SARS-COV2 VACCINATION RESPONSE IN PEDIATRIC ONCOLOGY PATIENTS

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Abstract

Background: There remains limited knowledge about the immune response to severe acute respiratory syndrome coronavirus 2 (SARS-COV2) vaccination in pediatric oncology patients, which is essential to provide counseling and risk adaptation in this vulnerable population. The goal of this study was to understand immunogenicity after vaccination in pediatric oncology patients and determine if certain clinical factors impacted response. **Methods:** Patients 0-25 years of age with a diagnosis of cancer and actively receiving therapy were enrolled on study. We excluded patients who were completely vaccinated prior to their cancer diagnosis. Blood samples were collected pre-vaccination, as well as 2, 4-6, and 8-12 weeks after vaccination. Healthy children who were fully vaccinated enrolled as controls. Clinical data and complete blood counts around time of vaccination were collected. To study B and T cell immunity, we measured neutralizing antibodies by enzyme-linked immunoassay and interferon gamma secretion by enzyme-linked immunospot, respectively. **Results:** 26 patients enrolled on study, for which 11 were evaluable oncology patients and 7 were healthy controls. Adequate B cell response was seen in 36.4% of patients and adequate T cell response in 77.8% of patients. Numbers were too small to detect differences based on malignancy type. There was no differences in immunity based on absolute lymphocyte count (ALC) or intensity of therapy. **Conclusion:** Pediatric oncology patients have a suboptimal immune response to SARS-COV2 vaccination. Booster doses will be imperative to provide optimal protection against COVID-19, however blood counts may not be a useful guide to optimize the time of administration.

INTRODUCTION

The burden from severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) has significantly impacted the healthcare system, and vaccination remains our best protection against this virus. Children with cancer have a higher morbidity and mortality from SARS-CoV2 infection compared to healthy children.[1,2] This includes need for oxygen administration, pleural effusions, pneumothorax, pulmonary arterial hypertension, bronchiolitis, diffuse alveolar hemorrhage, and septic shock.[1] Additionally, infection has been associated with postponement and modification of chemotherapy, which may impact outcome.

Unfortunately, immunocompromised patients were excluded from original vaccine studies, leaving the oncology community to speculate the level of protection achieved with vaccination. A few recent studies from Europe have identified an impaired immune response in pediatric oncology patients after the BNT162b2 COVID-19 vaccination, especially those with hematologic malignancies or those undergoing intensive therapy.[3,4] In the adult oncology literature, vaccination has high rates of seroconversion, with hematologic malignancies showing lower immunogenicity.[5–7] In solid organ transplant recipients, a third dose of an mRNA vaccine was needed to achieve a substantial immune response.[8,9]The primary goal of this study was to understand immunogenicity after vaccination with BNT162b2 or mRNA-1273 in pediatric oncology patients and determine if certain clinical factors impacted response.

Methods:

After institutional review board (IRB) approval, a prospective study analyzing immune response to COVID-19 vaccination in pediatric cancer patients was undertaken. Eligibility criteria included cancer patients aged 0-25 years of age actively receiving therapy, including chemotherapy, immunotherapy, and targeted therapy. We excluded patients who were completely vaccinated prior to their cancer diagnosis. Patients may have received any of the vaccines approved for emergency use by the U.S. Food and Drug Administration. This includes BNT162b2, developed by Pfizer and BioNTech, mRNA-1273, developed by Moderna. For the remainder of this paper, these vaccines will be referred to as Pfizer and Moderna. Blood samples were collected pre-vaccination, as well as 2, 4-6, and 8-12 weeks after completion of COVID vaccination. Completion was defined as completion of the two dose series. As a control group, we enrolled patients in our clinic aged 0-25 years who were not receiving cancer directed therapy, followed for a benign hematology issue, or were more than 6 months after completion of therapy for their cancer diagnosis. These participants had one sample drawn between 2 weeks and 6 months of completion of vaccination.

Demographic data was collected from the medical record, including age, gender, ethnicity, cancer diagnosis, personal history of COVID, current therapy, type of vaccine received, and dates of vaccination. Therapy details included if it was chemotherapy, immunotherapy, targeted therapy, or combination therapy. We also collected if therapy was intravenous, oral, or both. If available, we also collected complete blood count (CBC) prior to vaccination and CBCs within 4 days of all sample time points.

Once blood samples were obtained in clinic, they were transported to the lab and processed within two hours. In order to maximize T cell yield for analysis in patients who may have low lymphocyte counts due to therapy, participants blood samples were collected in Vacutainer® CPT Cell Preparation Tube (BD) and processed according to the manufacturer's instructions. The cells were then re-suspended in 1ml of freezing medium (RPMI containing 40% FBS and 12.5% DMSO) and stored in liquid nitrogen.

To measure immunogenicity, we performed two assays. First, we measured neutralizing IgG antibodies, which has been shown to be highly predictive of immune protection from symptomatic SARS-CoV2 infection.[10] This was done using the SARS-CoV2-Surrogate Virus Neutralization Test Kit (GenScript) according to the manufacturer's instructions. All the samples were run in duplicates. The quantitative detection range of this assay is between 47 to 185U/ml. Titers [?]60.8 U/ml (lower 10% of the quantifiable detection range) was taken as inadequate B-cell response. Titers [?]60.8U/ml was taken as adequate B-cell response. The second assay measures interferon gamma (IFNγ) secretion, which is a marker of T cell response to viral antigens and another established method of measuring cellular immune response after vaccination.[11] This is done using an enzyme-linked immunosorbent spot (ELISPOT) kit for IFNγ (Mabtech). Briefly, frozen peripheral blood mononuclear cells were thawed and then seeded onto anti-human IFNγ monoclonal antibody pre-coated plates at a concentration of 250,000 cells/well. These cells were then stimulated with SARS –CoV-2 spike peptide pools at a concentration of 2μg/ml by incubating for 36 hours in 5% CO incubator and sent to Mabtech, Inc. Ohio, USA, for quantitation. Results were reported as Spot forming units per million cells (SFU/10^6cells). SFU values [?] Mean- standard deviation (i.e. [?] 659 SFU/10^6 cells) of the control sample values were defined as an inadequate T-cell response.

When analyzing what clinical features may impact vaccine response, we utilized B cell response by neutralizing IgG antibodies to define our adequate and inadequate response groups. This was because this assay allowed for replicates to strengthen the validity of our results as compared to the T cell assay, which could be only done once. Statistical analysis was done using Graphpad Prism 8.0. When appropriate, unpaired t tests were obtained to evaluate for statistical significance, which was defined as p value <0.05.

Results

A total of 19 cancer patients enrolled on study. Of these patients, one died of disease prior to vaccination, 4 did not complete the series, and three only had blood draws after boosters, leaving 11 evaluable patients. Table 1 highlights the demographic and clinical characteristics of these patients. There were 7 control patients, including 5 who were $>$ 6 months from cancer diagnosis and 2 followed for a benign hematology issue. Within the evaluable cancer patients, the number of samples for each time-point were as follows:

6 pre-vaccination, 8 two weeks post vaccination, 8 four-six weeks post vaccination, and 11 eight to twelve weeks after vaccination.

Among the 11 cancer patients analyzed, only 36.4% (n=4) had an adequate B cell response (Figure 1). Of these four patients, one had Ewing sarcoma on oral chemotherapy and another had Hodgkin lymphoma on chemoimmunotherapy. The other two had leukemia, including a patient with chronic myelogenous leukemia (CML) on an oral tyrosine kinase inhibitor and one with acute lymphoblastic leukemia (ALL) on maintenance therapy. It's important to note that two of these four patients may have had subclinical COVID infection prior to vaccination based on their neutralizing antibodies. Patient 23 (with Hodgkin lymphoma), maintained very elevated neutralizing antibodies despite extremely low blood counts, however because we were unable to obtain a pre-vaccination sample, it's uncertain if he started with high antibody levels. The patient with CML (Patient 3) had elevated neutralizing antibodies prior to vaccination, suggestive of subclinical COVID infection. However his titers continued to decrease over the 8-12 week period after vaccination, suggestive of a waning response. The other seven subjects had an inadequate B cell response, including one patient with Ewing sarcoma on myelosuppressive intravenous chemotherapy. All other poor responders were ALL patients in various phases of therapy, including escalated IV methotrexate, delayed intensification, and oral maintenance chemotherapy. Additionally, one of those patients (Patient 26) had documented COVID infection in the middle of the vaccination series, and despite this, still had an inadequate response.

Next, we measured T cell response after vaccination in pediatric oncology patients. Of note, 9 patients had a sample for this analysis at 8-12 weeks. Adequate T cell response was seen in 77.8% of patients (n = 7). This includes Patients 3 and 21 with suspected subclinical COVID infection and both solid tumor patients. Two leukemia patients with an inadequate T cell response were in highly myelosuppressive phases of therapy (Patient 2 and 16).

In order to understand what clinical factors may impact response to vaccination, we analyzed vaccine response (by neutralizing antibody levels) based on cancer diagnosis (hematologic vs solid tumor), absolute lymphocyte count (ALC), and therapy regimen (Figure 2). Because the majority of patients had leukemia, it was not possible to determine if there were alterations in response based on cancer type. However, we did note that the majority of patients with leukemia had an inferior B cell response compared to healthy controls (Figure 2A). As previously mentioned, the two patient who maintained similar levels of neutralizing antibodies as the healthy controls (Patient 3 and 23) may have had subclinical COVID prior to vaccination. There was no correlation seen between ALC and B cell response, as ALC varied widely among both groups (Figure 2B). In order to evaluate the relationship between chemotherapy regimen and vaccine response, we reviewed the phase of therapy patients were on at the time of vaccination and divided them into highly myelosuppressive or less myelosuppressive (Figure 2C). We also focused on the patients with a hematologic malignancy since there were only two patients with solid tumors. Patients with highly myelosuppressive therapy included those receiving escalated methotrexate, delayed intensification, or early Continuation. Less myelosuppressive therapy included oral targeted therapy and maintenance chemotherapy. There was no difference in neutralizing antibody levels at any time point between the two groups, suggesting that leukemia therapy in general was associated with poor immune response to vaccination.

Discussion

Vaccination in oncology patients is typically limited to diseases that are highly prevalent with high risk for morbidity and mortality. This is largely because we know that efficacy of vaccination in immunocompromised patients is poor.[1] The analysis of our pediatric oncology cohort, albeit small, reiterates the findings seen in pediatric and adult oncology and transplant patients. Specifically, our cohort showed an inadequate immune response to SARS-COV2 vaccination compared to healthy controls. There was a notable difference between B cell response and T cell response, with less patients having an adequate B cell response. No patients received targeted B cell directed therapy, but we do not have lymphocyte subset data to quantitate B vs T lymphocytes. Another limiting factor is our ability to only complete the T cell assay once, which may impact the validity of that data compared to the B cell data which was repeated multiple times. Lastly, while no patients received steroids during within one week of SARS-COV2 vaccination, many of them had

steroids in that phase of therapy, which may have had a greater impact on T cell immunity.[12]

While previous studies have demonstrated worse immune response in patients with leukemia/lymphoma as compared to solid tumor malignancy,[4–7] our numbers were too small to detect differences between these two cohorts. Similarly, we did not find significant differences in immune response based on ALC or degree of myelosuppressive therapy, suggesting that ALC or phase of therapy may not be useful in guiding timing of vaccination or boosters.

There were two other studies to date that have also looked at immunity after SARS-COV2 vaccination in pediatric oncology patients. Both of these studies were from European countries, where only the BNT162b2 (Pfizer) vaccine was available. One study out of Germany included 21 pediatric oncology patients after receiving three doses (the two dose series and a booster).[4] The majority of these patients elicited both B and T cell immunity, which was stronger in patients with a solid tumor malignancy and in maintenance phase of therapy. The other study by a group in the Netherlands analyzed 73 patients who received either 2 or 3 doses and also included patients who received a hematopoietic stem cell transplant or CAR T-cell therapy.[3] This study demonstrated that time between last treatment and start of the vaccination series impacted immunity, with improved vaccination response in patients who were > 6 weeks from last treatment. Similar to the German study, they showed that three dose series was effective in increasing the humoral immune response. There are a few key differences between these studies and ours. First, there are differences in treatment regimens between North America and Europe that may impact immune response during therapy. Additionally, these other two studies did not include healthy patients as a control, but rather used cutoffs defined by the assays to determine response. Lastly, all of our patients only received a two vaccine series, with very few patients receiving boosters. Our data, taken in context of these other two studies, reiterate the value of booster shots in immunocompromised patients.

The major limitation of our study is a small cohort size, which impacted our ability to analyze how different clinical factors may impact response. Similarly, our minimal enrollment of patients with solid tumor malignancy did not allow us to assess how different malignancies may impact response to vaccination. The Children's Oncology Group has a study currently open to evaluate immunologic response to COVID-19 vaccination in pediatric oncology patients. This study should shed additional light on how immunocompromised children responds to vaccination, what factors may impact that response, and most importantly, how we should counsel our patients and families regarding risks of infection, importance and timing of boosters, and risk for serious sequelae.

Conclusions

Pediatric oncology patients have suboptimal response to COVID-19 vaccination compared to healthy controls. Booster shots should be encouraged to improve immunity and reduce risk of severe disease. Further work in larger cohorts of patients is needed to understand how malignancy type, blood counts, and intensity of therapy may impact immune response.

Conflict of Interest Statement : The authors have no conflicts of interest to discolose.

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Figures Legends :

Figure 1: B cell response by Neutralizing IgG Antibodies in Pediatric Cancer Patients compared to Healthy Controls. Quantitative neutralizing antibody response at each time point after vaccination compared to healthy controls. Please note that healthy controls only had one time point and for optimal visualization, is presented in a linear fashion over each time point (red line). **A.** Pediatric cancer patients with adequate B cell response. **B.** Pediatric cancer patients with inadequate B cell response.

Figure 2: Evaluation of clinical factors that may response to SARS-COV2 vaccination . **A** . B cell response after vaccination in leukemia/lymphoma patients (upper) and solid tumor patients (lower**). B.** Comparison of absolute lymphocyte counts prior to vaccination (0 week) and 8-12 weeks post vaccination in the adequate vs inadequate response groups. There was no statistical significance between any of the groups (Adequate response 0 wk and inadequate response 0 wk, $p = 0.86$; Adequate response 8 wk and inadequate response 8 wk, $p = 0.99$; adequate response 0 wk and adequate response 8 wk, $p = 0.75$; inadequate response 0 wk and adequate response 8 wk, $p = 0.79$).**C.** B cell response after vaccination in patients with highly myelosuppressive (upper) and less myelosuppressive (lower) chemotherapy. There was no statistical difference in neutralizing antibodies between both therapy groups (2 wk, $p = 0.859$; 4-6 wk, $p = 0.740$, 8-12 wk, $p = 0.758$).

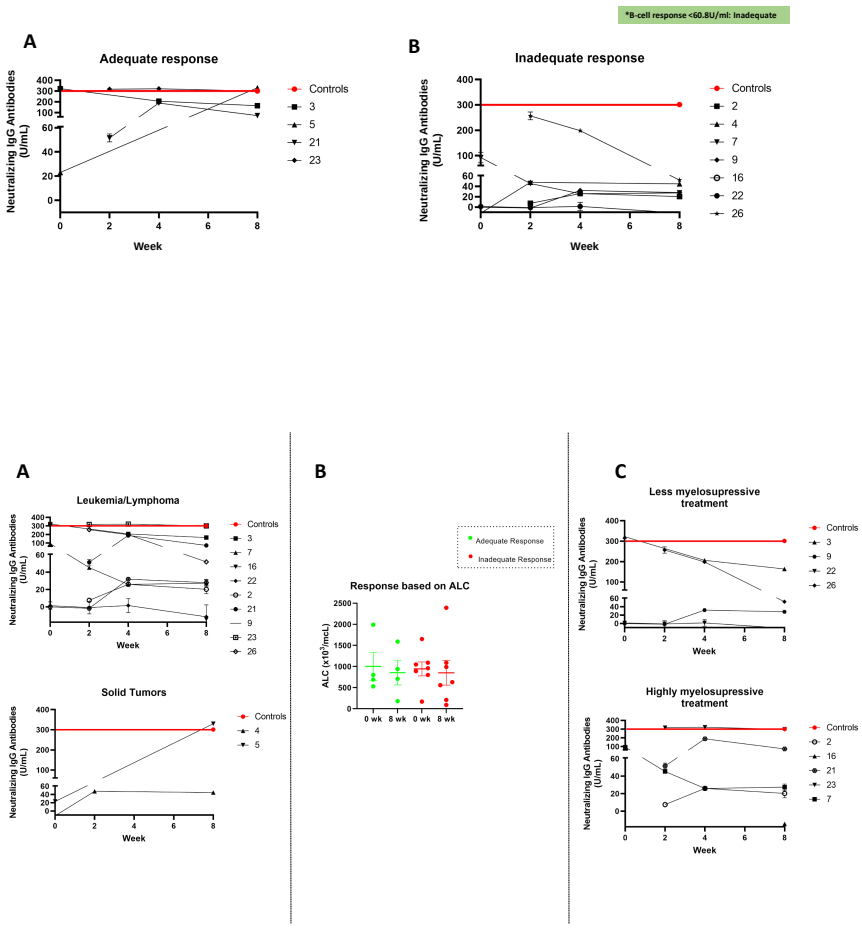

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Table 1 - Demographic and Clinical Data FINAL.docx available at https://authorea.com/users/671568/articles/671012-sars-cov2-vaccination-response-in-pediatric-oncology-patients