SARS-CoV-2 infection induces greater T-cell responses compared to vaccination in solid organ transplant recipients

Victor H. Ferreira[1,#], Tina Marinelli [1,2,#], Matthew Ierullo[1], Terrance Ku[1], Victoria G. Hall[1], Beata Majchrzak-Kita[1], Vathany Kulasingam[3], Atul Humar[1,*] and Deepali Kumar[1,*].

1 – Ajmera Transplant Centre, University Health Network, Toronto, Canada

2- Dept of Infectious Diseases, The Royal Prince Alfred Hospital, Sydney, Australia

3 - Laboratory Medicine Program, University Health Network, Toronto, Canada

- indicates co-first authorship

* - indicates co-senior authorship

Brief Summary: Solid organ transplant recipients mount antigen-specific T-cell responses after SARS-CoV-2 infection that correlate with antibodies and disease severity. Compared to natural infection, vaccine responses to two doses of mRNA vaccine result in comparably lower frequencies of antigen-specific CD4+ T-cells.

FOOTNOTES PAGE

Conflict of interest statement: A.H. has received clinical trials grants from Roche and Merck, and advisory fees from Merck. D.K. has received clinical trials grants from Roche and Merck, and advisory fees from Roche and Merck. V.K. is a consultant at Abbott Diagnostic Laboratories. No other authors have relevant conflicts to disclose.

Funding statement: This publication was supported in part by funding from the Public Health Agency of Canada, through the COVID-19 Immunity Task Force and Vaccine Surveillance Reference Group (DK, AH, VHF). This publication was also supported by University Health Network's PRESERVE-Pandemic Response Biobank for coronavirus samples, UHN Biospecimen Services, REB # 20-5364. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the University Health Network.

Meetings where data was presented: This data was presented, in part, at the 2021 American Transplant Congress, June 04-09, 2021; conference was held virtually.

Corresponding author:

Deepali Kumar MD, MSc, FRCPC

PMB 11-174, 585 University Avenue

Toronto, ON, Canada M5G 2N2

Tel: (416) 340-4241/Fax: (416) 340-4043

Email: Deepali.kumar@uhn.ca

ABSTRACT

T-cell immunity associated with SARS-CoV-2 infection or vaccination in solid organ transplant recipients (SOTRs) is poorly understood. To address this, we measured T-cell responses in 50 SOTRs with prior SARS-CoV-2 infection. The majority of patients mounted SARS-CoV-2-specific $CD4^+$ T-cell responses against spike (S), nucleocapsid (NP) and membrane proteins; $CD8^+$ T-cell responses were generated to a lesser extent. $CD4^+$ T-cell responses correlated with antibody levels. Severity of disease and mycophenolate dose were moderately associated with lower proportions of antigen-specific T-cells. Relative to non-transplant controls, SOTRs had perturbations in both total and antigen-specific T-cells, including higher frequencies of total $PD\text{-}1^+CD4^+$ T-cells. Vaccinated SOTRs (n=55) mounted significantly lower proportions of S-specific polyfunctional $CD4^+$ T-cells after two doses, relative to unvaccinated SOTRs with prior COVID-19. Together, these results suggest that SOTR generate robust T-cell responses following natural infection that correlate with disease severity but generate comparatively lower T-cell responses following mRNA vaccination.

Abstract Key words: transplantation, COVID-19, SARS-CoV-2, T-cells, vaccination.

INTRODUCTION

Solid organ transplant recipients (SOTRs) are at increased risk for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, with mortality rates ranging 10-30% [1-4]. Profound immune disturbances have been identified in immune competent individuals with acute COVID-19, including lymphopenia and decreased T-cell counts [5, 6]. Most immunocompetent individuals mount SARS-CoV-2-specific T-cells. While $CD4^+$ T-cell responses may outnumber $CD8^+$ T-cell responses in some studies [7, 8], both branches of T-cell immunity are induced following infection. A phenotype of T-cell exhaustion, associated with expression of specific cell-surface receptors, such as programmed cell death protein 1 (PD-1) or T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), has also been observed in severe cases [9]. The magnitude of SARS-CoV-2-specific T-cells ranges 0.01-1% of circulating T-cells [7, 10-15], and may be related to disease severity [16]. These cells primarily target spike (S) [10], and other SARS-CoV-2 antigens, including the nucleocapsid (NP) and membrane (Mb) proteins [7, 13-15]. Antigen-specific $CD4^+$ T-cells appear to be Th1-polarized, evidenced by production of IFN-γ and IL-2 as effectors [7, 10, 13, 17].

Although we have some understanding of antibody response following SARS-CoV-2 infection in the immunocompromised setting, we know far less about T-cell responses in SOTR [18]. Most studies describing T-cell responses in transplant patients are limited by small sample sizes, obviating the capacity to draw links with outcomes or clinical parameters, such as severity of disease. Most T-cell studies also suffer from severity bias with few studies evaluating the T-cell response in milder COVID disease. Few studies have also directly compared immune responses to SARS-CoV-2 between transplant recipients and the general population or examined how the magnitude of T-cell response in SOTR varies between natural infection and vaccination. In the general population, SARS-CoV-2 vaccination induces potent antibody and T-cell responses [19, 20]. Although there is evidence of decreased antibody responses in vaccinated SOTR [21-23], the impact on T-cell responses is less well understood. Here we provide a detailed look at the T-cell response in

50 SOTR with prior SARS-CoV-2 infection. We provide comparisons to previously infected non-transplant controls, and to vaccinated transplant recipients, and describe how T-cell responses during natural infection correlate with antibody responses, and severity of disease.

METHODS

Study design and ethics

This single-center study was performed at the University Health Network (UHN) Transplant Centre. The primary cohort comprised 50 SOTRs diagnosed with COVID-19 from March 2020-2021. Inpatients and outpatients were included if they had a positive nucleic acid test (NAT) for SARS-CoV-2 on a respiratory specimen. PBMCs and serum were collected at approximately 4-6 weeks after symptom onset. A second cohort of non-transplanted controls with prior COVID-19 was included for comparison. PBMCs were obtained during convalescence (>14 days post-symptom onset) from COVID-19 clinic outpatients at UHN (n=13), or via UHN's PRESERVE-Pandemic Response Biobank for coronavirus samples (n=7). All infected patients were followed for outcomes up to 90 days. The third cohort consisted of vaccinated SOTRs who had no previous history of COVID-19 (n=55). PBMCs were collected 4-6 weeks after the second dose of mRNA vaccine; design and ethical considerations for the vaccinated cohort are described elsewhere [24]. All vaccinated SOTR were negative for anti-RBD antibody before vaccination. The study was approved by the UHN research ethics board. All patients or their delegates provided informed consent.

T-Cell Assessment

A total of 10^6 cryopreserved PBMCs were thawed and rested for 2h prior to incubation with overlapping peptides (15-mers with 11 amino acid overlaps (PepTivator®, Miltenyi Biotec)) corresponding to SARS-CoV-2 S, NP or Mb proteins (final concentration 5 µg/mL per peptide, based on preliminary optimization experiments). Cells were incubated overnight with peptides, a co-stimulatory antibody cocktail (BD Biosciences) and a protein transport inhibitor (ThermoFisher Scientific). Intracellular cytokine staining was used to measure the frequency of SARS-CoV-2-specific T-cells, as has been done by others [20, 25, 26]. PMA/ionomycin was used as a positive control and cells treated with media alone were used as a negative (media) control. Following incubation at 37^{o}C, cells were stained with a viability dye (Zombie Aqua, Biolegend), Fc blocked (BD Biosciences) and incubated with a surface marker antibody cocktail (CD3, CD4, CD8, PD-1 and TIM-3). Cells were then fixed, permeabilized and incubated with an antibody cocktail to detect intracellular cytokines (IFN-γ, and IL-2). **Supplementary Table 1** lists the antibodies used in this study. Flow cytometry was performed on an LSR II BGRV (BD Biosciences) at the SickKids-UHN Flow Cytometry Facility. Representative gating is shown in **Supplementary Figure 1**. Frequencies of $CD4^+$ and $CD8^+$ T-cells were measured in terms of cells expressing IFN-γ and IL-2 alone, or both cytokines simultaneously (polyfunctional T-cells). The frequency of antigen-specific T-cells was determined by subtracting the frequency of cytokine positive T-cells in untreated comparators from the frequency in peptide-stimulated samples. A positive T-cell response was defined as a frequency exceeding 0.01%, the limit of quantitation (LOQ) for this study. Results below this threshold were set to 0.005%, or 50 cells per

10^6 $CD4^+/CD8^+$ T-cells. A minimum number of 100,000 live, $CD3^+$ T-cells were required for samples to be included in the flow analysis. Vaccine-specific T-cell responses were assessed by stimulating isolated PBMCs with S peptides using the same protocol described above. Total $CD4^+$ and $CD8^+$ T-cells were assessed using non-peptide stimulated PBMCs (media controls). Total T-cells were used to characterize cell surface markers associated with T-cell exhaustion (PD-1, TIM-3).

Antibody testing

Serologic testing for anti-SARS-CoV-2 antibody was performed using an anti-NP chemiluminescent microparticle immunoassay (Abbott Laboratories, USA) [27] and an anti-S RBD electrochemiluminescent immunoassay (Roche, Switzerland) [28]. Index measurements of ≥1.4 and ≥0.8 U/mL were considered positive for anti-NP and anti-S antibodies, respectively.

Statistics

Demographics were analyzed using descriptive statistics. Categorical variables were compared using a two-tailed Fisher's exact test. Continuous variables were compared using Mann Whitney U test, the Kruskal–Wallis (K-W) test or Spearman's correlation. Dunn's correction for multiple comparisons was used when performing the K-W test. Statistical significance was defined at the level of $p<0.05$. All statistical analyses were performed with Prism (version 9, GraphPad Software, USA. Data is available upon reasonable request.

RESULTS

Patient Demographics

Fifty SOTR diagnosed with COVID-19 had PBMCs collected and tested at a median of 38.5 days (IQR: 36.0-51.3) from symptom onset. Demographic information for the 50 SOTR, and 20 non-transplant controls, are described in **Table 1**. SOTR were primarily male (72.0%) with a median age of 55.5 years. Kidney transplant recipients comprised 48.0% of the cohort. The median time from transplant to COVID-19 diagnosis was 5.9 years (IQR: 1.8-9.4 years). At diagnosis, most SOTRs (98%) were treated with calcineurin inhibitors (CNIs), primarily tacrolimus (70%), along with anti-metabolites (78.0%) and prednisone (76.0%). Hospitalization for COVID-19 occurred in 46.0% (n=23) of cases, with oxygen supplementation, ICU admission and mechanical ventilation occurring in 24%, 6.0%, and 2.0%, respectively. No deaths were recorded in this SOTR cohort.

SARS-CoV-2-specific T-cells in SOT recipients.

The frequency of SARS-CoV-2 specific T-cells in SOTRs were measured after stimulation with SARS-CoV-2 S, NP, or Mb protein peptides. The proportions of SOTR who mounted S-reactive $CD4^+$ T-cell was 58.0% (IFN-γ monofunctional), 86.0% (IL-2 monofunctional) and 72.0% (polyfunctional) **(Figure 1A)**. The proportion that had detectable NP-specific $CD4^+$ T-cells was 68.0% (IFN-γ monofunctional), 86.0% (IL-2 monofunctional) and 68.0% (polyfunctional). Lastly the proportion of individuals with detectable Mb-specific $CD4^+$ T-cells was 50.0% (IFN-γ monofunctional), 76.0% (IL-2 monofunctional) and 56% (polyfunctional). The percent of SOTR with at least one positive SARS-CoV-2 reactive $CD4^+$ T-cell population was 92% for S, 90% for NP and 84% for Mb **(Figure 1C),** representing the overall proportion of individuals with detectable $CD4^+$ T-cell responses against each antigen. The percent positive for all three cytokine populations following S, NP or Mb stimulation were 50%, 58% and 38%, respectively.

The SARS-CoV-2 directed $CD8^+$ T-cell response was less pronounced **(Figure 1B)**. The proportion of SOTRs who mounted S-reactive $CD8^+$ T-cell was 26.0% (IFN-γ monofunctional), 54.0% (IL-2 monofunctional) and 2.0% (polyfunctional). The proportion that had detectable NP-specific $CD8^+$ T-cells was 36.0% (IFN-γ monofunctional), 52.0% (IL-2 monofunctional) and 12.0% (polyfunctional). Lastly the proportion of individuals with detectable Mb-specific $CD8^+$ T-cells was 28.0% (IFN-γ monofunctional) and 38.0% (IL-2 monofunctional). No polyfunctional Mb-directed $CD8^+$ T-cells were detected in the SOTR group. The percent of SOTR with at least one positive SARS-CoV-2 reactive $CD8^+$ T-cell population was 64% for S, 68% for NP and 54% for Mb **(Figure 1C)**. The percentage of patients who were positive for all three cytokine populations following S, NP or Mb stimulation were 2%, 10% and 0%, respectively. Together, these data suggest the majority of SOTR generate SARS-CoV-2 specific T-cell responses following natural infection.

Relationship Between antigen-specific T-cell and antibody responses in SOTR.

Previously, we measured SARS-CoV-2-specific antibody responses in the 50 SOTR in our cohort. Those results, including analysis of factors associated with antibody response, are published elsewhere [2]. Anti-NP and anti-S receptor biding domain (RBD) antibody levels in sera were measured at the same time as T-cell responses were assessed. Antibody levels were compared with proportions of S-, or NP-directed $CD4^+$ T-cell responses. This was done in order to identify antigen-specific $CD4^+$ T-cells that may be important for driving antibody responses. Six of the 50 naturally infected patients (12%) did not develop anti-S antibodies at sampling time. All six of these patients mounted anti-S T-cell responses. A larger proportion were anti-NP negative at sampling time (13/50, 26%). T-cell responses were found in all but three (10/13, 76.9%) of these patients.

Proportions of S-specific $CD4^+$ T-cells correlated only moderately with levels of anti-S RBD antibodies, particularly among IL-2 monofunctional (p=0.052) and polyfunctional

(p=0.041) cells **(Figure 2 A-C)**. We found a similar relationship with respect to NP, where the magnitude of NP-specific IL-2 monofunctional (p=0.025) or polyfunctional $CD4^+$ T-cells (p=0.086) had a trend towards correlating with anti-NP antibody levels in blood **(Figure 2 D-F)**. Interestingly, IFN-γ monofunctional $CD4^+$ T-cells poorly correlated with antibody responses.

T-cell responses and severity of SARS-CoV-2 Infection in SOTR

SOTR were categorized according to severity of clinical COVID-19 disease and compared with respect to total and antigen-specific T-cell responses. Those not receiving oxygen supplementation were considered to have milder COVID-19, consistent with WHO severity scores of 1-4, and those requiring oxygen supplementation, or any other higher level of hospital care (n=12; 24%), comprised the moderate-to-severe SOTR group, consistent with WHO severity scores of 5-9 [2].

No differences in total $CD4^+$ and $CD8^+$ T-cells were found with respect to disease severity **(Supplementary Figure 2A-C)**.We also found no differences in frequencies of $CD4^+$ or $CD8^+$ T-cells expressing markers associated with T-cell exhaustion, namely PD-1 and TIM-3 **(Supplementary Figure 2D-G).**

We next compared frequencies of SARS-CoV-2-specific $CD4^+$ **(Figure 3)** and $CD8^+$ **(Figure 4)** T-cells according to disease severity. In general, those with higher WHO disease scores had lower proportions of antigen-specific $CD4^+$ and $CD8^+$ T-cells, with significant differences observed among S-specific IFN-γ expressing $CD4^+$ T-cells (**Figure 3A**; p=0.038), and Mb-specific $CD4^+$ T-cells expressing IL-2 alone (**Figure 3H**; p=0.017), or Mb-specific polyfunctional $CD4^+$ T-cells (**Figure 3I**; p=0.047). Among antigen-specific $CD8^+$ T-cells, we noted a similar pattern; in particular, the proportions of S-specific IL-2 expressing $CD8^+$ T-cells (**Figure 4B**; p=0.027) were significantly lower in SOTR with higher disease scores.

Impact of immunosuppression on SARS-CoV-2-specific T-cells in SOTR.

Next, we investigated the impact of immunosuppression at diagnosis on SARS-CoV-2-specific T-cells. To minimize the number of comparisons, we only analyzed the impact of immunosuppression on S-specific T-cells. These T-cells were assessed in composite: proportions of monofunctional and polyfunctional T-cells were pooled together and expressed as *total* S-reactive $CD4^+$ or $CD8^+$ T-cells.

The majority of SOTR received a CNI (98%). We compared the magnitude of total S-specific T-cell responses according to type of CNI and found no significant differences for both $CD4^+$ and $CD8^+$ T-cells **(Supplementary Figure 3A & 4B).** We also found no correlation between the blood level of tacrolimus, the most commonly used CNI, and the magnitude of total S-specific $CD4^+$ or $CD8^+$ T-cells (**Supplementary Figure 3C**). Use of anti-metabolites, namely mycophenolate or azathioprine, did not significantly impact proportions of total S-specific $CD4^+$ or $CD8^+$ T-cells **(Supplementary Figure 3D & 3E)**, however, total daily dose (TDD) of mycophenolate, had a weak inverse correlation with the magnitude of total S-specific $CD4^+$ T-cells (Spearman r = -0.35, p=0.048, **Supplementary Figure 3F**). Regarding steroids, no significant differences in total S-specific T-cells were measured according to use, or TDD of prednisone (**Supplementary Figure 3G-I**).

Bulk and SARS-CoV-2 specific T-cells responses in SOTR compared to non-transplant controls

We next compared T-cell responses in 50 SOTR and 20 non-transplant controls, similar in age, sex, and time from symptom onset to blood collection (**Table 1**). Although SOTR and controls had similar proportions of $CD3^+$ T-cells in the peripheral blood, we noted a significantly lower frequency of total $CD4^+$ T-cells, and a significantly higher proportion of total $CD8^+$ T-cells in SOTRs **(Supplementary Figure 4).** We also compared the proportions of total PD-1^+ or TIM-3^+ T-cells between groups; SOTR were characterized by significantly higher frequencies of PD-1-expressing total $CD4^+$ T-cells relative to controls **(p=0.008; Figure 5A)**. No differences were found with respect to TIM-3^+ $CD4^+$ T-cells, or $CD8^+$ T-cells expressing markers of exhaustion (**Figure 5B-D)**.

Lastly, we examined whether the magnitude of the SARS-CoV-2-specific T-cell response varied between SOTR and controls. To minimize number of comparisons, we analyzed only antigen-specific polyfunctional $CD4^+$ T-cells, and $CD8^+$ IFN-γ monofunctional T-cells as these are common subsets used to assess quality of T-cell response during natural infection and in vaccine studies [29]. S-specific polyfunctional $CD4^+$ T-cells were more proportionally abundant in SOTR compared to controls (**Figure 5E**, p=0.046), but no differences were seen with respect to NP-specific or Mb-specific polyfunctional $CD4^+$ T-cells **(Figure 5F & 5G)**. With respect to $CD8^+$ T-cells, antigen-specific T-cells were consistently more abundant in controls than in SOTR, particularly among S- (p=0.0059) or NP-directed (p=0.0099) T-cell responses **(Figure 5H-J)**. All together, these data suggest that immune responses to SARS-CoV-2 vary between SOTR and non-transplant controls at the global and antigen-specific T-cell level.

Magnitude of T-cell response between SARS-CoV-2 natural infection and vaccination in SOTR.

Lastly, we compared the magnitude of the T-cell response between naturally infected SOTR and SOTR receiving mRNA-based SARS-CoV-2 vaccination. The demographics of the vaccine cohort is found in **Supplementary Table 2**. Both groups were similar with respect to sex and time from transplant. The vaccinated group was significantly older (55.5 vs 65.5 years, p<0.0001). Seven SOTR received two doses of BNT162b2 (Pfizer), and 48 received two doses mRNA-1273 (Moderna). Factors determining vaccine response to mRNA-1273 have been described elsewhere [24]. For the purpose of this study, we specifically compared S-specific polyfunctional $CD4^+$ T-cells between groups as this type of cellular response is commonly used to assess immunogenicity [29-31].

Relative to naturally infected SOTR, SOTR receiving mRNA vaccination mounted proportionally less abundant polyfunctional $CD4^+$ T-cell responses **(p=0.011; Figure 6)**. A total of 47.3% of SOTR had detectable antigen-specific T-cell responses at 4-6 weeks after second dose, compared to 72.0% of SOTR who has detectable polyfunctional S-specific responses after recovery from natural infection. Neither the use, nor the dose or level of immunosuppression (CNIs, anti-metabolites, prednisone) were statistically associated with spike-specific $CD4^+$ T-cells within the vaccinated cohort (**Supplementary Figure 5).** Further, no differences in immunosuppression were measured between vaccinated and naturally infected SOTR (p>0.05 for all comparisons, data not shown). These results suggest that the T-cell responses are comparatively lower in SOTR vaccinated with two doses of mRNA vaccine, with a greater proportional response in naturally infected SOTR.

DISCUSSION

Our study provides a number of novel and key findings. SARS-CoV-2 specific $CD4^+$ T-cell responses were generated in most SOTR with natural infection (84-92%). Anti-S and anti-NP responses were most prominent, but Mb-directed $CD4^+$ T-cell responses were regularly detected. As has been found for the general population [7, 10], the $CD8^+$ T-cell response in SOTR was lower. The overall magnitude of antigen-specific $CD4^+$ and $CD8^+$ T-cell response measured in our study is similar to the proportion observed for the general population [7, 10-15], along with the directionality of T-cell responses against S and NP antigens. Other studies have reported vigorous SARS-CoV-2 specific T-cell responses against S, NP, Mb in SOTR in convalescence [32, 33]; in one of the larger studies, the proportion of liver transplant recipients who developed SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses was 90.3% and 83.9%, respectively, by 103 days post COVID diagnosis [32].

In our study, the functionality of the $CD4^+$ T-cell response was primarily driven by IL-2 producing $CD4^+$ T-cells, and IFN-γ^+IL-2^+ polyfunctional responses, but IFN-γ monofunctional responses were also commonly identified. Unlike $CD4^+$ T-cells, $CD8^+$ polyfunctional T-cell responses were uncommonly detected. In the literature there is often a preponderance of using IFN-γ-related readouts to assess T-cell responses following infection or vaccination. Our results suggest that a significant portion of antigen-specific T-cell responses in SOTR may be missed if IL-2 is not taken into consideration. IL-2 producing $CD4^+$ T-cells – represented in both IL-2-monofunctional and polyfunctional T-cells - correlated with anti-S RBD and anti-NP antibody levels, while monofunctional IFN-γ producing $CD4^+$ T-cells did not. Further, of the T-cell responses that were significantly less abundant in SOTR who developed severe disease, many were IL-2 expressing, further underscoring the need to consider IL-2, and potentially other effectors, in assays that assess T-cell responses.

In addition to lower proportions of T-cell populations expressing IL-2, higher severity scores in SOTR were also associated with lower frequencies of S-specific IFN-γ monofunctional $CD4^+$ T-cells. These results suggest that a balance of antigen-specific T-cell responses may be required for optimal control of infection in SOTR. Many drugs in the SOTR setting target IL-2 and IFN-γ, such as mycophenolate, a potent T- and B-cell inhibitor. In line with our results, others have reported on the potentially negative impact of mycophenolate on anti-SARS-CoV-2 responses, both in terms of infection and vaccination [2, 21, 34-37]. Our results suggest that reducing the dosage of immunosuppression, specifically for mycophenolate, may be an advantageous step toward maximizing the induction of SARS-CoV-2 specific T-cells during natural infection, but this needs to be weighed carefully in light of risk for graft rejection.

In our study, SOTRs experienced several disturbances in total T-cells, notably increased frequencies of PD-1-expressing $CD4^+$ T-cells relative to non-transplant controls. In acutely infected immune competent patients, severity of COVID-19, including death, was associated with PD-1 expression on T-cells [9, 38]. Although PD-1 is implicated as a marker of T-cell exhaustion, it's exact role here is unknown, and could also be associated with an activated cell state, or immune suppression relating to transplantation. Recently Rha et al. [39] showed that PD-1 expressing SARS-CoV-2 specific T-cells were not exhausted, but functional in both acutely infected and convalescent immune-competent persons. While it is possible that the increased frequency of PD-1 expressing cells may in turn negatively regulate SARS-CoV-2-specific, or other antigen-specific T-cell responses, future studies will need to directly evaluate the role of PD-1 and other exhaustion markers in the pathogenesis of COVID-19 in SOTR.

Importantly, our study directly compares T-cell responses post-vaccination to post-infection in the immunocompromised setting. We identify that the proportion of SOTR that generate T-cell responses after two-dose SARS-CoV-2 mRNA vaccination (47.3%) was significantly lower than the proportion of SOTR that generate comparable T-cell responses

post natural infection (72.0%). This is in contrast to the general population where mRNA vaccine generates a greater antibody and T-cell response relative to natural infection. Several studies have now shown that humoral and cellular vaccine responses are diminished in immunocompromised populations [21, 24, 34, 40-45]. Specific immunosuppressives such as mycophenolate may contribute to this, although our data suggests this may not be the case. Further interventions to expand antibody and T-cell immunogenicity, such as additional vaccine doses, may be required to optimize vaccine immunity in this cohort. One limitation of this data is that the natural infection cohort was significantly younger than the vaccine cohort; therefore, it is possible that the lower responses seen in the vaccinated cohort may be partly due to older age. Also, while our data show a quantifiable difference in T-cell response, these data do not suggest that those with natural infection are more likely than vaccinated patients to be protected against subsequent viral challenge. Furthermore, the authors discourage deliberate exposure to SARS-CoV-2 in immunologically naïve SOTRs because of the high risk for serious COVID-19 complications and continue to strongly encourage all SOTRs to be vaccinated.

Our study is limited by a lack of longitudinal follow-up data, owing to the cross-sectional nature of the study. Uneven numbers of organ transplant types limited our ability to look at role of type of transplant on T-cell responses. Further, we only examined effect of baseline immunosuppression, and it's possible that changes to immunosuppression during the course of illness may have also impacted T-cell responses. We also did not assess the maturation subtypes of antigen-specific T-cells. Since we did not always correct for multiple comparisons, we recognize the preliminary nature of our data. Future studies with larger cohorts of SOTRs will be required to confirm these observations. However, these limitations are countered by several strengths. We believe this study provides important information to the scientific community and fills many gaps in knowledge with respect to our understanding of T-cell responses in immunocompromised persons with COVID-19.

REFERNCES

1. Elias M, Pievani D, Randoux C, et al. COVID-19 Infection in Kidney Transplant Recipients: Disease Incidence and Clinical Outcomes. J Am Soc Nephrol **2020**; 31:2413-23.

2. Marinelli T, Ferreira VH, Ierullo M, et al. Prospective Clinical, Virologic, and Immunologic Assessment of COVID-19 in Transplant Recipients. Transplantation **2021**.

3. Pereira MR, Mohan S, Cohen DJ, et al. COVID-19 in solid organ transplant recipients: Initial report from the US epicenter. Am J Transplant **2020**; 20:1800-8.

4. Kates OS, Haydel BM, Florman SS, et al. COVID-19 in solid organ transplant: A multi-center cohort study. Clin Infect Dis **2020**.

5. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet **2020**; 395:497-506.

6. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest **2020**; 130:2620-9.

7. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell **2020**; 181:1489-501 e15.

8. Law JC, Koh WH, Budylowski P, et al. Systematic Examination of Antigen-Specific Recall T Cell Responses to SARS-CoV-2 versus Influenza Virus Reveals a Distinct Inflammatory Profile. J Immunol **2021**; 206:37-50.

9. Diao B, Wang C, Tan Y, et al. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). Front Immunol **2020**; 11:827.

10. Weiskopf D, Schmitz KS, Raadsen MP, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol **2020**; 5.

11. Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature **2020**; 587:270-4.

12. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell **2020**; 183:996-1012 e19.

13. Sattler A, Angermair S, Stockmann H, et al. SARS-CoV-2 specific T-cell responses and correlations with COVID-19 patient predisposition. J Clin Invest **2020**.

14. Peng Y, Mentzer AJ, Liu G, et al. Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol **2020**; 21:1336-45.

15. Jung JH, Rha MS, Sa M, et al. SARS-CoV-2-specific T cell memory is sustained in COVID-19 convalescent patients for 10 months with successful development of stem cell-like memory T cells. Nat Commun **2021**; 12:4043.

16. Chen Z, John Wherry E. T cell responses in patients with COVID-19. Nat Rev Immunol **2020**; 20:529-36.

17. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity **2020**; 52:971-7 e3.

18. Phadke VK, Scanlon N, Jordan SC, Rouphael NG. Immune Responses to SARS-CoV-2 in Solid Organ Transplant Recipients. Curr Transplant Rep **2021**:1-13.

19. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature **2020**; 586:594-9.

20. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med **2020**; 383:1920-31.

21. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients. JAMA **2021**; 325:1784-6.

22. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients. JAMA **2021**; 325:2204-6.

23. Marion O, Del Bello A, Abravanel F, et al. Safety and Immunogenicity of Anti-SARS-CoV-2 Messenger RNA Vaccines in Recipients of Solid Organ Transplants. Ann Intern Med **2021**.

24. Hall VG, Ferreira VH, Ierullo M, et al. Humoral and cellular immune response and safety of two-dose SARS-CoV-2 mRNA-1273 vaccine in solid organ transplant recipients. Am J Transplant **2021**.

25. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature **2020**; 584:457-62.

26. Anderson EJ, Rouphael NG, Widge AT, et al. Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. N Engl J Med **2020**; 383:2427-38.

27. Bryan A, Pepper G, Wener MH, et al. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. J Clin Microbiol **2020**.

28. National S-C-SAEG. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis **2020**.

29. Panagioti E, Klenerman P, Lee LN, van der Burg SH, Arens R. Features of Effective T Cell-Inducing Vaccines against Chronic Viral Infections. Front Immunol **2018**; 9:276.

30. L'Huillier AG, Hirzel C, Ferreira VH, et al. Evaluation of Recombinant Herpes Zoster Vaccine for Primary Immunization of Varicella-seronegative Transplant Recipients. Transplantation **2021**.

31. Hirzel C, L'Huillier AG, Ferreira VH, et al. Safety and immunogenicity of adjuvanted recombinant subunit herpes zoster vaccine in lung transplant recipients. Am J Transplant **2021**.

32. Fernandez-Ruiz M, Olea B, Almendro-Vazquez P, et al. T cell-mediated response to SARS-CoV-2 in liver transplant recipients with prior COVID-19. Am J Transplant **2021**.

33. Candon S, Guerrot D, Drouot L, et al. T cell and antibody responses to SARS-CoV-2: Experience from a French transplantation and hemodialysis center during the COVID-19 pandemic. Am J Transplant **2021**; 21:854-63.

34. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients. JAMA **2021**.

35. Benotmane I, Gautier-Vargas G, Cognard N, et al. Weak anti-SARS-CoV-2 antibody response after the first injection of an mRNA COVID-19 vaccine in kidney transplant recipients. Kidney Int **2021**; 99:1487-9.

36. Colmenero J, Rodriguez-Peralvarez M, Salcedo M, et al. Epidemiological pattern, incidence, and outcomes of COVID-19 in liver transplant patients. J Hepatol **2021**; 74:148-55.

37. Rozen-Zvi B, Yahav D, Agur T, et al. Antibody response to SARS-CoV-2 mRNA vaccine among kidney transplant recipients: a prospective cohort study. Clin Microbiol Infect **2021**.

38. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol **2020**; 17:533-5.

39. Rha MS, Jeong HW, Ko JH, et al. PD-1-Expressing SARS-CoV-2-Specific CD8(+) T Cells Are Not Exhausted, but Functional in Patients with COVID-19. Immunity **2021**; 54:44-52 e3.

40. Monin L, Laing AG, Muñoz-Ruiz M, et al. Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. Lancet Oncol **2021**: 22(6):765-78.

41. Boyarsky BJ, Ruddy JA, Connolly CM, et al. Antibody response to a single dose of SARS-CoV-2 mRNA vaccine in patients with rheumatic and musculoskeletal diseases. Ann Rheum Dis **2021**.

42. Schramm R, Costard-Jackle A, Rivinius R, et al. Poor humoral and T-cell response to two-dose SARS-CoV-2 messenger RNA vaccine BNT162b2 in cardiothoracic transplant recipients. Clin Res Cardiol **2021**.

43. Sattler A, Schrezenmeier E, Weber UA, et al. Impaired humoral and cellular immunity after SARS-CoV2 BNT162b2 (Tozinameran) prime-boost vaccination in kidney transplant recipients. J Clin Invest **2021**.

44. Cucchiari D, Egri N, Bodro M, et al. Cellular and humoral response after MRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients. Am J Transplant **2021**; 21:2727-39.

45. Herrera S, Colmenero J, Pascal M, et al. Cellular and humoral immune response after mRNA-1273 SARS-CoV-2 vaccine in liver and heart transplant recipients. Am J Transplant **2021**.

FIGURE LEGENDS

Figure 1 - Antigen-specific T-cells in peptide-stimulated CD4+ and CD8+ T-cells. Proportions of S-, NP- and Mb-specific CD4+ (A) and CD8+ (B) T-cells are shown. Individual patients are shown by coloured dots. The fraction underneath each bar corresponds to the proportion of SOTRs positive for each corresponding cytokine population. Bars show median. Horizontal dotted line indicates limit of quantification, 0.01%. (C) Proportion of SOTR with SARS-CoV-2 reactive T-cells. All data shown is collected from n=50 SOTRs. Abbreviations - SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SOTR: solid organ transplant recipients, IFN-γ: interferon gamma, IL2: interleukin 2.

Figure 2 - Correlation of antibody levels and magnitude of antigen-specific T-cell response in SOTR. Proportions of S-specific IFN-γ monofunctional (A), IL-2 monofunctional (B), or IFN-γ and IL-2 polyfunctional (C) CD4+ T-cells relative to levels of anti-S RBD antibodies in U/mL. Similar plots are shown for of NP-specific IFN-γ monofunctional (D), IL-2 monofunctional (E) or IFN-γ and IL-2 polyfunctional (F) CD4+ T-cells relative to levels of anti-NP antibody level in U/mL. Each dot corresponds to one participating SOTR. Spearman r for each comparison is shown in the top right of each plot, along with the corresponding p-value. Abbreviations - NP: nucleocapsid, RBD: spike receptor binding domain, IFN-γ: interferon gamma, IL2: interleukin 2, SOTR: solid organ transplant recipient.

Figure 3 - Impact of disease severity on antigen-specific $CD4^+$ T-cells according to cytokine subpopulations. Proportions of S-specific (A-C), NP-specific (D-F) and Mb-specific (G-I) $CD4^+$ T-cells were compared with respect to disease severity: WHO scores 1-4 (milder COVID-19) vs 5-9 (moderate-to-severe COVID-19). The proportion of IFN-γ monofunctional (A, D, G), IL-2

monofunctional (B, E, H), or IFN-γ and IL-2 polyfunctional (C, F, I) T-cells are shown with each SOTR represented by a dot. Bars show median ± interquartile range. Dotted line indicates the limit of quantification, 0.01%. Abbreviations - SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, S: spike, NP: nucleocapsid, Mb: membrane protein, SOTR: solid organ transplant recipient, IFN-γ: interferon gamma, IL2: interleukin 2, WHO: world health organization.

Figure 4 - Impact of disease severity on antigen-specific $CD8^+$ T-cells according to cytokine subpopulations. Proportions of S-specific (A-C), NP-specific (D-F) and Mb-specific (G-I) $CD8^+$ T-cells were compared with respect to disease severity: WHO scores 1-4 (milder COVID-19) vs 5-9 (moderate-to-severe COVID-19). The proportion of IFN-γ monofunctional (A, D, G), IL-2 monofunctional (B, E, H), or IFN-γ and IL-2 polyfunctional (C, F, I) T-cells are shown with each SOTR represented by a dot. Bars show median ± interquartile range. Dotted line indicates the limit of quantification, 0.01%. Abbreviations - SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, S: spike, NP: nucleocapsid, Mb: membrane protein, SOTR: solid organ transplant recipient, IFN-γ: interferon gamma, IL2: interleukin 2, WHO: world health organization.

Figure 5 - PD-1 or TIM-3 expressing Total T-cells, and SARS-CoV-2 specific T-cells between SOTR and controls. PD-1 (A) or TIM-3 (B) expressing total $CD4^+$ T-cells, and PD-1 (C) or TIM-3 (D) expressing total $CD8^+$ T-cells between n=50 SOTR and n=20 non-transplant controls. Proportion of spike- (E), NP- (F) or Mb-specific (G) polyfunctional $CD4^+$ T-cells, and total spike- (H), NP- (I) or Mb-specific (J) IFN-γ monofunctional $CD8^+$ T-cells between SOTR and non-transplant controls. Each patient is presented by a coloured dot. Bars show median ± interquartile range. Dotted line indicated the limit of quantification, 0.01%. Abbreviations - SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SOTR: solid organ transplant

recipient, S: spike, NP: nucleocapsid, Mb: membrane protein, IFN-γ: interferon gamma, PD-1: programmed cell death protein 1, TIM-3: T cell immunoglobulin and mucin domain-containing protein 3.

Figure 6 – Spike-specific polyfunctional $CD4^+$ T-cell response in recovered SOTRs with natural infection vs. SOTRs vaccinated against SARS-CoV-2. Polyfunctional $CD4^+$ T-cell responses were measured in 50 SOTR with natural infection and 55 SOTR vaccinated with two doses of mRNA vaccines. Each patient is represented by a coloured dot. Bars show median ± interquartile range. The fraction beneath each box whisker plot indicates the number of SOTR with positive T-cell responses in each group. Dotted line indicates the limit of quantification, 0.01%. Abbreviations: SOTR: solid organ transplant recipient, IFN-γ: interferon gamma, IL2: interleukin 2.

	SOTR	Controls	p-value
N	50	20	
Age in years, median	55.5 (IQR: 47.0-61.5)	52.5 (IQR: 36.3-56.8)	0.18
Sex, n	Male: 36 (72.0%) Female: 14 (28.0%)	Male: 14 (70.0%) Female: 6 (30.0%)	>0.99
Time from symptom onset to sample in days, median	38.5 (IQR: 36.0-51.3)	41.5 (IQR: 20.5-53.8)	0.49
Time from transplant to COVID-19 in years, median	5.9 (IQR: 1.8-9.4)	--	--
Disease Severity:			
WHO Score 1-4	38 (76.0%)	6 (30.0%)	0.76
WHO Score 5-9	12 (24.0%)	14 (70.0%)	
Type of Transplant			
Kidney	24 (48.0%)	--	--
Kidney-pancreas	3 (6.0%)	--	--
Heart	3 (6.0%)	--	--
Liver	13 (26.0%)	--	--
Lung	7 (14.0%)	--	--
Immunosuppression at time of COVID-19			
Calcineurin inhibitor	49 (98.0%)	--	--
Cyclosporin	14 (28.0%)	--	--

Tacrolimus	35 (70.0%)	--	--
Anti-metabolite	39 (78.0%)	--	--
Azathioprine	6 (12.0%)	--	--
Mycophenolate	33 (66.0%)	--	--
Steroid		--	--
Prednisone	38 (76.0%)	--	--

Table 1 – Patient and Control Demographics. Abbreviations: IQR: interquartile range, ICU: intensive care unit, SOTR: solid organ transplant recipient; COVID-19: coronavirus infectious disease 2019, ICU: intensive care unit, WHO: world health organization.

Figure 1

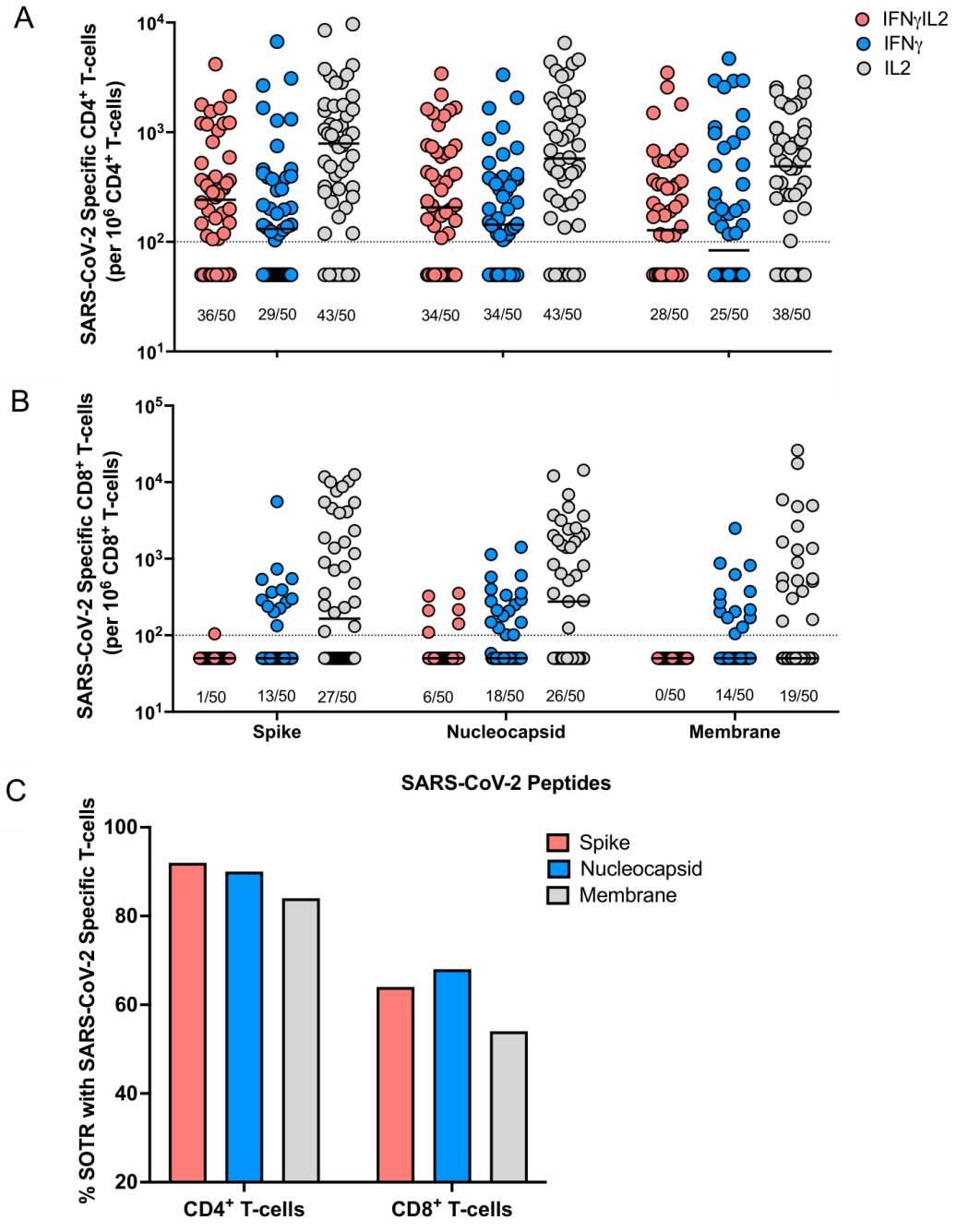

Figure 2

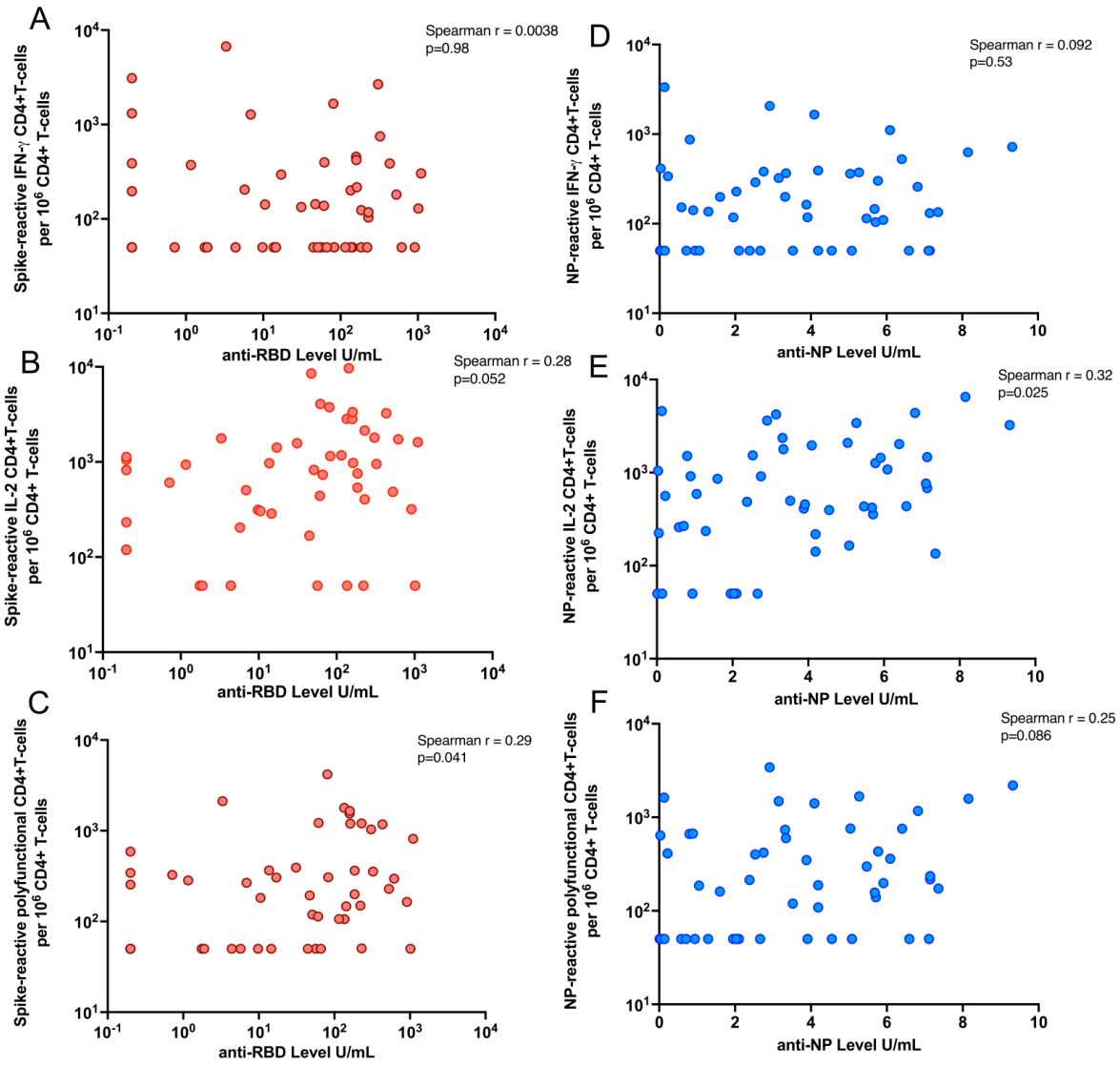

Figure 3

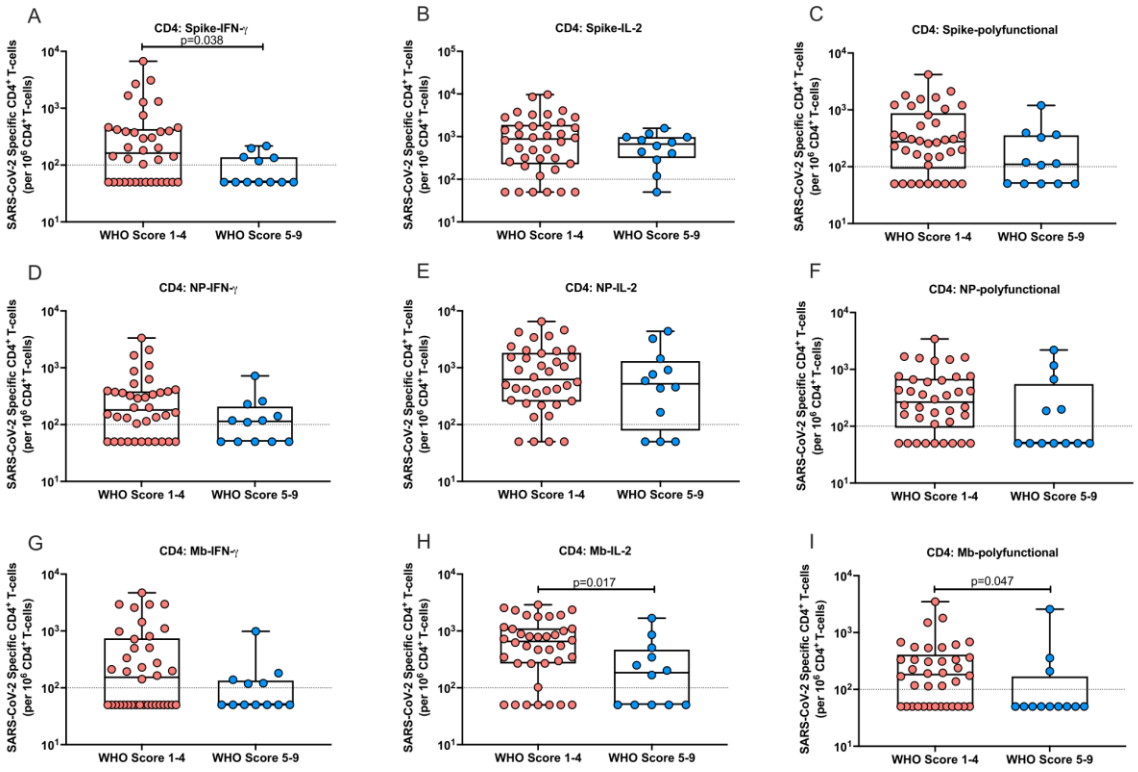

Figure 4

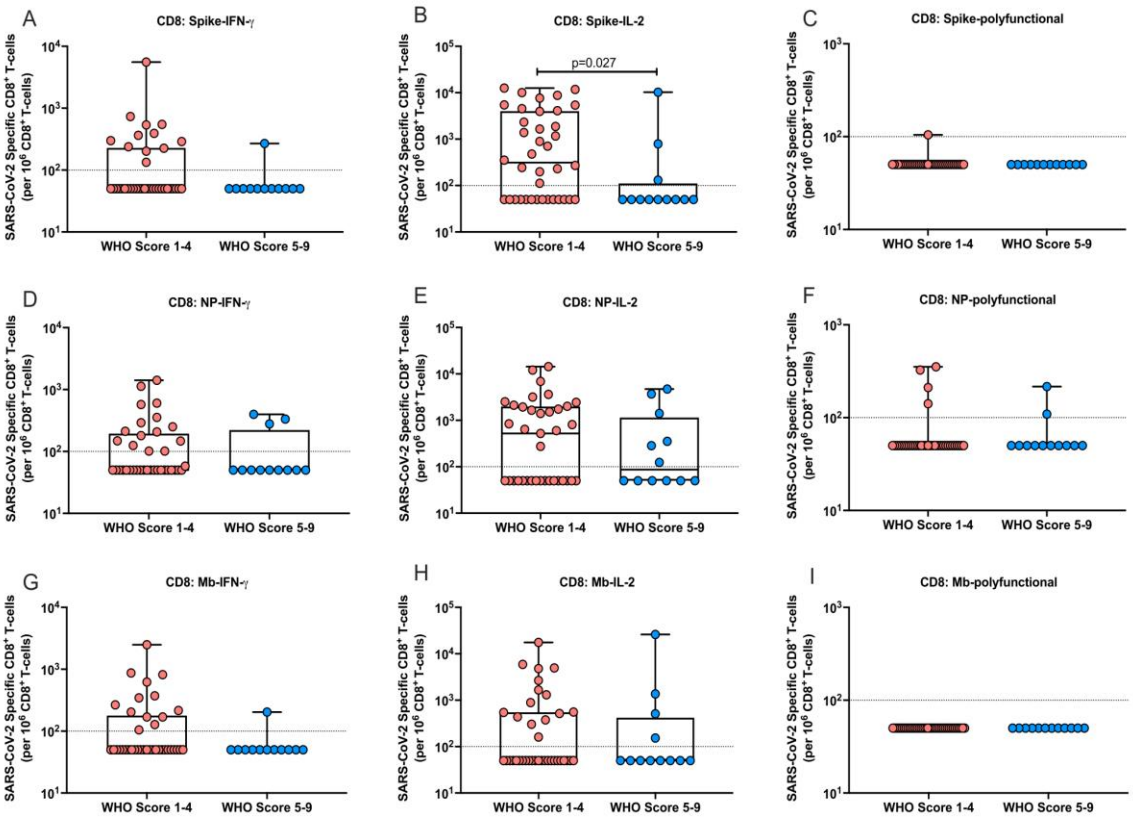

Figure 5

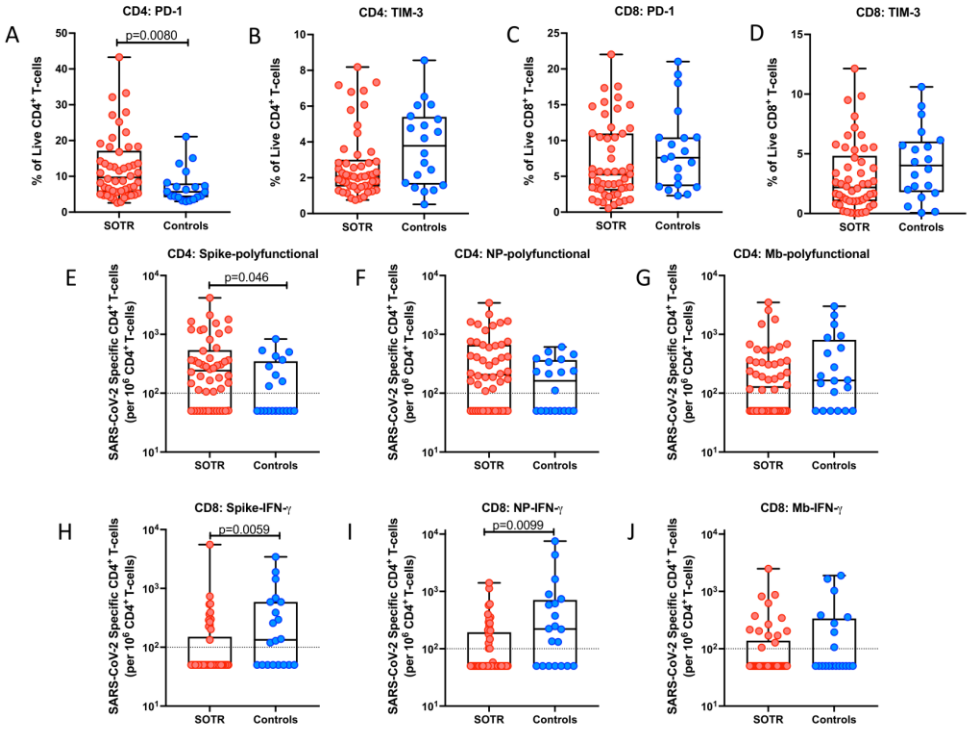

Figure 6