of IgG against SARS-CoV-2 nucleoprotein (NP) and spike protein receptor binding domain (RBD) using a microsphere-based antibody assay as described previously (Fong et al., 2020). IgM was not measured in this assay. Briefly, cloning and purification of SARS-CoV-2 NP and spike RBD were performed as described previously (To et al., 2020a, n.d.). Both proteins were biotinylated using EZ-link™ Sulfo-NHS-Biotin (ThermoFisher Scientific, MA, USA). SuperAvidinTM coated microspheres (Bangs Laboratories, Indiana, USA) were coated with biotinylated NP or spike RBD and then mixed with serum at a dilution of 1:400. Bound antibodies were detected with Alexa Fluor ® 647 AffinPure Fab fragment goat anti-human IgG. Flow cytometry was performed on a BD LSR Fortessa analyzer (BD Biosciences, San Jose, CA, USA) and data were analyzed using FlowJo v10.6.2 (FlowJo LLC, Ashland, OR, USA).

3.3. Microneutralization (MN) assay

Virus culture and MN assay were performed as previously described (To et al., n.d., 2020b). Briefly, serum samples (50 μL) were prepared in minimum essential medium and mixed with SARS-CoV-2 (50 μL) to give a serum dilution of 1:10 and a final virus inoculum of 100 $TCID_{50}$. The serum-virus mixture was incubated at 37 °C for 1 hour and then added to VeroE6 cells and incubated at 37 °C and 5% CO_2 for 72 hours. Cytopathic effects were determined under inversion microscopy. The MN antibody titer was determined as the highest dilution showing 50% inhibition of CPE. An MN titer ≥10 was considered positive as described previously (Okba et al., 2020).

4. Results

Three Chinese children, who had no epidemiological links with COVID-19 patients were diagnosed with typical KD during the peak of COVID-19 outbreak in Hong Kong (Table 1). They were tested negative for SARS-CoV-2 and other common respiratory pathogens in nasopharyngeal aspirate polymerase chain reaction (PCR), including respiratory syncitial virus, adenovirus, human metapneumovirus, influenza A and B, parainfluenza virus type 1, 2, 3, 4, rhinovirus, bocavirus, enterovirus, coronavirus 229E, NL63, HKU1, and OC43, *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. They achieved complete recovery with one dose of intravenous immunoglobulins at 2g/kg, high-dose aspirin at 30–50 mg/kg per day until 2 days after defervescence, followed by low-dose aspirin at 3–5 mg/kg per day for 8 weeks. In view of the possible association between KD and COVID-19 infection, they were called back to test for SARS-CoV-2 anti-NP and anti-RBD antibodies 60–90 days after the diagnosis of KD. Patient 1 tested positive for SARS-CoV-2 anti-RBD IgG, whereas both patients 2 and 3 tested positive for SARS-CoV-2 anti-NP and anti-RBD IgG. However, all 3 patients tested negative with the microneutralization assay, suggesting that the IgG results were false positives.

5. Discussion

To the best of our knowledge, this is the first report demonstrating false-positive SARS-CoV-2 serology among KD children. The 3 patients reported in this study did not report any epidemiological links to individuals with COVID-19 or any travel history in areas with COVID-19 outbreaks. They did not report any symptoms or signs of SARS-CoV-2 infection prior to admission for KD. Only SARS-CoV-2 anti-RBD IgG was detected in 1 patient, whereas both anti-RBD and anti-NP IgG were detected in 2 patients. However, no neutralizing antibodies were detected in any of the patients by MN assay, suggesting the antibodies detected in the serology assay were unlikely to be related to a prior SARS-CoV-2 infection. The serological assay used in this study has sensitivity of 89.8% for ant-NP IgG and 79.5% for anti-RBD IgG, as well as specificity of 100% for anti-NP IgG and 98.9% for anti-RBD IgG when evaluated using sera collected from influenza patients or organ donors before 2020 (Fong et al., 2020). The false-positive results from the serological testing could possibly be due to the presence of cross-reactive antibodies elicited by other triggers, such as non-specific antibodies triggered by Kawasaki Disease reacting to NP, RBD or any reagents in the blocking buffer; or cross-reactive antibodies triggered by other coronaviruses. False-positive results have been well reported in serological testing for immune responses against viral infections, such as false positives in hepatitis A and cytomegalovirus serologies from Epstein-Barr virus infection (Miendje et al., 2000; Valota et al., 2019). We believe the false positive SARS-CoV-2 serology results were unrelated to the administration of IVIG for treating KD. First, the time interval between IVIG administration and serology testing ranged between 60 and 90 days, whereas the median half-life of IVIG is 21 days (Koleba and Ensom, 2006). Second, the IVIG treatment, Intragam® P (CSL Behring Asia Pacific Limited), was manufactured from the pooled plasma of healthy Hong Kong blood donors and had been made months before its use (Barahona Afonso and João, 2016), and therefore unlikely to contain any plasma from donors with COVID-19, as the first case of COVID-19 was diagnosed in Hong Kong in late January 2020 (Leung et al., 2020).

The association between KD and COVID-19 remains unclear. To date, a total of 4 case series and reports on 40 patients have proposed a possible link between COVID-19 and KD (Jones et al., 2020; Verdoni et al., n.d.; Toubiana et al., 2020; Riphagen et al., 2020). Although 11 patients were confirmed to have COVID-19 by a positive SARS-CoV-2 PCR in respiratory specimens, the other patients were reported to have positive SARS-CoV-2 serology without confirmation by microneutralization assay (Table 2). Thus, the possibility that these patients might have false positive results cannot be ruled out. Clinically, not all the patients in these case series presented with KD symptoms reported in cases in Asia, which typically involved younger children under 4 years and

Table 1
Summary of 3 Chinese Kawasaki Disease patients with false positive SARS-CoV-2 serology.

No.	Age/ Gender	Significant Past Health	COVID-19 Contact	Symptoms	Respiratory Virus PCR[#]	SARS-CoV-2 PCR[※]	Echo	Serology (Number of Days taken after IVIG)	MN	Treatment	Outcome
1	3 months/F	None	None	• Rhinorrhea • Blocked Nose • 7 days of fever • Conjunctivitis • Cracked lips • MP rash	Negative	Negative	Perivascular echogenicity and non-tapering coronary arteries	Anti-RBD IgG positive (90 days)	Negative	IVIG 2 g/kg Aspirin[*]	Resolution of fever and KD features. Normal coronary arteries at 12-week follow-up.
2	6 months/F	None	None	• Cough • Rhinorrhea • 6 days of fever • Conjunctivitis • MP rash • Erythematous lips	EV/RV	Negative	Perivascular echogenicity and non-tapering coronary arteries	Anti-RBD and anti-NP IgG positive (87 days)	Negative	IVIG 2 g/kg Aspirin[*]	Resolution of fever and KD features. Normal coronary arteries at 8-week follow-up.
3	3 months/M	None	None	• 5 days of fever • Cough and • Rhinorrhoea • Conjunctivitis • Cervical lymphadenopathy • MP rash • Erythematous Lips • Swelling of hands and feet • Erythema of BCG scar	Negative	Negative	Normal	Anti-RBD and anti-NP IgG positive (60 days)	Negative	IVIG 2 g/kg Aspirin[*]	Resolution of fever and KD features. Normal coronary arteries at 2-week follow-up.

Echo = echocardiogram, EV/RV = enterovirus/rhinovirus, IVIG = intravenous immunoglobulin, MN = microneutralization assay, MP = maculopapular, NP = nucleoprotein, RBD = receptor binding domain.
[*] Initial high-dose aspirin at 30–50 mg/kg per day until 2 days after defervescence, followed by low-dose aspirin at 3–5 mg/kg per day for 8 weeks.
[#] Nasopharyngeal swab specimen.
[※] Pooled nasopharyngeal and throat swab specimens.

Table 2
Summary of case series and reports on Kawasaki Disease possibly associated with SARS-CoV-2 infection.

Study/Country	No. of Cases Reported	Age Range (years)	Parents' Ethnicity	Diagnoses	IgG assay	No. of cases with suspected or confirmed COVID-19 contact	No. of cases with positive respiratory pathogens from respiratory specimen	No. of cases with positive SARS-CoV-2 RT-PCR from respiratory specimen	No. of cases with positive SARS-CoV-2 IgM	No. of cases with positive SARS-CoV-2 IgG	Was MN assay performed?
(Jones et al., 2020)/USA	1	0.5	N/A	Typical KD	Not done	0	0	1	N/A	N/A	N/A
(Verdoni et al., n.d.)/Italy	10	2.9–16	N/A	5 typical KD 5 KDSS	Lateral flow chromatographic immunoassay	5	0	2	3	8	No
(Toubiana et al., 2020)/France	21	3.7–16.6	Sub-Saharan Africa/ Caribbean islands Asian European Middle East	11 typical KD 10 incomplete KD 12 KDSS	Architect SARS-CoV-2 chemiluminescent microparticle immunoassay	10	0	8	N/A	19	No
(Riphagen et al., 2020)/UK	8	6–14	Afro-Caribbean	All considered as atypical KD, KDSS or toxic shock syndrome	N/A	4	1 (Adenovirus, enterovirus)	0	2 were antibody positive (no mention of whether IgM or IgG)		No

COVID-19 = Coronavirus Disease 2019, KD = Kawasaki Disease, KDSS = Kawasaki Disease Shock Syndrome, MN = Microneutralization assay, N/A = not available, RT-PCR = reverse transcription polymerase chain reaction, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

commonly included symptoms such as erythema and swelling of BCG scars (Nagata, 2019). Epidemiologically, there was a 30-fold increase in the monthly incidence of KD in Italy during the COVID-19 pandemic, whereas the incidence of KD in Hong Kong remained the same (unpublished data). Furthermore, we reviewed all NPS samples from KD patients admitted to public hospitals in Hong Kong, which were all negative for SARS-CoV-2 PCR. Data from Wuhan Children's Hospital in China reported 244 children with COVID-19, but none showed any symptoms and signs of KD (submitted and under review). Large-scale observational studies are needed to determine whether the reported cases belong to classical KD or another form of hyperinflammatory disease, recently termed pediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS). Frontline clinicians should be aware of the potential false-positive IgG results. Patients with positive SARS-CoV-2 serology but negative PCR should be further tested by MN assay for the presence of neutralizing antibodies.

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Declaration of interest

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