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Original Article

Age and prior vaccination determine the antibody level in children with primary SARS-CoV-2 Omicron infection

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Abstract *Background:* Protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reinfection relies on immunity generated after primary infection. However, humoral immunity following primary infection with the Omicron variant is not well understood. *Methods:* We prospectively recruited children <19 years with virologically-confirmed SARS-CoV-2 infection at National Cheng Kung University Hospital from February 2022 to September 2022 during the first wave of Omicron BA.2 outbreak in Taiwan. Serum samples were collected one month after acute infection to measure anti-spike protein receptor binding domain

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infection

antibody levels and surrogate virus neutralizing antibody (NAb) levels against wild type disease and variants.

Results: Of the 164 patients enrolled, most were under 5 years (65.2%) with a diagnosis of upper respiratory tract infection. Children under 6 months with maternal coronavirus disease 2019 (COVID-19) vaccination had higher levels of both anti-SARS-CoV-2 spike antibody (119.0 vs 27.4 U/ml, $p < 0.05$) and anti-wild type NAb (56.9% vs 27.6% inhibition, $p = 0.001$) than those without. Children aged 5—12 years with prior vaccination had higher anti-spike antibody, anti-wild type, and anti-Omicron BA.2 NAb levels than those without (all $p < 0.05$). In previously naïve children without maternal or self-vaccination, those 6 months to 2 years had the highest antibody levels. Multivariable linear regression analysis showed age was the only independent factor associated with antibody level.

Conclusions: In our study, children aged 6 months to 2 years have the highest antibody responses to SARS-CoV-2 Omicron variant infection. Age and prior vaccination are the main factors influencing the immunogenicity of SARS-CoV-2 infection.

Introduction

Compared to adults, children with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have relatively mild symptoms and are less likely to develop severe complications.[1] However, children with immunocompromised status and comorbidities, such as diabetes, heart, chronic lung and neurological disease, are at higher risk of severe coronavirus disease 2019 (COVID-19).[2] Meanwhile, the multisystem inflammatory syndrome in children (MIS-C, also referred to as pediatric multisystem inflammatory syndrome) is a new multi-organ inflammatory disease developing 2—6 weeks after acute COVID-19, which is also more common in children than in adults. Although the incidence rate is relatively low, MIS-C can lead to shock, impaired organ function, and even organ failure; its mortality rate is much higher than that of uncomplicated COVID-19 (1% vs 0.09%).[3] Neurologic complications, including seizure and acute encephalitis/encephalopathy, could be manifestations of both MIS-C and acute COVID-19 in children.[4] Both direct viral effects on the nervous system and downstream effects of post-infectious inflammation have been proposed as potential causes of these neurological manifestations, which also raises the appropriateness of using both antiviral and anti-inflammatory drugs in severe cases.[5]

Anti-spike antibody (both binding and neutralizing antibody [NAb]) generated either through natural infection or vaccination are thought to provide protective immunity from re-infection and symptomatic COVID-19, and to prevent disease progression.[6,7] In one study exploring the antibody response to SARS-CoV-2 in children with COVID-19, antibody levels were relatively low in the first two weeks, peaked at 28 to <56 days, then lasted for at least 3 months with statistically significantly decreasing antibody titers.[8] However, whether children can generate an adequate and durable immune response after SARS-CoV-2 infection and which factors contribute to immunity remains debatable. Khaitan et al. found the NAb response to SARS-CoV-2 infection in children after symptomatic or asymptomatic infection to be at least as strong as in adults, and lasting longer in children.[8,9] Another study found that children aged 0—4 years had both higher binding antibody and NAb levels than adults following community infection.[10] Although some studies showed NAb levels were correlated with age, others showed children younger than 4 years had higher NAb levels against SARS-CoV-2 during the acute phase compared to older children.[8,10—12] All these studies were conducted in the pre-Omicron period, which prompts the question whether children with the Omicron variant infection can generated a similar immune response as those found against pre-Omicron variants.[13]

Currently, COVID vaccine is not approved for infants under 6 months, which means these infants must rely only on antenatal maternal Immunoglobulin G (IgG) via placenta transfer and "cocoon vaccination strategy" to resist SARS-CoV-2 infection.[14] A previous study demonstrated a high placental transfer of anti-SARS-CoV-2 NAb to the fetus in fully vaccinated pregnant women.[15] However, there is limited evidence regarding the durability of the maternal antibody and its influence on acquired immunity post SARS-CoV-2 infection in infants. Thus, it is necessary to extensively elucidate the antibody response in children with COVID-19 and determine the associated host factors.

In the present study, we analyzed the clinical manifestations in previous infection-naïve children with SARS-CoV-2 Omicron infection during the first wave of endemic outbreak in Taiwan. Also, we explored the humoral immune response, cross-reactivity, and possible contributing host factors.

Methods

Study design and clinical definitions

Children aged under 19 years with SARS-CoV-2 infection from February 2022 to September 2022 in National Cheng

Kung University Hospital were prospectively recruited into this study. SARS-CoV-2 infection was defined as microbiological confirmation through polymerase chain reaction test or rapid antigen test. Participants and their guardians were provided with informed consent upon recruitment. The protocol of this study was approved by the Institutional Review Board of National Cheng Kung University Hospital (No. A-BR-110-051). The procedures used in this study adhered to the tenets of the Declaration of Helsinki. All participants had blood samples taken 1 month after acute SARS-CoV-2 infection for antibody measurement; they also received outpatient clinic follow-up for 3 months to monitor any long COVID symptoms. Demographic and clinical information was retrieved from the electronic medical record. As to the clinical diagnosis, acute myositis was defined as myalgia or muscle weakness with elevated serum creatinine kinase level. COVID-19 encephalitis in COVID-19 children was defined as neurological dysfunction lasting over 24 h and showed evidence of CNS inflammation based on clinical, laboratory, or neuro-radiological features. Neurological dysfunction was defined as decreased or altered level of consciousness, lethargy, or personality changes, seizures, or focal neurological findings.[16,17] COVID-19 related febrile seizure was defined as seizure occurring in COVID-19 children associated with a fever (body temperature $>38\ ^{\circ}C$) not attributed to an infection of the central nervous system.[18] To ensure specificity, we excluded patients with a history of neonatal seizures, unprovoked seizures, or other acute symptomatic seizures from the febrile seizure diagnosis.[18]

SARS-CoV-2 anti-spike protein antibody measurement and anti-nucleocapsid protein antibody

The SARS-CoV-2 anti-spike (S) antibodies were tested using the Roche Elecsys anti-SARS-CoV-2 S test (Roche Diagnostics, Rotkreuz, Switzerland), with electrochemiluminescence immunoassay for in vitro qualitative detection of antibodies to the SARS-CoV-2 S protein receptor binding domain (RBD). The assay uses a Cobas® e601 analyzer. The process used followed manufacturer's instructions. We also randomly selected 17 patients from our study to test anti-nucleocapsid protein antibody (anti-N antibody) by Elecsys Anti-SARS-CoV-2 test (Roche Diagnostics Basel, Switzerland).

SARS-CoV-2 NAb detection: wild type, delta, Omicron BA.1, and BA.2 variant

CPass SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript, Piscataway, NJ) was used to detect the level of NAbs for the wild type, Delta, and Omicron BA.1 variant. The kit uses a Blocking Enzyme-Linked Immunosorbent Assay for qualitative and semi-quantitative direct detection of total neutralizing antibodies to SARS-CoV-2. For the SARS-CoV-2 Omicron BA.2 variant, the Anti-SARS-CoV-2 (BA.2) Neutralizing Antibody Titer Serologic Assay Kit (ACROBiosystems, Newark, DE) was used for qualitative detection. All the above processes were conducted following the manufacturer's instructions.

Statistical analysis

The antibody titer was presented as median (interquartile range [IQR]). We used the Mann—Whitney U test for calculating statistical significance between antibody titer and concentration percentage. The Kruskal—Wallis test was used for comparison of more than two groups, and the Mann—Whitney U test was used in post hoc tests. To adjust p values for multiple comparisons between groups, Bonferroni's correction was used. The linear regression model was used to analyze antibody titer difference in naïve patients by age group (the group aged 12—18 years was eliminated due to insufficient case number). We used the group aged 6 months to 2 years as the reference for the multiple linear regression model, adjusting for potential cofounding factors (including sex, C-reactive protein [CRP], white blood cell counts [WBC], and drug usage). Because anti-spike antibodies were not normally distributed, the data was analyzed after log transformation, and the data was normally distributed after log transformation. NAb concentration was analyzed with raw data, since it was already normally distributed. Statistical significance was set as $p < 0.05$. All analyses were performed using SAS software 9.4 version (SAS Institute, Inc., Cary, NC) and figures were generated using Graphpad Prism 9.5.1 (Dotmatics, Boston, MA).

Results

Epidemiology and clinical characteristics

Monthly distribution of the COVID-19 cases in the current study, along with the nationwide COVID-19 cases, are shown in Fig. 1. According to the Taiwan Centers for Disease Control (CDC) disease notification data, the endemic COVID-19 outbreak started from January 2022, and was predominantly caused by the Omicron variant since then (over 98% BA.2 strain after April 2022).[19,20] Our case distribution coincided with the curve of the national COVID-19 epidemic distribution.

In total, 164 pediatric COVID-19 cases were enrolled, and the demographics, clinical findings and laboratory data are summarized in Table 1. The mean age of these cases was 4.3 ± 4.1 years (range 1 month—18.8 years) and 57.3% of patients were male. During the clinical recruitment period, the COVID-19 vaccination timeline for children aged between 6 months and 5 years was officially commenced on July 21st, 2022, as stated by Taiwan CDC. However, we had evaluated medical history of children aged under 5 years in our study and none of them had vaccination history. Overall, the COVID-19 vaccination rate in current study was only 18.9% among all cases (49.0% in those 5—12 years; 87.5% in those 12—18 years). For children aged under 2 years, 33.0% of their mothers had received at least one dose of COVID-19 vaccination before delivery (63.6% of those 0—6 months; 19.1% of those 6 months to 2 years). Around 75.6% of patients were admitted to the hospital (mean hospitalization days 4.3 ± 3.1), and 16.5% of patients had an Intensive Care Unit admission. The systemic underlying diseases rate was 12.9%, including neurological disorders (4.9%), prematurity (2.4%), congenital heart disease (3.7%), diabetes mellitus

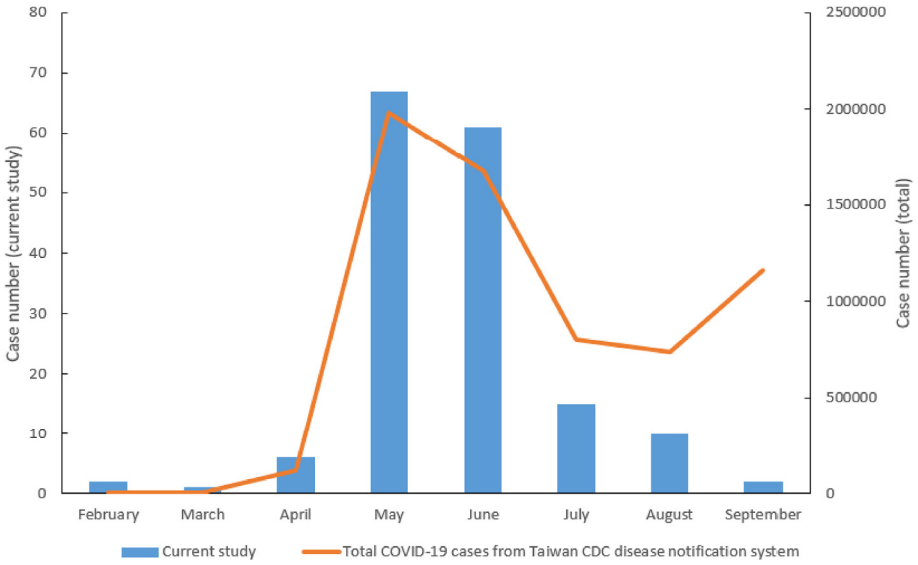

Figure 1. Monthly distribution of coronavirus disease 2019 (COVID-19) cases in the current study and in the Taiwan Centers for Disease Control (CDC) disease notification system during February to September 2022.

(0.6%), and other (5.5%, including asthma, genetic disorder, vesicoureteral reflux, psoriasis). The most common clinical presentations were fever (70.1%), cough (40.2%), and rhinorrhea (22.0%).

The most common clinical diagnoses were unspecific upper respiratory tract infection (URTI) (51.2%) and croup (12.8%). Croup presented more often in those aged under 5 years, a result compatible with the age of its usual occurrence. Combined bacterial infection was more often diagnosed in those under 2 years; the most common type was urinary tract infection (9 cases). Of the 6.7% of participants diagnosed with encephalitis, a high proportion were aged 5—12 years (14.3%). Febrile convulsions were historically seen in children aged 6 months to 5 years. However, recent studies show that older children can also experience them with the Omicron variant.[21,22] To capture this shift, we included patients over 5 years in our calculations, offering a comprehensive view of the disease manifestation, particularly in the context of the Omicron variant.

Serological response during convalescent period

During the convalescent period, 144 patients had a blood sample drawn, and the time between diagnosis and blood sampling was around 38 days (median 38.3, range 13—87 days). There was no significant correlation between the timing of blood sampling and NAb level during the study period (Supplementary Fig. 1). The anti-spike antibody level by age group is shown in Table 2. Children aged under 6 months with maternal vaccination had a higher anti-spike antibody level than those without maternal vaccination (119.0 vs 27.4 IU/ml, $p = 0.023$), but children aged 6 months to 2 years did not have this difference. Children aged 5—12 years who had any dose of COVID-19 vaccine before infection had a higher anti-spike antibody level than those who did not receive COVID-19 vaccine (431.1 vs 17.4 IU/ml, $p < 0.001$).

NAb against wild type disease and variants of concern

Since these patients were most likely infected with Omicron BA.2 variant according to the Taiwan CDC epidemiological surveillance database, we also measured the neutralizing antibody level (inhibition %) against wild type and Omicron BA.2 in these patients (Fig. 2). For children under 6 months old, the NAb inhibition percentage against wild type was significantly higher in those with maternal vaccination than in those without (56.9% vs 27.6%, $p = 0.001$), but no significant difference was noted in BA.2 variant neutralization activity. Also, maternal vaccination had no impact on the NAb inhibition percentage generated in children aged 6 months to 2 years. Children 5—12 years who had prior COVID-19 vaccination had a higher NAb inhibition percentage against both wild type (91.4% vs 23.6%, $p < 0.001$) and BA.2 variant (47.8% vs 25.8%, $p = 0.002$) than those without prior vaccination.

Binding antibody and NAb levels in previously naïve children

According to CDC, Taiwan, the incidence of COVID-19 cases before 2022 remained relatively low in comparison to the global trend (69.46 cases per 100,000 population was infected in Taiwan during 2021),[23] which suggest that a majority of the Taiwanese population were likely immunologically naive to the virus before 2022. We meticulously inquired about each patient's past medical history and records to exclude any possibility of prior infection. We also randomly selected 17 patients and examined their acute stage anti-N antibodies using blood samples taken upon admission. None of these patients displayed positive anti-N antibodies during testing. We excluded children aged under 2 years with maternal vaccination and children with their

Table 1 Demographic and clinical characteristics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected children by age group (n = 164).

	0—6 months (n = 22)	6 months to 2 years (n = 47)	2—5 years (n = 38)	5—12 years (n = 49)	12—18 years (n = 8)	Total (n = 164)
Male sex	11 (50.0)	26 (55.3)	24 (63.1)	30 (62.5)	3 (7.5)	94 (57.3)
Age (years), mean ± SD[c]	0.3 ± 0.1	1.2 ± 0.4	3.4 ± 1.0	8.0 ± 1.9	15.2 ± 2.6	4.3 ± 4.1
Vaccination status						
Maternal vaccination[a],[c]	14 (63.6)	9 (19.1)	0 (0.0)	0 (0.0)	0 (0.0)	23 (14.0)
Vaccination[b],[c]	0 (0.0)	0 (0.0)	0 (0.0)	24 (49.0)	7 (87.5)	31 (18.9)
Hospitalization	22 (100.0)	42 (89.4)	30 (78.9)	25 (51.0)	5 (62.5)	124 (75.6)
Days (mean ± SD)	3.6 ± 1.3	3.4 ± 1.8	4.1 ± 2.2	6.1 ± 5.3	6.6 ± 3.8	4.3 ± 3.1
ICU admission	3 (13.6)	4 (8.5)	6 (15.8)	11 (22.4)	3 (37.5)	27 (16.5)
Underlying diseases	4 (18.2)	6 (12.8)	2 (5.3)	7 (14.3)	2 (25.0)	21 (12.9)
Neurological disorders	0 (0)	1 (2.1)	1 (2.6)	3 (6.1)	1 (12.5)	8 (4.9)
Prematurity	0 (0)	2 (4.3)	1 (2.6)	1 (2.0)	0 (0)	4 (2.4)
Congenital heart disease	2 (9.1)	2 (4.3)	0 (0)	2 (4.1)	0 (0)	6 (3.7)
Diabetes mellitus	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	1 (0.6)
Others	2 (9.1)	2 (4.3)	2 (5.3)	2 (4.1)	1 (12.5)	9 (5.5)
Clinical symptoms						
Fever	18 (81.8)	40 (85.1)	26 (68.4)	27 (55.1)	4 (50.0)	115 (70.1)
Cough	11 (50.0)	23 (48.9)	15 (39.5)	15 (30.6)	2 (25.0)	66 (40.2)
Rhinorrhea	2 (9.1)	14 (29.8)	11 (28.9)	7 (14.3)	2 (25.0)	26 (22.0)
Sore throat	0 (0.0)	1 (2.1)	1 (2.6)	2 (4.1)	1 (12.5)	5 (3.0)
Vomiting/anorexia	1 (4.5)	10 (21.3)	5 (13.2)	8 (16.3)	1 (12.5)	25 (15.2)
Diarrhea	5 (22.7)	14 (29.8)	4 (10.5)	4 (8.2)	0 (0)	27 (16.5)
Abdominal pain[c]	0 (0)	0 (0)	0 (0)	8 (16.3)	0 (0)	8 (4.9)
Skin rash	5 (22.7)	7 (14.9)	4 (10.5)	5 (10.2)	0 (0)	21 (12.8)
Muscle pain[c]	0 (0)	0 (0)	2 (5.3)	5 (10.2)	0 (0)	7 (4.3)
Seizure	0 (0)	12 (25.5)	3 (7.9)	8 (16.3)	0 (0)	24 (14.6)
Bizarre behavior[c]	0 (0)	0 (0)	1 (2.6)	4 (8.2)	1 (12.5)	6 (3.7)
Diagnosis						
URTI	14 (63.6)	17 (36.2)	19 (50.0)	32 (65.3)	2 (25)	84 (51.2)
Croup[c]	3 (13.6)	14 (29.8)	4 (10.5)	0 (0)	0 (0)	21 (12.8)
Myositis	0 (0)	0 (0)	1 (2.6)	1 (2.0)	0 (0)	2 (1.2)
Febrile convulsion	0 (0)	6 (12.8)	3 (7.9)	2 (4.1)	0 (0)	11 (6.7)
Encephalitis[c]	0 (0)	1 (2.1)	2 (5.3)	7 (14.3)	1 (12.5)	11 (6.7)
LRTI	0 (0)	1 (2.1)	2 (5.3)	2 (4.1)	0 (0)	5 (3.0)
MIS-C	0 (0)	0 (0)	2 (5.3)	0 (0)	1 (12.5)	3 (1.8)
Bacterial infection[c]	4 (18.2)	6 (12.8)	3 (7.9)	1 (2.0)	1 (12.5)	15 (9.1)
Other	1 (4.5)	2 (4.3)	2 (5.3)	4 (8.2)	3 (37.5)	12 (7.3)
Laboratory data, mean ± SD						
WBC (× 10^9/L)	7.7 ± 2.6	8.6 ± 3.2	7.4 ± 3.1	7.1 ± 3.4	7.8 ± 3.3	7.8 ± 3.1
Segment (%)[c]	36.6 ± 20.1	46.1 ± 20.1	56.7 ± 19.4	69.3 ± 14.7	71.8 ± 15.4	53.3 ± 21.8
Lymphocyte (%)[c]	47.5 ± 25.6	36.8 ± 18.1	27.0 ± 17.3	16.1 ± 12.8	17.1 ± 14.5	30.8 ± 20.9
Monocyte (%)[c]	14.0 ± 6.1	11.9 ± 5.4	10.4 ± 5.2	9.1 ± 5.1	8.5 ± 3.5	11.1 ± 5.5
Platelet (× 10^9/L)[c]	377 ± 115	270 ± 130	251 ± 118	215 ± 71	285 ± 150	272 ± 125
CRP (mg/L)	3.3 ± 4.4	17.5 ± 24.9	23.1 ± 31.8	14.3 ± 21.7	16.4 ± 27.4	16.2 ± 25.1

[a] Maternal vaccination: mother received at least one dose, any brand of SARS-CoV2 vaccine before delivery.
[b] Vaccination: participants received at least one dose, any brand of SARS-CoV2 vaccine before coronavirus disease 2019 (COVID-19) diagnosis.
[c] Significant different between age groups (p value < 0.05).
Data is shown as no. (%), unless indicated otherwise.
Abbreviations: SD: standard deviation; ICU, intensive care unit; URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; MIS-C, multi-system inflammatory syndrome in children; WBC, white blood count; CRP, C-reactive protein.
Other: includes urticaria, viral exanthema, shock, hepatitis, asthma, acute bronchiolitis, pericardial effusion, and syncope.

Table 2 The anti-spike antibody level generated after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron infection in children, by age and vaccination status (n = 109).

	0–6 months			6 months to 2 years			5–12 years		
	Without maternal vaccination (n = 8)	Maternal vaccination (n = 13)	*p*-value	Without maternal vaccination (n = 38)	Maternal vaccination (n = 8)	*p*-value	Without vaccination (n = 21)	Vaccination (n = 21)	*p*-value
Anti-spike antibody (U/mL), Median (IQR)	27.4 (19.5–43.4)	119.0 (43.0–228.3)	0.023	86.5 (45.6–135.1)	50.5 (22.5–99.5)	0.102	17.4 (4.9–22.4)	431.1 (217.2–4039.0)	<0.001

IQR: interquartile range.

own vaccination, then re-evaluated the anti-spike antibody and NAb levels in these previously naïve children (Fig. 3 and Supplementary Table 1). The anti-spike antibody and the NAb levels against the wild type, Delta, Omicron BA.1, and BA.2 variants were highest in children aged 6 months to 2 years. Post hoc tests showed differences in the levels of all NAbs between children aged 6 months to 2 years and those aged 5–12 years. However, not all groups had statistical significance in all NAb results.

Linear regression

We adjusted our linear regression model for the potential cofounding factors of sex, CRP, WBC, anti-viral drug use, underlying disease, bacterial infection and encephalitis on anti-spike antibody levels (Table 3). Both CRP and WBC were positively associated with anti-spike antibody level, but lost significance in the multivariable linear regression model. With the anti-spike antibody level in children aged 6 months to 2 years used as the reference, all other age groups were negatively associated with anti-spike antibody level in the univariate linear regression model. In the multivariable regression model, only age 5–12 years was significantly negatively associated with anti-spike antibody level (β coefficient -1.75, $p < 0.001$) after adjusting other variables.

Discussion

In the present study, the most common clinical manifestation in previous infection-naïve children with primary SARS-CoV-2 Omicron infection is mild, uncomplicated URTI. In serum recovered from these patients, the amount of both the anti-spike antibody and NAb is influenced by prior vaccination status, either through antenatal maternal vaccination (children younger than 6 months) or self-vaccination (children aged 5–12 years), although self-vaccination contributes more. In all infection-naïve children without maternal or self-vaccination, children aged 6 months to 2 years demonstrated the highest levels of anti-spike antibody and NAb. Age was the independent factor contributing to anti-spike antibody level, after adjustment for sex, CRP, WBC, and antiviral drug use.

In general, children with COVID-19 present with milder symptoms and are at lower risk of hospitalization and life-threatening complications than adults.[24] In our study, croup was the most common clinical diagnosis (12.8%), after URTI (51.2%). Two retrospective analyses found that the incidence of croup co-occurring with SARS-CoV-2 infection sharply increased with the emergence of the Omicron variant.[25,26] Another multicenter retrospective cohort study found that the proportion of patients with croup was significant higher during the Omicron period than the Delta period (11.0% vs 0.5%).[27] The neurologic features associated with SARS-CoV-2 infection in children are diverse. A cross-sectional multicenter study found neurologic complications in 7% of children hospitalized with COVID-19, most commonly febrile seizures (3.8%), followed by non-febrile seizures (2.3%) and encephalopathy (2.1%).[4] Another multicenter retrospective study conducted by the Spanish Society of Neurology reported encephalitis in 2.2% of the

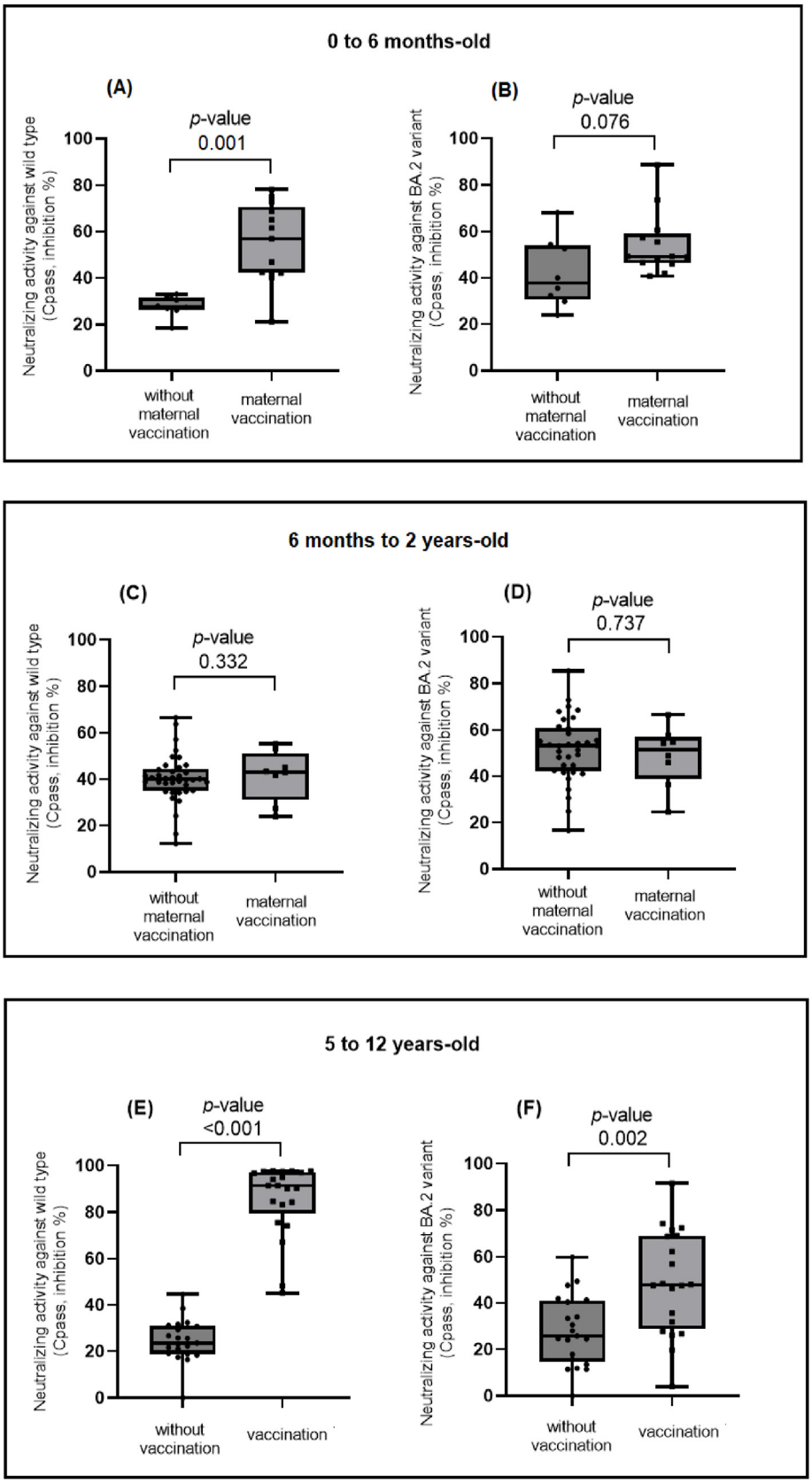

Figure 2. The neutralizing antibody activity level against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wild type disease and the Omicron BA.2 variant in children with primary Omicron BA.2 infection, by age and vaccination status. Box-and-whiskers plot of SARS-CoV-2 neutralizing antibody level (inhibition percentage) against wild type and Omicron BA.2 variant by vaccination status. The neutralizing antibody against wild type disease in children aged 0—6 months is shown in (A) and against

COVID-19 patients with neurological symptoms.[28] In our study, up to 6.7% of patients had encephalitis, a higher rate than previously reported. The differences in age, population characteristics, study period dates, and disease definitions may have contributed to these rate differences. For example, our definition of COVID-19 encephalitis/encephalopathy used only clinical symptoms for diagnosis (defined as visual or auditory hallucination, bizarre behavior, or altered consciousness, with or without seizure), which may lead to over-diagnosis. Also, the higher rate of more severe patients in a tertiary hospital may also have contributed to the higher complication rate found in our study.

In our study, children younger than 6 months with maternal vaccination demonstrated significant higher NAb levels against wild type, but not against Omicron BA.2. This result demonstrates the lower cross-reactivity against the BA.2 variant from maternal vaccination. For children older than 6 months, maternal vaccination has no significant influence on antibody level, a result compatible to a previous study showing that passive maternal IgG levels decreased obviously after 6 months.[29] Another study revealed that at 6 months of age, 57% (16 out of 28) of infants born to vaccinated mothers retained detectable antibodies, while only 8% (1 out of 12) of infants born to infected mothers exhibited detectable antibodies.[30] These collective findings suggest that maternal antibody durability may not endure for an extended period and could vary depending on the source of immunity, either from infection or vaccination. Meanwhile, the influence of maternal antibodies on the infant's response to vaccines remains poorly understood. Existing studies on other vaccines have suggested that infants with higher levels of maternal antibodies might exhibit reduced responses to vaccines, a phenomenon known as immune interference.[31] Such interference could potentially impact the development of acquired immunity in infants. However, there is lack of relevant research regarding the interaction between COVID-19 vaccines and maternal antibodies in infants. This may be attributed to the fact that vaccines for children under 6 months old have not yet received approval. Additionally, previous studies indicate a decreased effect of maternal antibodies in children older than 6 months.[32] Further investigations are needed to gain a comprehensive understanding of the complex dynamics between maternal antibodies and vaccine responses in infants. Meanwhile, children aged 5—12 years with self-vaccination generated significant higher NAb levels against both wild type disease and the Omicron BA.2 variant, relative to than those without self-vaccination. Active vaccination not only enhances neutralizing antibody production against wild type disease, but also broadens the cross-reactivity against variants. Thus, for better protection against SARS-CoV-2, children should receive vaccination, whether or not their mother did so before delivery.

Previous studies have not determined whether antibody levels are correlated with age.[10—12,33—35] Our study found that children aged 6 months to 2 years had higher levels of

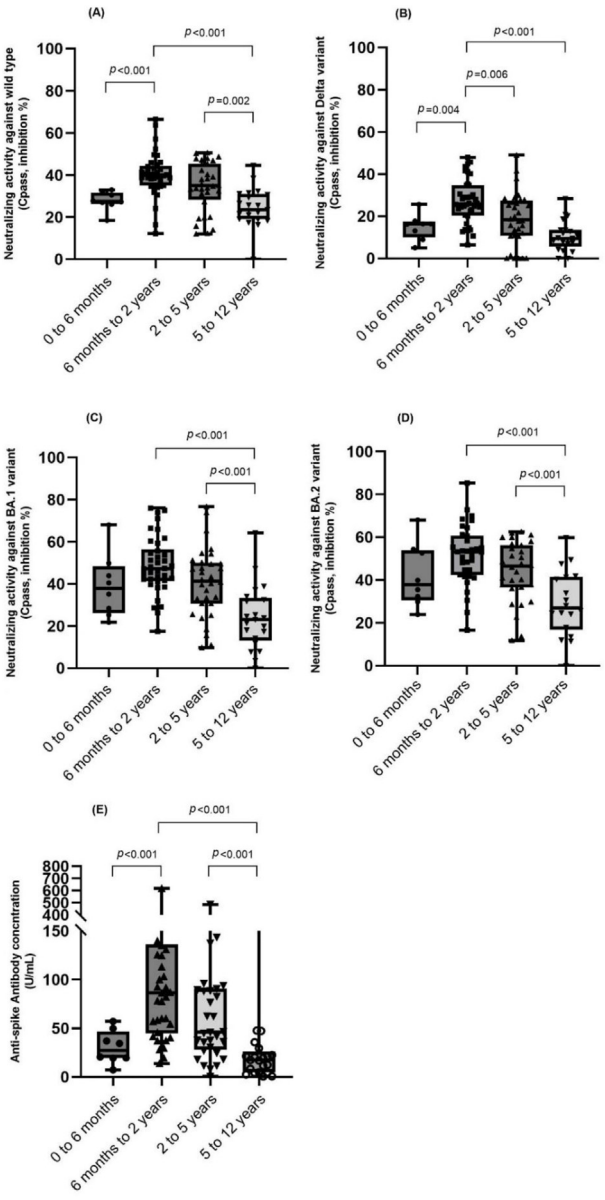

Figure 3. The anti-spike antibody and neutralizing antibody activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wild type, Delta Omicron BA.1, and BA.2 variants in previous disease-naïve children with primary Omicron BA.2 infection.

Box-and-whiskers plot of SARS-CoV-2 anti-spike antibody and neutralizing antibody activity (inhibition percentage) against wild type disease, Delta, Omicron BA.1, and Omicron BA.2 variants in previous disease-naïve children is shown. Panels show the neutralizing antibody activity against the wild type (A), delta variant (B), BA.1 variant (C), and BA.2 variant (D) of the disease. The anti-spike antibody is shown in (E). Statistical differences in neutralizing antibody activity between groups were compared using the Mann-Whitney-U test, and only statistically significant results were marked on the graph.

Omicron BA.2 in (B); in children aged 6 months to 2 years old against wild type disease is shown in (C) and against Omicron BA.2 in (D); and in children aged 5—12 years against wild type disease is shown in (E) and against Omicron BA.2 in (F). Statistical differences between groups in neutralizing antibody level were compared using the Mann-Whitney-U test.

Table 3 Univariable and multivariable linear regression analysis to evaluate the association of anti-spike antibody generation and clinical variables.

Clinical variables	Univariable linear regression		Multivariable linear regression	
	β± SE	*p*-value	β± SE	*p*-value
Age group				
0—6 months	−1.16 ± 0.42	0.007*	−0.94 ± 0.49	0.058
6 months to 2 years	Ref.		Ref.	
2—5 years	−0.60 ± 0.26	0.023*	−0.30 ± 0.27	0.262
5—12 years	−1.94 ± 0.29	<0.001*	−1.75 ± 0.39	<0.001*
Male sex	0.31 ± 0.27	0.254	0.41 ± 0.25	0.109
CRP ≥7 (mg/L)	0.93 ± 0.27	0.001*	0.41 ± 0.24	0.096
WBC (× 10^9/L)	0.12 ± 0.04	0.003*	0.07 ± 0.04	0.060
Anti-viral drug use	−0.92 ± 0.50	0.069	−0.37 ± 0.46	0.423
Underlying disease	−0.28 ± 0.76	0.712	0.13 ± 0.59	0.824
Bacterial infection	−0.09 ± 0.48	0.860	0.08 ± 0.43	0.845
Encephalitis	−0.78 ± 0.76	0.310	−0.42 ± 0.63	0.513

Abbreviations: β±SE, β coefficient ± standard error; Ref., reference group; WBC, white blood count; CRP, C-reactive protein.

anti-spike antibody and NAb than other age groups, contrary to the assumption that older children have a stronger immune response. The reason for this result is unknown, but some previous research has suggested that the higher levels of SARS-CoV-2 antibodies observed in children may be due to a booster effect from cross-reactivity with other human coronaviruses. However, this theory contradicts the finding that older children had lower anti-spike antibody levels. Although the innate immune response may be higher in younger children,[10,33,34,36] this finding could not fully explain the higher antibody level in this specific age range. We speculate that the result may be related to heterologous immunity from previous vaccinations, as children under 2 years have recently received bacillus Calmette—Guérin; measles, mumps, and rubella; tetanus, diphtheria, pertussis; and other vaccines.[37—40]

Given that children are recognized as major spreaders of respiratory viruses in the community, their heightened antibody response to SARS-CoV-2 plays a crucial role in controlling virus replication and curbing further transmission. Consequently, our findings carry important implications for achieving herd immunity and understanding the role of children in the transmission of SARS-CoV-2. The relatively high level of antibodies observed 3—5 months after acute infection in young children may confer protection against re-infection and transmission, particularly compared to older children. However, it is essential to acknowledge that despite the stronger antibody response demonstrated by younger children, the antibody levels generated after natural infection are still lower than those induced by vaccination. Furthermore, with the emergence of evolving Omicron sub-variants and their associated immune escape mechanisms, a comprehensive assessment of the true re-infection rate in this population is warranted.

One study demonstrated that anti-SARS-CoV-2 IgG levels positively correlate with those of inflammatory markers (including interleukin-6, lactate dehydrogenase, erythrocyte sedimentation rate, and CRP) and blood cell count (percentages of granulocytes).[41] Thus, we adjusted our linear regression analysis for the potential cofounding factors of male sex, CRP, WBC count, and antiviral drug use. However, in this study, only age group had a significant effect in the multivariable linear regression analysis, indicating that age is likely the most relevant factor for the magnitude of antibody level. Although natural infection induced substantial immunity against SARS-CoV-2, the antibody levels were relatively lower in those with primary infection compared to those with previous vaccination. On a positive note, children previously infected with SARS-CoV-2 may develop potent hybrid immunity upon receiving vaccination, similar to what has been observed in adults. Therefore, vaccination may be still warranted in these previously infected children to protect against reinfection.

There are some limitations to our study. First, the antibody level was measured only once at 1 month post infection, and the duration and decline rate of antibody level were not tested. Although antibody level at this time point may not fully represent long-term protection, a higher antibody level was found to possess better durability in a previous study.[7] Second, the study was conducted in a tertiary medical center where disease severity was higher than in the general population, which may influence the antibody concentration levels. Previous studies found that symptomatic COVID-19 patients, especially extremely severe cases, had lower antibody levels than those with less severe disease.[8,11,42] Therefore, antibody level in our study may be slightly over-estimated relative to those in patients not needing hospitalization. Lastly, the study period coincided with the prevalence of the SARS-CoV-2 BA.2 variant in Taiwan, but there was no actual evidence of BA.2 infection in each patient, which may lead to under- or over-estimation of the level of Omicron BA.2 infection.

Conclusion

In conclusion, this study demonstrates that younger children develop higher anti-spike antibody and NAb levels with SARS-CoV-2 infection, peaking at 6 months to 2 years. Children younger than 6 months with maternal

immunization and children with self-vaccination had higher antibody levels than those without. Age and prior vaccination are the major factors influencing the magnitude of antibody level after infection.

Author contributions

CFS, CCL, and PCH are the guarantors of the content of this manuscript, had full access to all the data in the study, and take responsibility for the integrity of the data. CFS, CCS, and CMC contributed to the study conception and design, as well as the collection of data. PCH and TYL organized the database and collected the clinical information. SWS and BYT organized and performed the experiment. CFS and PCH wrote and revised the draft. CCC, YFT, PJT, and WCK contributed to the data interpretation and to the critical review of the manuscript. All authors contributed to manuscript revision, and have read and approved the submitted version.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.08.006.