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Original Article

Immunogenicity of SARS-CoV-2 vaccination in adolescents with cardiac disease

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Abstract

Background: Although widely reported to affect older adults more, coronavirus disease 2019 also affects adolescents especially with co-morbidities, including heart diseases. The safety and efficacy of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines was established in healthy adolescents, yet there were few data for humoral and cellular immunogenicity in adolescents with cardiac diseases.

Methods: We evaluated anti-spike antibodies, neutralizing activities, and interferon-gamma production prior to and post SARS-CoV-2 vaccination in adolescents with cardiac diseases and healthy controls.

Results: Five healthy adolescents and 26 patients cardiac diseases including congenital heart disease (CHD, n=10), dilated cardiomyopathy (DCM, n=4), idiopathic pulmonary arterial hypertension (IPAH, n=4), and post-heart transplantation (HTx, n=8) were enrolled. No severe adverse events including myocarditis and pericarditis were noted, even in patients with severe heart failure. Febrile events were noted in 21 of 62 injections (34%). All the healthy adolescents and 21 of the 26 patients (81%) showed sufficient elevation of neutralizing antibodies after the second dose of vaccination. Neutralizing

antibodies and cellular immunity were absent in four of the eight post-HTx patients and one with CHD of single ventricle. There was no correlation between the anti-spike and neutralizing antibody titers and interferon-gamma levels. When comparing the clinical characteristics of the patients post-HTx who did or did not acquire antibodies, there were no significant differences in the immunosuppressant types and trough levels.

Conclusion: SARS-CoV-2 mRNA vaccine has efficient immunogenicity for adolescents with CHD, IPAH, and DCM. Half of post-HTx patients could not acquire sufficient humoral immunity.

Keywords: antibody, COVID-19, congenital heart disease, SARS-CoV-2, quantiferon, vaccination,

Introduction

Although the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been widely reported to affect the geriatric population more, it also affects children and adolescents, especially those with co-morbidities including immunodeficiency, congenital heart disease (CHD), lung diseases, and obesity. [1] In Japan, there are two mRNA vaccines available for adolescents who are aged 12 years or older (BNT162b2 [Pfizer-BioNTech] and mRNA-1273 [Moderna]). Their safety and efficacy has been established in clinical trials involving healthy adolescents. [2,3] However, the humoral and cellular immunity after vaccination have not been clarified, especially in adolescents post-heart transplantation (HTx), or with CHD, heart failure, and single ventricle physiology. Thus, we aimed to investigate the immunogenicity of the SARS-CoV-2 vaccine among adolescents with heart disease.

Subjects and Methods

Study design and protocol

Twenty-six patients with cardiac disease, including CHD after surgical repair, dilated

cardiomyopathy (DCM), idiopathic pulmonary arterial hypertension (IPAH), and post-HTx, who were aged <20 years, followed up at Osaka University Hospital; and who received SARS-CoV-2 mRNA vaccination between July 2021 and November 2021 were included for participation in this study. Additionally, five healthy volunteers who were aged <18 years and received vaccination during the same period were also included. This study was approved by the Osaka University Clinical Research Review Committee (no. 21203). Written informed consent was obtained from all the participants and their parents.

Blood samples were collected 1–7 days before the vaccination, 2–3 weeks after the first dose (before the second dose), and 3–4 weeks after the second dose. At the time of sampling, information about adverse events and the types of vaccines (BNT162b2 or mRNA-1273) were collected. Clinical information of the patients with cardiac disease was collected from the clinical records and included the patient's medical history, cardiac operation, medications, total immunoglobulin G (IgG) levels, and immunosuppressant trough levels.

Anti-spike IgG titer determination using an enzyme linked immunosorbent assay

(ELISA)

The anti-spike IgG titer was evaluated using an ELISA. [4] Briefly, 96-well plates were coated with recombinant SARS-CoV-2 spike protein (Cell Signaling Technology, Danvers, MA, USA; 1 µg/mL) at 4 °C overnight. The following day, after blocking with goat serum (Thermo Fisher Scientific, Waltham, MA, USA) for 2 h at room temperature (RT), the wells were incubated with the diluted sera (10- to 31 250-fold in goat serum) at 4 °C overnight. The wells were washed seven times with wash buffer (phosphate-buffered saline containing 0.05% Tween 20) and incubated with a secondary antibody (horseradish peroxidase-conjugated anti-human IgG, Abcam, Cambridge, UK) at RT for 3 h. After washing with wash buffer four times, the wells were incubated with peroxidase chromogenic substrate 3,3'-5,5'-tetramethyl benzidine (MilliporeSigma, Burlington, MA, USA) at RT for 30 min. The reaction was halted with 0.5 N sulfuric acid (MilliporeSigma). The absorbance of the wells was immediately measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The half-maximum antibody titers were calculated from the highest absorbance in the dilution range of sera using Prism 8 software (GraphPad Software, San Diego, CA, USA). The World Health Organization's International Standard for anti- SARS-CoV-2 immunoglobulin (NIBSC Code 20-136)

was used for the analysis and represented the IgG titer as binding antibodies units per mL

(BAU/mL). [5] Based on pre-vaccination sample range, the cutoff value was determined to

be 100 BAU/mL (Supplemental Table S1).

Whole blood interferon-gamma (IFN-γ) release immune assay (IGRA) for SARS-CoV-2 specific T cell responses using the QuantiFERON assay

The SARS-CoV-2 specific cluster of differentiation CD4/CD8 T cell immune responses

were evaluated using the SARS-CoV-2 QuantiFERON assay (QIAGEN, Hilden,

Germany), according to the manufacturer's instructions. [6] Whole blood samples mixed

with 1 mL of heparin were incubated in Ag_2 tubes coated with SARS-CoV-2 CD4+/CD8+

T cell epitopes at 37 °C for 22–24 hours. Plasma samples were obtained by centrifugation

at 3000 × g for 15 min, as well as from the Nil tube (negative control) and the Mito tube

(positive control). Plasma IFN-γ concentrations derived from SARS-CoV-2 specifically

activated T cells were measured using an ELISA (Qiagen). SARS-CoV-2-specific

secreted IFN-γ levels (IU/mL) were calculated by subtracting the value from the Nil tube

(negative control). As previously described, the cutoff value was set at 0.15 IU/mL. [7]

Pseudovirus production and Pseudovirus neutralization assay

For SARS-CoV-2 pseudo-typed virus construction, spike genes (GenBank: QZC47358.1) were codon-optimized for human cells and cloned into the eukaryotic expression plasmid pCAGG to generate the envelope recombinant plasmid pCAGG-SARS-CoV-2-Wuhan. The obtained construct was transfected into HEK293T cells using TransIT-LT1 (Mirus Bio). After 24 h, the cells were infected with VSVΔG-Luc/G, in which the G envelope gene was replaced with the luciferase gene and pseudo-typed with the VSV-G glycoprotein. [8, 9] The virus was absorbed for 2 h at 37 °C, and then extensively washed four times with Dulbecco's Modified Eagle Medium (DMEM). After 24 h, the culture supernatants were collected, centrifuged to remove cell debris, and stored at -80 °C until use.

Vero cells (1.5×10^4 cells/well) were seeded on 96-well plates overnight. Serum samples were inactivated at 56 °C for 30 min and diluted to 10–40 960 dilution with DMEM. Sixty microliters of pseudo-typed virus, equivalent to 2.5×10^6 RLU/mL, was incubated with an equal volume of diluted serum samples at 37 °C for 1 h. After incubation, 100 µL of

the mixture was added to the Vero cells and incubated for 24 h at 37 °C. The luciferase

activity of cell lysates was activated using the Luciferase Assay System (Promega,

Madison, WI, USA) and measured using Synergy LC (Agilent, Santa Clara, CA, USA).

The percent neutralization was calculated using Prism 8. The percentage of RLU

reduction (inhibition rate) was calculated as follows: 1- (RLU of samples – RLU of

pseudo-typed virus only wells)/(RLU from medium only wells)) × 100 (%). The

neutralization titer was calculated as 50% inhibitory dilution (ID50). Based on a previous

study, the cut-off value was determined to be 64. [10]

Statistical analysis

All analyses were performed by using JMP Pro14 software. Fisher's exact test was used

for comparison of the two groups of nominal variables. Mann–Whitney U test was

conducted for two group comparison of continuous variables. The linear regression

equations were used to analyze correlations between the anti-spike protein IgG,

neutralizing antibody, and IFN-γ values. $P<0.05$ was considered as statistically significant.

Results

Ten patients had CHD with bi-ventricular repair (BVR, n = 3) and uni-ventricular repair (UVR, n = 7), four had IPAH, four had DCM, and eight were post-HTx. The median age was 15 (range: 12–19) years. The clinical characteristics of ten patients with CHD are summarized in Supplemental Table S2. No patients had right isomerism, chromosomal abnormalities, including 22q11.2 deletion, early thymectomy, or splenectomy. Two patients had protein-losing enteropathy (PLE) after the Fontan operation. One patient had severe PLE and frequently required hospital admission due to pleural effusion and ascites. Although the other patient has relatively mild PLE and did not frequently require admission, her serum albumin level was reduced a few times per year. One patient with a single ventricle and atrium with left isomerism showed severe cyanosis. He had complications with a right congenital diaphragmatic hernia; therefore, he did not undergo the Glenn and Fontan operation.

Twenty-nine patients received BNT162b2 and two received mRNA-1273. No severe adverse events including myocarditis, pericarditis, and graft rejection were noted, even in the severe heart failure patients with DCM, single ventricle physiology, or post-HTx

(Table 1). Febrile events were noted in 21 of 62 injections (34%); however, all improved within 2 days. Another major complication was pain in the injected arm (45%). Headache, gastrointestinal manifestations, and muscle or joint pain were relatively rare as compared to the previous reports. [2, 3] There was no significant correlation between the incidence of adverse events and cardiac disease.

The median [interquartile range (IQR)] anti-spike antibody titers after the second dose of vaccination were 7696 (7684–8863) BAU/mL in healthy adolescents and 5826 (802-12577) BAU/mL in all the cardiac disease patients (cut off 100 BAU/mL). The median (IQR) neutralizing activities were 3578 (2340–4244) (ID50) in healthy adolescents and 1997 (396-3623) (ID50) in all the cardiac disease patients (cut off 64). All the healthy adolescents and 21 of the 26 patients with cardiac disease (81%) showed sufficient elevation of both the anti-spike and neutralizing antibodies after the second vaccination dose. However, neutralizing antibodies was absent in four of the eight post-HTx patients and one with UVR (Figure 1A and 1B). The patient with UVR experienced Fontan failure with severe PLE and growth retardation.

The median (IQR) IFN-γ values evaluated by QuantiFERON were 0.77 (0.40-1.42)

IU/mL in healthy adolescents and 0.86 (0.14–1.92) IU/mL in all the cardiac disease patients (cut off 0.15 IU/mL). Despite sufficient neutralizing antibody titers after immunization, two patients post-HTx lacked cellular immunity (Figure 1C).

When comparing the clinical characteristics of the patients post-HTx with respect to acquiring sufficient neutralizing antibodies, there were no significant differences in sex, age, type of vaccine, age at HTx, time after HTx, adverse events of vaccination, types and trough levels of immunosuppressant, or history of rejection. However, serum levels of total IgG were significantly lower in the group with absent neutralizing antibody (Table 2).

Finally, we assessed the correlation between each value of humoral and cellular immunity. Even with a low determination coefficient, there was a significant correlation between anti-spike IgG levels and the neutralizing activity. However, there was no correlation between the anti-spike antibody titers or neutralizing activities and IFN-γ values (Figure 2).

Discussion

This is the first study to investigate both humoral and cellular immunogenicity in adolescents after SARS-CoV-2 mRNA vaccination. There were no significant adverse events among the healthy adolescents or patients with various cardiac diseases. Previous studies with large cohorts, which assessed the safety and efficacy of BNT162b2 and mRNA-1273 vaccines, revealed that fever above 38.0°C and fatigue was observed in 50–70% and 10–20% of the healthy adolescents, respectively.[2,3] Although the sample size in this study is relatively small, it is suggested that the incidence of adverse events in patients with cardiac disease might not be higher than that in healthy adolescents, regardless of their heart condition.

In this study, both humoral and cellular immunity can be successfully acquired in most patients with CHD, IPAH, and DCM, despite cyanosis or heart failure. Moreover, we found a relatively large variation of anti-spike IgG titers and neutralizing activities, which corroborates the findings of previous studies. [11, 12] One patient with UVR failed to acquire immunity. The patient was hypoplastic left heart syndrome and experienced chronic pleural effusion, ascites, and severe growth retardation caused by repeated PLE after Fontan procedure. Although serum albumin and total IgG levels were preserved at the time of vaccination, both humoral and cellular immunogenicity were lacking.

Immunosuppressants were reported to affect the humoral immunogenicity of SARS-CoV-2 vaccination. A previous study involving pediatric patients post-HTx reported that 12 of 33 (36%) who were SARS-CoV-2 naïve could not acquire sufficient anti-spike antibodies. [13] Similarly, three of eight (38%) patients, in this study, failed to acquire anti-spike IgG. We evaluated the neutralizing activities for pseudovirus, and found that one post-HTx patient showed lack of neutralizing antibody despite the sufficient titer of anti-spike antibody. However, the anti-spike antibody titers and neutralizing activities were significantly correlated (Figure 2A).

All the patients post-HTx received tacrolimus or cyclosporine plus mycophenolate mofetil in this study. Additionally, five of eight patients received everolimus. Several previous studies have reported that mycophenolate mofetil may weaken humoral immunogenicity after SARS-CoV-2 vaccination. [14, 15] However, here no differences were noted in the types and trough levels of immunosuppressants used. There was no significant difference in incidence and severity of adverse events of vaccination. On the other hand, we identified that serum level of total IgG was significantly lower in the post-HTx patients with absent neutralizing antibodies. The previous studies regarding post-HTx patients in children and adults did not concern the total IgG levels, [13, 14] whereas

another study demonstrated that total IgG levels were not associated with humoral immunogenicity in the primary immunodeficiency patients. [15]

Cellular immunogenicity after SARS-CoV-2 mRNA vaccination in adolescents has not been reported until now. We evaluated the SARS-CoV-2 specific cluster of differentiation CD4/CD8 T cell immune responses using the SARS-CoV-2 QuantiFERON assay. Cellular immunogenicity was absent in one CHD patient who experienced Fontan failure with PLE, and six of eight (75%) patients post-HTx. Previous studies revealed that both humoral and cellular immunogenicity in adult cardiothoracic transplant recipients were poorer as compared to those in healthy controls. [16, 17] The correlation of humoral and cellular immunogenicity still remains to be elucidated. One previous report suggested that low antibody levels generally showed limited cellular immunogenicity in the general population. [18] In our study, there were no significant correlations between anti-spike antibody titers or neutralizing activities and IFN-γ values. We did not evaluate the ratio of B cell and T cell, as well as CD4+ and CD8+ T cells. Meanwhile, a previous study investigating patients with human immunodeficiency virus demonstrated that fewer CD4+ T-cells were associated with humoral and cell-mediated immunogenicity after SARS-CoV-2 vaccination. [19]

The major limitation of this study is a small sample size. Further studies are necessary to uncover the factors which could affect humoral and cellular immunogenicity post SARS-CoV-2 mRNA vaccination, especially in immunocompromised children.

In conclusion, no severe adverse events were noted in SARS-CoV-2 mRNA vaccination for our small population of adolescents including various heart diseases. Both humoral and cellular immunogenicity of SARS-CoV-2 mRNA vaccines were sufficient in those adolescents, however immunosuppressants could weaken the immunogenicity.

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Author contribution statement

H.H. evaluated the antibody titers, neutralizing activity, and QuantiFERON interferon-gamma production, and wrote the manuscript. H.I., Y.Y., K.O., and H.N. designed the study, and wrote and revised the manuscript. J.N., R.I., M.H., and K.H. collected blood samples from the patients and initially prepared the samples for the experimental measurements, and wrote the manuscript. All authors approved the final manuscript for submission and agreed to be accountable for all aspects of the work.

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Table 1. The participants' characteristics and adverse events.

	All (n=31, 62 injections)	Healthy (n=5, 10 injections)	All heart diseases (n=26, 52 injections)	CHD with BVR (n=3, 6 injections)	CHD with UVR (n=7, 14 injections)	IPAH (n=4, 8 injections)	DCM (n=4, 8 injections)	Post-HTx (n=8, 16 injections)
Male, No. (%)	15, (48%)	1, (20%)	14, (54%)	1, (33%)	3, (43%)	1, (25%)	3, (75%)	6, (75%)
Age at vaccination, median (range)	15 (12-19)	14 (12-17)	15 (12-19)	13 (12-17)	14 (12-15)	15.5 (13-19)	13.5 (13-17)	18 (12-19)
Pre-history of COVID-19, No. (%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)
Adverse events								
Pain at injection site, No. (%)	28, (45%)	2, (20%)	26, (50%)	1, (17%)	6, (43%)	4, (50%)	5, (63%)	10, (56%)
Fever	21, (34%)	4, (40%)	17, (33%)	3, (50%)	4, (29%)	2, (25%)	1, (13%)	7, (44%)
Below 37.9°C, No. (%)	11, (18%)	1, (10%)	10, (19%)	3, (50%)	2, (14%)	0, (0%)	1, (13%)	4, (25%)
Above 38.0°C, No. (%)	10, (16%)	3, (30%)	7, (13%)	0, (0%)	2, (14%)	2, (25%)	0, (0%)	3, (19%)
Fatigue, No. (%)	7, (11%)	1, (10%)	6, (12%)	0, (0%)	0, (0%)	2, (25%)	1, (13%)	3, (19%)
Headache, No. (%)	1, (2%)	0, (0%)	1, (2%)	0, (0%)	0, (0%)	0, (0%)	1, (13%)	0, (0%)
Vomiting, No. (%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)
Diarrhea, No. (%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)
Muscle or joint pain, No. (%)	1, (2%)	0, (0%)	1, (2%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	1, (6%)
COVID-19 occurrence after 2nd injection, No. (%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)	0, (0%)

CHD, congenital heart disease; BVR, bi-ventricular repair; UVR, uni-ventricular repair; IPAH, idiopathic pulmonary arterial hypertension; DCM, dilated cardiomyopathy; HTx, heart transplantation; COVID-19, coronavirus disease 2019.

Table 2. Clinical characteristics of post-heart transplant adolescents after the second dose of SARS-CoV-2 mRNA vaccination

	Neutralizing antibody present (n=4)	Neutralizing antibody absent (n=4)	P value
Male, No. (%)	4 (100%)	2 (50%)	0.426
Age at vaccination (yr), median (range)	18 (15-19)	18 (12-19)	0.882
Type of vaccine, BNT162b2, No. (%)	3 (75%)	3 (75%)	1.00
Age at transplantation (yr), median (range)	12.5 (12-14)	15 (10-17)	0.381
Time after transplantation (yr), median (range)	4.5 (2-6)	2 (2-5)	0.216
Adverse events either 1st or 2nd injection			
Pain at injection site, No. (%)	3 (75%)	3 (75%)	1.00
Fever below 37.9°C, No. (%)	1 (25%)	2 (50%)	1.00
Fever above 38.0°C, No. (%)	1 (25%)	2 (50%)	1.00
Fatigue, No. (%)	2 (50%)	1 (25%)	1.00
Immunosuppressant			
Tacrolimus, No. (%)	3 (75%)	2 (50%)	1.00
Everolimus, No. (%)	2 (50%)	3 (75%)	1.00
Micophenolate mofetil, No. (%)	4 (100%)	4 (100%)	1.00
Cyclosporine A, No. (%)	1 (25%)	2 (50%)	1.00
Predonisolone, No. (%)	0 (0%)	0 (0%)	1.00
Trough level of immunosuppressant			
Tacrolimus (ng/mL), median (range)	7.3 (3.7-8.6)	6.5 (4.5-8.5)	1.00
Everolimus (ng/mL), median (range)	5.0 (4.5-5.5)	5.1 (3.5-5.7)	1.00
Micophenolate mofetil (µg/mL), median (range)	2.3 (0.9-3.0)	2.0 (1.2-4.6)	0.827
Cyclosporine A (ng/mL), median (range)	75	119 (115-123)	0.540
Serum level of total immunoglobulin G (mg/dL), median (range)	1158 (1079-1296)	864.5 (560-1092)	0.043¶
History of acute cellular rejection, No. (%)	1 (25%)	2 (50%)	1.00
History of antibody mediated rejection, No. (%)	0 (0%)	0 (0%)	1.00

¶P<0.05

Figure legend

Figure 1. Humoral and cell-mediated immunogenicity after SARS-CoV-2 mRNA vaccination in adolescents.

(A) Anti-spike IgG titers before and after the first and second vaccination doses. (B) Neutralizing activity for pseudo-virus Wuhan before the first and after the second vaccination dose. (C) IFN-γ levels determined using the SARS-CoV-2 QuantiFERON assay before and after the first and second vaccination doses. All the bars represent medians with interquartile ranges.

Figure 2. Scatter plots of the anti-spike IgG titers, neutralizing antibodies, and IFN-γ levels.

(A) Scatter plots of the anti-spike IgG titers and neutralizing activities. (B) Scatter plots of the anti-spike IgG titers and IFN-γ levels by QuantiFERON. (C) Scatter plots of the neutralizing activities and IFN-γ levels by QuantiFERON. The linear regression equations were used to analyze correlations between each value.
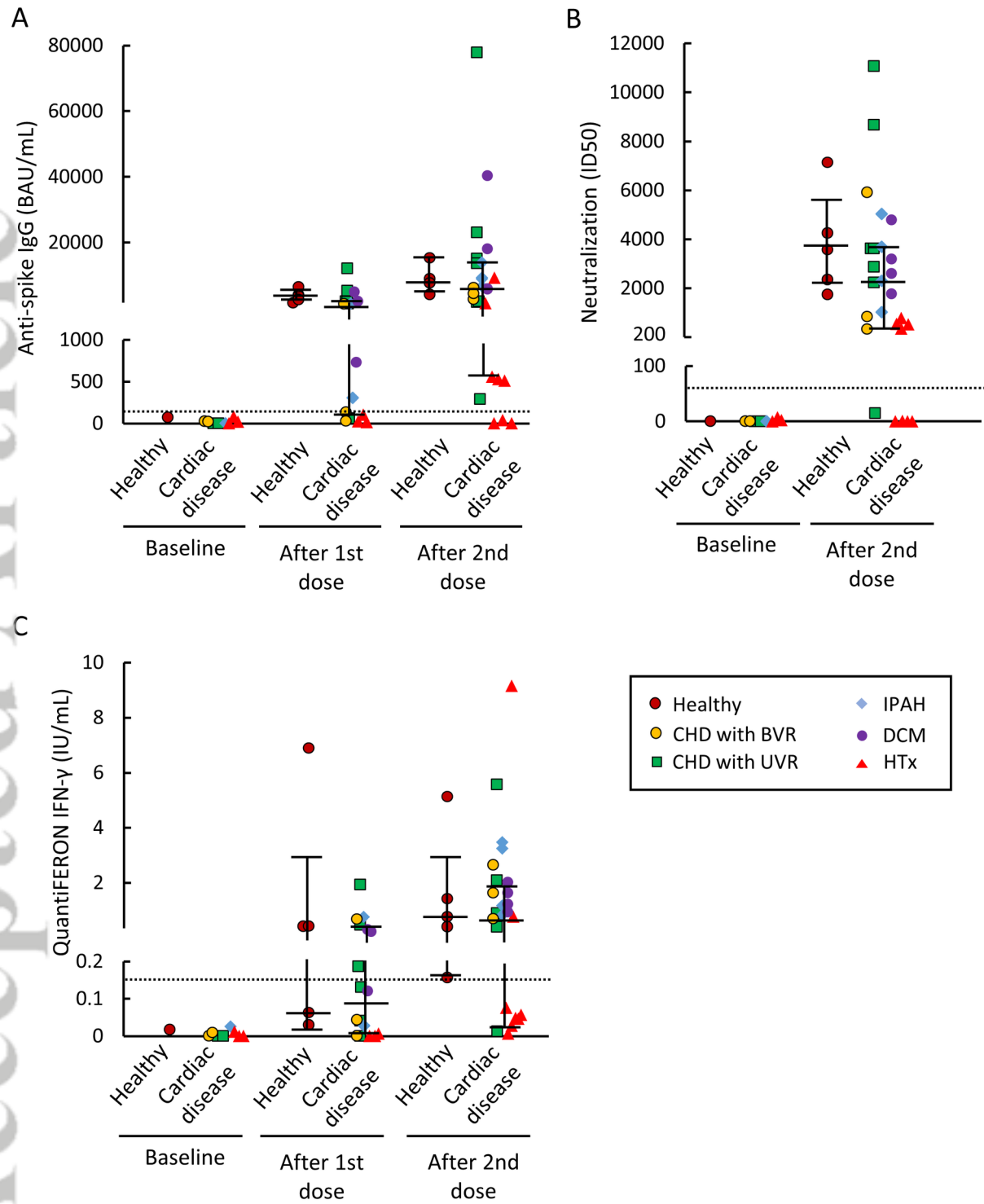
Figure 1

A

B

C

Legend:
- Healthy
- CHD with BVR
- CHD with UVR
- IPAH
- DCM
- HTx

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Figure 2