## Antibody testing.

Measurement for COVID antibodies was performed on a Diasorin LIASON® SARS-CoV-2 S1/S2 IgG assay on the LIASON® XL analyzer which tests for antibodies to spike proteins. This gives a qualitative detection of IgG antibodies to SARS-CoV-2 spike protein in human serum and plasma.

## Immunophenotyping.

Blood was obtained when SARS-CoV-2 antibody or PCR positivity was first documented (or within 7-days in some instances, specifically, 4 patients had blood obtained within 24-hours of a positive test result: 4 patients within 4-days of a positive test result, 3 patients within 7-days of a positive test result) for immunophenotyping. Stored peripheral blood mononuclear cells (PBMC) from healthy pediatric SOT patients (collected pre-pandemic) was used as healthy controls.

***Antibodies and reagents*.**

**Panel 1:** anti-CD3 (clone OKT3; Cat# 317331; BioLegend), anti-CD4 (clone A161A1; Cat# 357417; BioLegend), anti-CD8 (clone SK1; Cat# 344704; BioLegend), anti-CD19 (clone HIB19; Cat# 302237; BioLegend), anti-CD20 (clone 2 H7; Cat# 302355; BioLegend), anti-CD21 (clone Bu32; Cat# 354911; BioLegend), anti-CD45 (clone HI30; Cat# 304049; BioLegend), anti-CD45RA (clone HI00; Cat# 563429; BD Biosciences), anti-CD45RO (clone UCHL1; Cat# 304237; BioLegend), anti-CD317 (BST2, Tetherin)(clone RS38E; Cat# 348410; BioLegend), anti-CD197 (CCR7)(cline G043H7; Cat# 353203; BioLegend), anti-CD185 (CXCR5)(clone J252D4; Cat# 356917; BioLegend), anti-CD278 (ICOS)(clone G043H7; Cat# 313531; BioLegend).

**Panel 2:** anti-CD4 (clone A161A1; Cat# 357413; BioLegend), anti-CD8 (clone RPA-T8; Cat# 301041; BioLegend), same as Panel 1 (anti-CD3, anti-CD45, anti-CD317), anti-HLA-DR (clone L243; Cat# 307637; BioLegend), anti-CD303 (BDCA-2)(clone 201A; Cat# 354221; BioLegend), anti-IFN-α (clone REA1013; Cat# 130-116-995; Miltenyi Biotec), anti-IFN-γ (clone B27; Cat# 506516; BioLegend), anti-IL17 (clone BL168; Cat# 512321; BioLegend), anti-IL6 (clone MQ2-13A5; Cat# 25-7069-42; eBioscience), anti-Granzyme B (clone GB11; Cat# 560211; BD Biosciences), anti-G-CSF (clone 5008; Cat# IC214P; R & D Systems).

**Panel 3:** anti-CD45, anti-HLA-DR, anti-BDCA-2, anti-CD11c (clone Bu15; Cat# 337205; BioLegend), anti-CD123 (clone 6H6; Cat# 306033; BioLegend), anti-lineage cocktail (CD3, CD14, CD16, CD19, CD20, CD56) (clone UCHT1, HCD14, 3G8, HIB19, 2H7, HCD56; Cat# 348803; BioLegend).

For cell viability, Zombie NIR Fixable Viability Kit (Cat# 423105; BioLegend) was used.

For cell stimulation, cell activation cocktail (Cat# 423301; BioLegend) and protein transport inhibitor (containing Monensin) (Cat# 554724; BD Biosciences) were used.

***Cell surface staining*.**

PBMCs were isolated from whole blood obtained from study participants at enrollment by Ficoll-Paque Plus (GE Healthcare) gradient centrifugation. Isolated PBMCs were washed twice with Phosphate Buffer Saline (PBS) and cell count was performed by Countess II Automated Cell Counter (ThermoFisher Scientific). PBMCs ($1\times10^6$ cells) were suspended in 200 0.2 µL Zombie NIR PBS with 2% fetal bovine serum (FBS) and incubated for 15 minutes with 0.2 µL Zombie NIR and stained using antibodies described above for Panels 1 and 3.

***Stimulation of PBMCs and intracellular staining*.**

PBMCs ($1\times10^6$ cells) were suspended in 1mL of RPMI complete media (RPMI with 10% FBS and 1% antibiotic-antifungal) and stimulated with 2 µL/mL cell activation cocktail (pre-mixed cocktail of PMA / Ionomycin) for 5 hours. 1 µL/mL protein transport inhibitor was added in the last 4 hours. Cells were then centrifuged at 2000 rpm for 5 minutes and the cell pellet was suspended in 200 µL PBS with 2% FBS. Cell surface staining was performed as described above using Panel 2 antibodies. Following fixing and perm (Fix/Perm; eBioscience), intracellular staining was performed using FOXP3 staining buffers (eBioscience) per manufacturer's recommendations for the following antibodies: IFN-α, IFN-γ, IL-17A, IL-6, Granzyme B, G-CSF for 30–45 minutes.

Data was acquired using BD LSRFortessa™ cell analyzer (BD Biosciences) and analyzed with FlowJo software (BD Biosciences).

## Quantitative RT-PCR.

Nasopharyngeal specimens were subjected to qRT-PCR for quantification of viral load using the Lyra SARS-CoV-2 assay. Liver tissue obtained at time of detection of *SARS-CoV-2* PCR or IgG positivity was subjected to qRT-PCR. Briefly, *Coronavirus* RNA was isolated and purified from nasopharyngeal swab samples. Viral nucleic acid was extracted by the MagNA Pure 96 IVD instrument (ROCHE Diagnostics), and real-time PCR performed using the Thermo Fisher® ABI Real-Time PCR platform. Viral nucleic acid was detected in patient samples by the

identification of *SARS-CoV-2* and EAV (RNA extraction control) using target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of *SARS-CoV-2* and EAV[1].

Liver tissue obtained at time of detection of *SARS-CoV-2* PCR or IgG positivity was also subjected to qRT-PCR. Briefly, *Coronavirus* RNA was manually isolated using Trizol/chloroform. Real-Time PCR was performed using the ABI 7500 Real-Time Fast (Thermo Fisher) or ABI Quant Studio Dx or ROCHE COBRAS Z480. Viral nucleic acid was detected in patient samples by the identification of *nCOV* (COVID-19) and PRC (RNA processing control) using target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of *nCOV* and PRC[1-4].

## Multiplex Immunohistochemistry.

Liver or small bowel tissue obtained at time of detection of *SARS-CoV-2* PCR or IgG positivity was stained for tissue resident memory ($T_{RM}$) cells defined as $CD69^+$ expressing CD4 T cells or $CD103^+$ expressing CD8 T cells.

Briefly, paraffin slides of liver biopsies (or graft small intestine biopsies for intestinal transplant patients) obtained preceding *SARS-CoV-2* PCR or IgG positivity, and paraffin slides of liver biopsies (or graft small intestine biopsies for intestinal transplant patients) obtained at detection of *SARS-CoV-2* PCR or IgG positivity were baked at 60°, deparaffinized in xylene, rehydrated, washed in DI water and incubated with 10% neutral buffered formalin (NBF) for an additional twenty minutes to increase tissue-slide retention. Epitope retrieval/microwave treatment (MWT) for all antibodies was performed by boiling slides in Antigen Retrieval buffer 6 or 9 (AR6 pH 6, Akoya AR6001KT and AR9 pH 9, Akoya AR9001KT). Protein blocking was performed using antibody diluent/blocking buffer (Akoya, AR1001EA) for ten minutes at room temperature. Primary antibody/OPAL dye pairings, staining order, and incubation conditions for CD4, CD69, CD103, and CD8 antibodies are detailed in **Supplementary Table 1**.

Slides were counterstained with spectral DAPI (Akoya FP1490) for five minutes, mounted with ProLong Diamond Antifade (Thermofisher, P36961) using StatLab #1 coverslips (CV102450), and subsequently scanned using Vectra 3.0 Automated Quantitative Pathology Imaging System (Perkin Elmer / Akoya). Whole slide scans were viewed with Phenochart (Perkin Elmer / Akoya). Seventeen to twenty-three multispectral image ROIs (669 μm X 500 μm) were captured on each slide for a total of 85 multispectral images across six slides. Multispectral images were unmixed using a spectral library built from images of single stained control tissues for each OPAL dye using the inForm Advanced Image Analysis software (inForm 2.4.6, Perkin Elmer / Akoya). A selection of ten to fifteen representative multispectral images spanning all tissue sections was used to train the inForm software. Settings applied to the training images were saved within an algorithm to allow batch analysis of all the multispectral images. All raw data was consolidated in PhenoptrReports (Akoya).

## Reference


1. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. Jan 2020;25(3)doi:10.2807/1560-7917.ES.2020.25.3.2000045
2. Corp Q. Lyra SARS-CoV-2 assay for the qualitative detection of human coronavirus SARS-CoV-2 viral RNA extracted from nasopharyngeal and oropharyngeal swab specimens. March 2020;
3. *Viral Culture; Approved Guidelines*. Clinical and Laboratory Standards Institute CLSA document M41-A 945. 2006.
4. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Ann Intern Med*. May 5 2020;172(9):577-582. doi:10.7326/M20-0504

Title of supplemental figures and tables

**Supplemental Figure 1**. **Absolute lymphocyte count**.

**Supplemental Figure 2**. **Trough tacrolimus level**.

**Supplemental Figure 3**. **Kaplan-Meier plot**.

**Supplemental Figure 4**. **Summary plots**. CD3, CD20, CD4, and CD8 cell frequency.

**Supplemental Figure 5**. **Summary plots**. CD4CD45RA naïve cell frequency.

**Supplemental Figure 6**. **Gating strategy**.

**Supplemental Table 1. Primary antibody/OPAL dye pairings, staining order, and incubation conditions for CD4, CD69, CD103, and CD8 antibodies.**

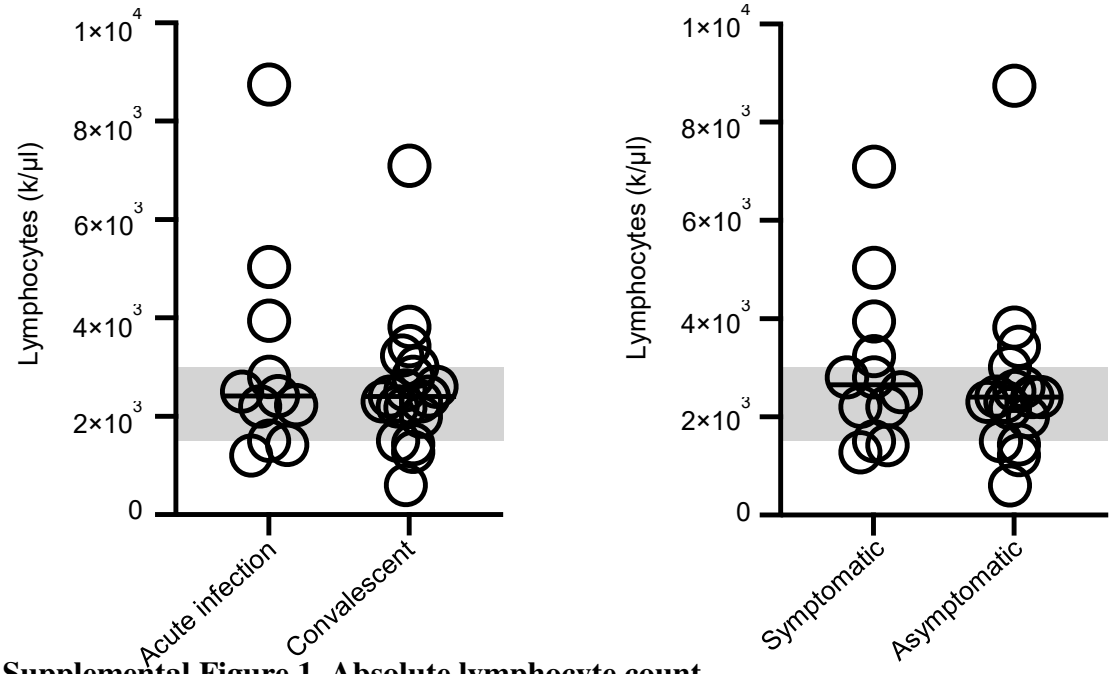
**Supplemental Table 2. Patient data stratified by acute infection vs. convalescence.**

**Supplemental Table 3. Demographics of symptomatic & asymptomatic patients.**

**Supplemental Table 4. Patient data stratified by symptomatic vs. asymptomatic disease.**

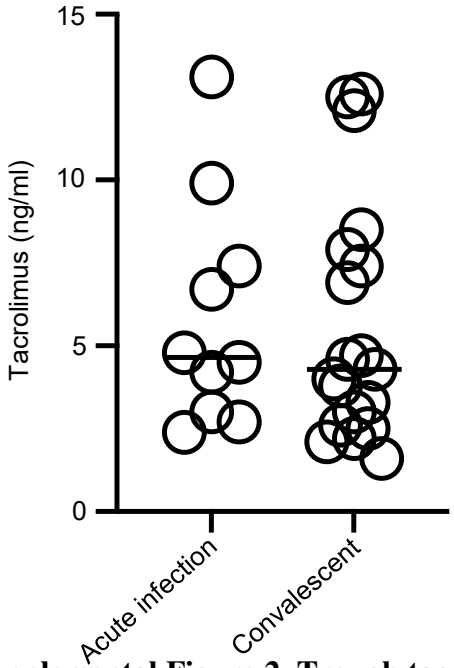
**Supplemental Table 5. Demographic data in patients with blood samples for immunophenotyping. Data stratified by COVID-19 positive vs. healthy control.**

**Supplemental Table 6. Flow parameters between healthy controls and COVID samples.**




**Supplemental Figure 1. Absolute lymphocyte count**.

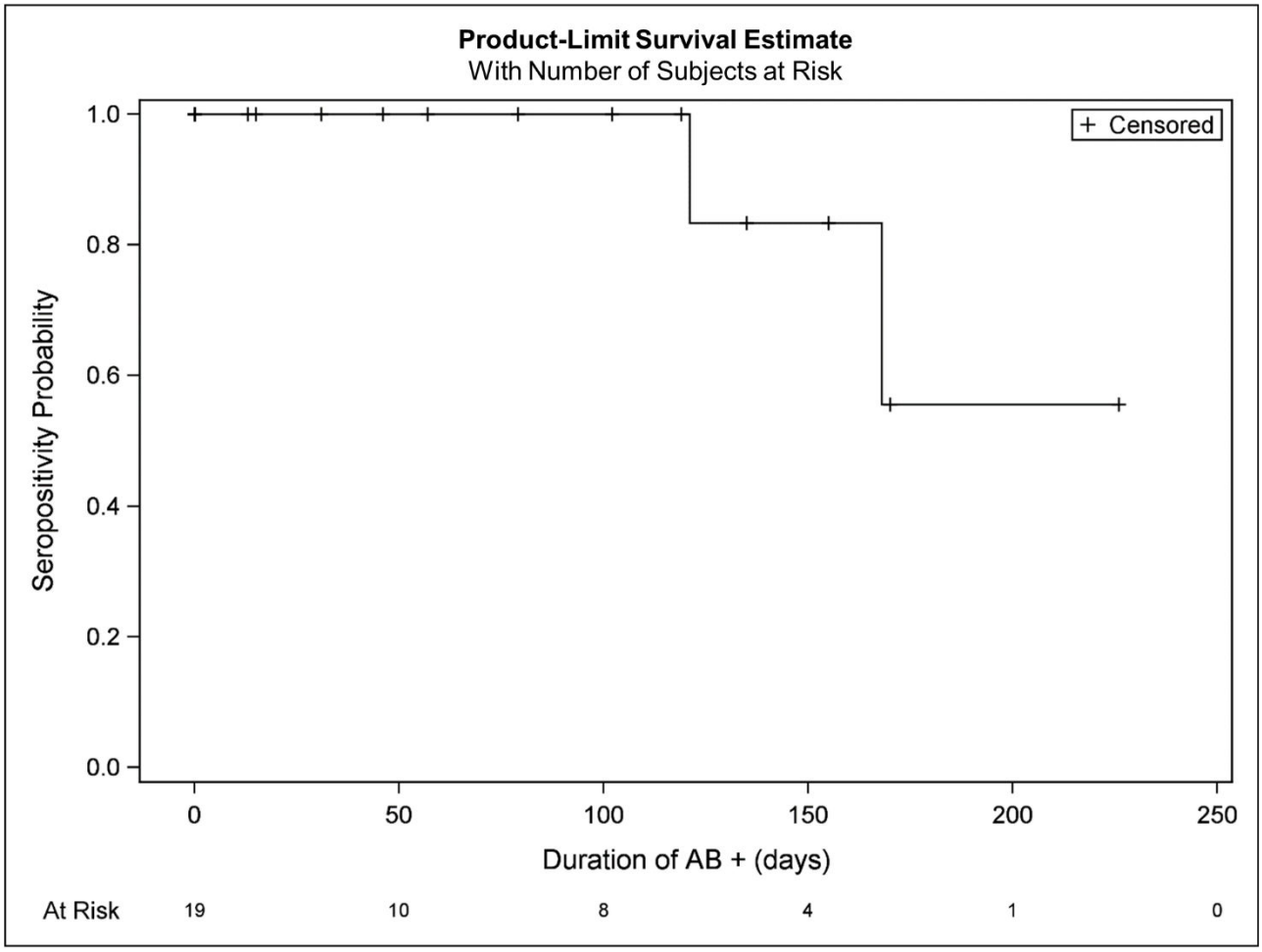
Absolute lymphocyte count at diagnosis of acute infection vs. convalescent and symptomatic vs. asymptomatic. Gray zone indicates the lower and upper limit of normal for absolute lymphocyte count in children. [Nelson's Textbook of Pediatrics, 21st Ed].




**Supplemental Figure 2. Trough tacrolimus level**.

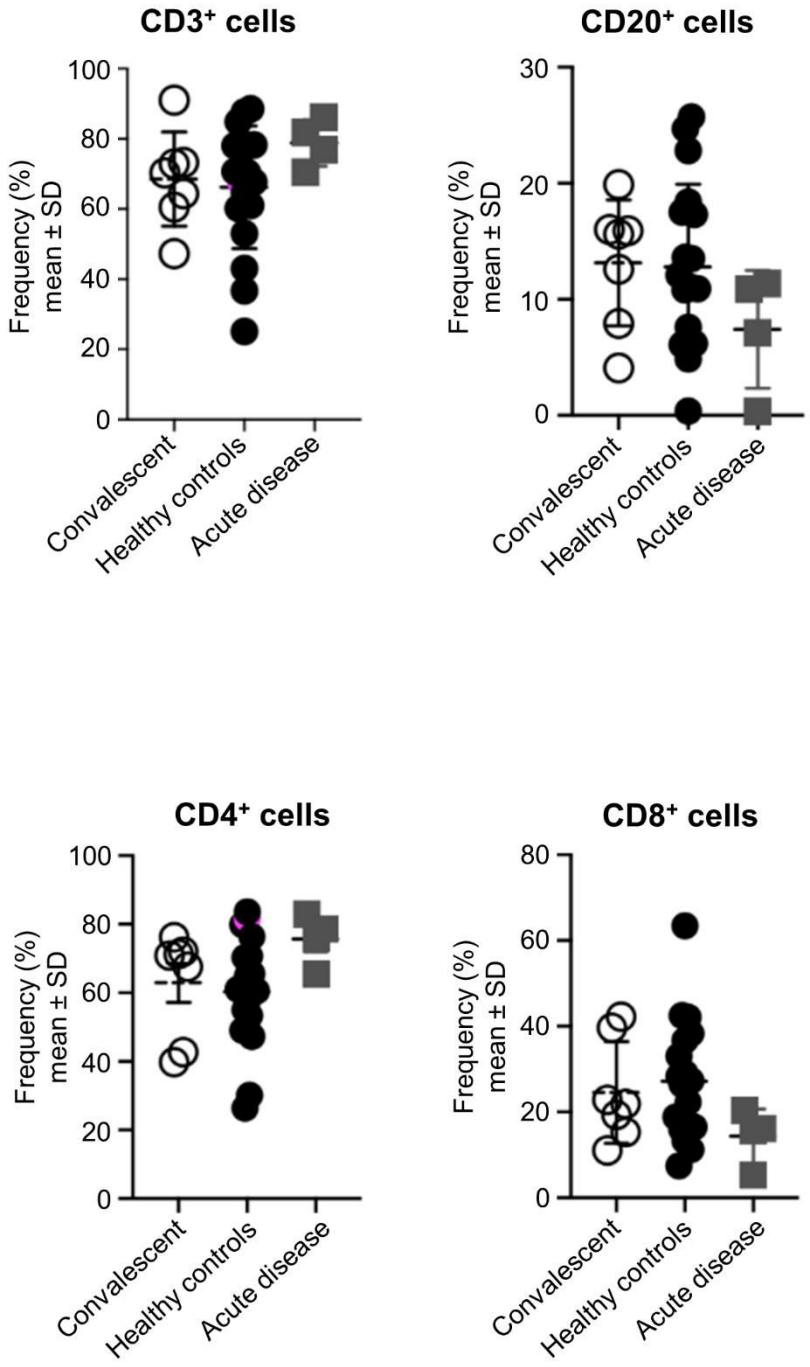
Trough tacrolimus level at diagnosis of acute infection or positive SARS-CoV-2 IgG (convalescence).

**Supplemental Figure 3. Kaplan-Meier plot**.



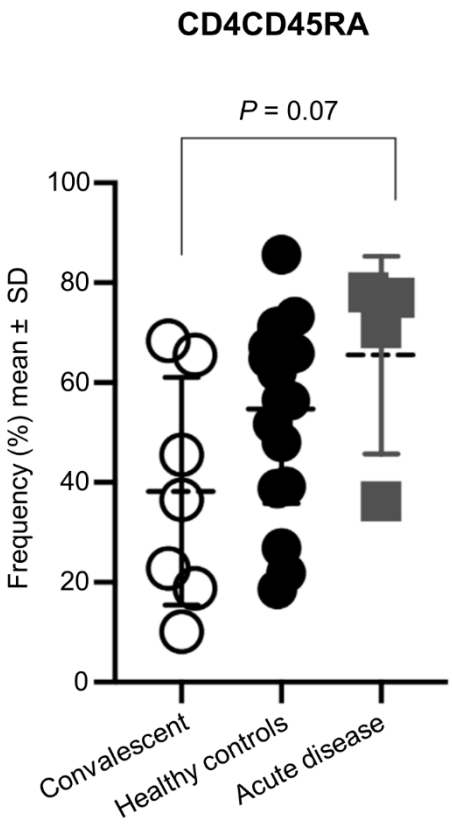

Proportion of children with persistent SARS-CoV-2 IgG following initial detection.

**Supplemental Figure 4. Summary plots**.



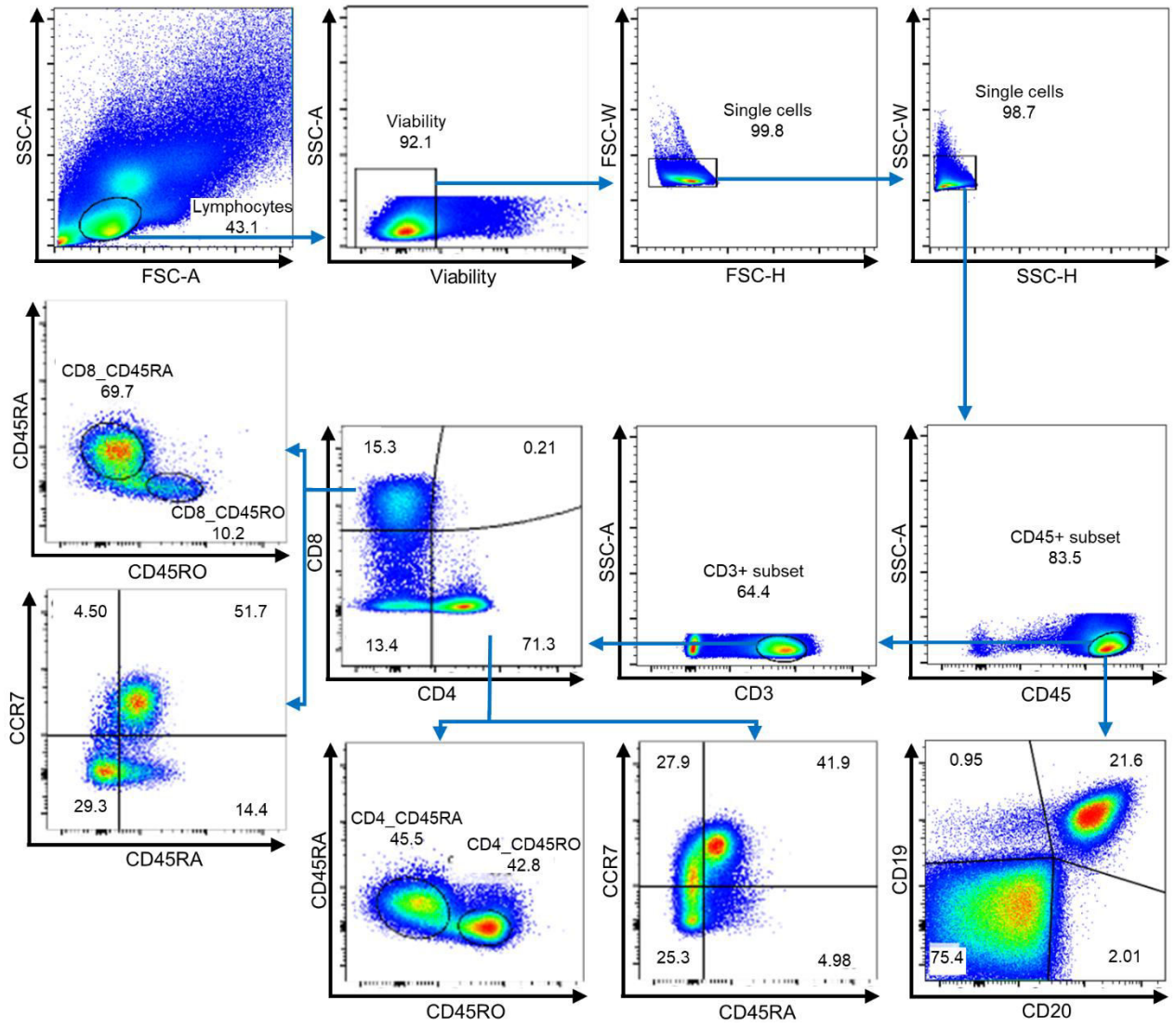

CD3, CD20, CD4, and CD8 cell frequency (acute infection n=4, convalescent n=7, healthy controls n=17). Analysis performed by ANOVA.

**Supplemental Figure 5. Summary plots**.




CD4CD45RA naïve cell frequency (acute infection n=4, convalescent n=7, healthy controls n=17). Analysis performed by Mann Whitney test.

**Supplemental Figure 6. Gating strategy**.




Lymphocytes identified by forward and side scatter. Viable cells gated upon, single cells gated on, $CD45^+$ cells gated on, and $CD20^+$ cells identified. $CD3^+$ cells identified from $CD45^+$ cells. $CD4^+$ and $CD8^+$ cells identified from $CD3^+$ cells. Gating on $CD4^+$ cells, $CD4^+CD45RA^+$ naïve cells and $CD4^+CD45RO^+$ memory cells identified. CD4 $T_{CM}$, $T_{EM}$, $T_N$, $T_{EMRA}$ identified as $CCR7^+CD45RA^-$, $CCR7^-CD45RA^-$, $CCR7^+CD45RA^+$, $CCR7^-CD45RA^+$ respectively. Gating on $CD8^+$ cells, $CD8^+CD45RA^+$ naïve cells and $CD8^+CD45RO^+$ memory cells identified. CD8 $T_{CM}$, $T_{EM}$, $T_N$, $T_{EMRA}$ identified as $CCR7^+CD45RA^-$, $CCR7^-CD45RA^-$, $CCR7^+CD45RA^+$, $CCR7^-CD45RA^+$ respectively.

**Supplemental Table 1. Primary antibody/OPAL dye pairings, staining order, and incubation conditions for CD4, CD69, CD103, and CD8 antibodies.**

| | *Antibody 1* | *Antibody 2* | *Antibody 3* | *Antibody 4* |
|---|---|---|---|---|
| ***Antigen*** | CD4 | CD69 | CD103 | CD8 |
| ***Company*** | Cell Marque | Abcam | Abcam | Agilent |
| ***Catalogue #*** | 104R-15 | ab233396 | ab129202 | M7103 |
| ***Species*** | Rabbit | Rabbit | Rabbit | Mouse |
| ***Dilution*** | 1:300 | 1:600 | 1:500 | 1:50 |
| ***Incubation time*** | overnight | 1-hour | 1-hour | 1-hour |
| ***Incubation temp.*** | 4ºC | Room temp. | Room temp. | Room temp. |
| ***Control tissue*** | Tonsil 8818 | Tonsil 8818 | Colon 3926 | Tonsil 8818 |
| ***OPAL Fluor.*** | 690 | 540 | 620 | 520 |
| ***OPAL Conc.*** | 1:75 | 1:400 | 1:750 | 1:600 |
| ***Antigen retrieval*** | AR6 | AR9 | AR9 | AR9 |

**Supplemental Table 2. Patient data stratified by acute infection vs. convalescence.**

| | Acute_infection (N = 11) | Convalescent (N = 19) | P-value |
|---|---|---|---|
| **Median (IQR) age at diagnosis (yrs.)** | | | *P* = 0.4010 |
| Median (IQR) | 6.13 (2.99, 6.56) | 7.33 (4.96, 11.26) | |
| **Median (IQR) duration from transplant at diagnosis (yrs.)** | | | *P* = 0.0902 |
| Median (IQR) | 2.07 (1.36, 3.90) | 5.09 (4.29, 8.93) | |
| **Gender** | | | *P* = 1.0000 |
| Female | 4 (36) | 6 (32) | |
| Male | 7 (64) | 13 (68) | |

**Supplemental Table 3. Demographics of symptomatic & asymptomatic patients.**

*Symptomatic patients*

| | Gender | Tx Type[a] | Co-morbidities[b] | IS[c] | Past corona (not covid-19) virus infection |
|---|---|---|---|---|---|
| 001 | F | LT | No | T, C | No |
| 002 | M | LT | H | T, P | HKU1 |
| 003 | F | LT | No | T, S | No |
| 004 | M | MVT | No | T, P | No |
| 005 | M | LT | No | T | No |
| 006 | M | MVT | D | T, P, S | No |
| 007 | F | MVT | H | T, P, S | No |
| 008 | M | LT | No | T | No |
| 009 | F | MVT | H | T, P | HKU1 |
| 010 | M | SB | H, DM | T, P | No |
| 011 | M | LT | No | T, S | No |
| 012 | M | LT | H | T, P | No |
| 013 | M | SB | No | T, P, S | No |
| 014[d] | F | n/a | No | n/a | |

*Asymptomatic patients*

| | Gender | Tx Type[a] | Co-morbidities[b] | IS[c] | Past corona (not covid-19) virus infection |
|---|---|---|---|---|---|
| 001 | M | SLK | DM, H | T, P, S | No |
| 002 | M | MVT | No | T, P | OC43 |
| 003 | F | LT | No | T | No |
| 004 | F | LT | No | T, C | No |
| 005 | M | LT | No | T, C | No |
| 006 | F | LT | No | T | No |
| 007 | M | LT | No | T | No |
| 008 | F | LT | O | T | No |
| 009 | M | MVT | No | T, P | No |
| 010 | M | LT | H | T, S | No |
| 011 | M | SB | H, O | T, P, R | No |
| 012 | F | LT | No | T, C | No |
| 013 | F | LT | No | T | No |
| 014 | M | LT | No | T, C | No |
| 015 | M | LT | No | T, C | No |
| 016 | M | LT | No | T | No |
| 017 | M | MVT | H | T, P | No |

*Key:*
a: LT: liver transplant; MVT: multi-visceral transplant; SB: small bowel transplant
b: H: hypertension; D: dialysis; DM: diabetes; O: obesity
c: T: tacrolimus; P: prednisolone; C: cellcept; S: sirolimus; R: ruxolitinib
d: listed patient. Not transplanted

**Supplemental Table 4. Patient data stratified by symptomatic vs. asymptomatic disease.**

| | asymptomatic (N = 17) | symptomatic (N = 13) | P-value |
|---|---|---|---|
| **Median (IQR) age at diagnosis (yrs.)** | | | *P* = 0.2068 |
| Median (IQR) | 6.40 (5.03, 12.44) | 6.13 (3.46, 9.99) | |
| **Median (IQR) duration from transplant at diagnosis (yrs.)** | | | *P* = 0.3929 |
| Median (IQR) | 5.03 (4.29, 6.14) | 3.12 (1.54, 5.80) | |
| **Gender** | | | *P* = 1.0000 |
| Female | 6 (35) | 4 (31) | |
| Male | 11 (65) | 9 (69) | |
| **Transplant Type** | | | *P* = 0.5126 |
| LT | 12 (71) | 7 (54) | |
| MVT/SB | 4 (24) | 6 (46) | |
| SLK | 1 (6) | 0 (0) | |
| **Number of Immunosuppressive drugs** | | | *P* = 0.4155 |
| 1 | 6 (35) | 2 (15) | |
| 2 | 9 (53) | 8 (62) | |
| 3 | 2 (12) | 3 (23) | |
| **Number of Co-morbidities** | | | *P* = 0.4393 |
| 0 | 12 (71) | 7 (54) | |
| 1 | 3 (18) | 5 (38) | |
| 2 | 2 (12) | 1 (8) | |

**Supplemental Table 5. Demographic data in patients with blood samples for immunophenotyping. Data stratified by COVID-19 positive vs. healthy control.**

| Variable | COVID positive n=11 | Healthy control[#] n=17 | P value |
|---|---|---|---|
| # of IS drugs* | | | |
| 1 IS | 2 (20%) | 8 (47.1%) | 0.65 |
| ≥2 IS | 8 (80.0%) | 9 (52.9%) | |
| Tacrolimus level (ng/ml) Median (IQR)* | 7.0 (4.2, 9.1) | 4.6 (3.2, 6.5) | 0.19 |
| Age (years) | 6.0 (3.0, 10.0) | 2.0 (0.8, 8.0) | 0.18 |
| Gender | | | |
| Male | 6 (55.5%) | 8 (47.1%) | >0.99 |
| Female | 5 (45.5%) | 9 (52.9%) | |
| Duration from TX at blood draw (years)* | 2.59 (1.01, 5.15) | 5.1 (1.03, 7.87) | 0.38 |
| Race | | | |
| AA | 4 (36.4%) | 5 (29.4%) | >0.99 |
| non-AA | 7 (63.6%) | 12 (70.6%) | |

* Listed patient not included

# Healthy control refers to pediatric solid organ transplant recipients with no exposure/infection with COVID-19 whose blood samples predated 2020.

| | 17 Healthy controls vs. 4 Acute disease | | 17 Healthy controls vs. 6 Convalescents | |
|---|---|---|---|---|
| **Name** | **$log_2$ Fold Change** | **p-value** | **$log_2$ Fold Change** | **p-value** |
| CD20 (%) | -0.520 | 0.362 | -0.226 | 0.516 |
| CD21 dim B cells (%) | 0.703 | 0.203 | 0.361 | 0.516 |
| CD3 (%) | -2.414 | 0.165 | 0.070 | 0.649 |
| CD3+CD317- | 0.036 | 0.897 | -0.222 | 0.609 |

**Supplemental Table 6: Flow parameters between healthy controls and COVID samples.**

| | | | | |
|---|---|---|---|---|
| CD3+CD317+ | -1.552 | 0.527 | 0.494 | 0.776 |
| CD317- CD4 Tcell | 0.180 | 0.130 | 0.003 | 0.580 |
| CD317- CD8 Tcell | 0.516 | 0.065 | 0.224 | 0.392 |
| CD317+ CD4 Tcell | -1.234 | 0.275 | 0.604 | 0.183 |
| CD317+ CD8 Tcell | -0.869 | 0.812 | -0.188 | 0.244 |
| CD4 (%) | 0.281 | 0.275 | -0.213 | 0.177 |
| CD4 Memory (%) | 0.789 | 0.144 | 1.754 | 0.117 |
| CD4 Na√Øve (%) | 0.061 | 0.275 | 0.401 | 0.256 |
| CD4 TCM (%) | -0.933 | 0.122 | -0.198 | 0.712 |
| CD4 TEM (%) | 0.577 | 0.040 | -0.207 | 0.516 |
| CD4 TEMRA (%) | 0.155 | 0.263 | -0.090 | 0.462 |
| CD4 TN (%) | -0.927 | 0.081 | -0.114 | 0.812 |
| CD4+ G-CSF+ | 0.236 | 0.148 | 0.019 | 0.854 |
| CD4+ Granzyme B+ | "-Inf" | 0.255 | 0.336 | 0.176 |
| CD4+ HLADR+ | 0.297 | 0.089 | 0.003 | 0.759 |
| CD4+ IFNa+ | -3.040 | 0.019 | -1.482 | 0.112 |
| CD4+ IFNg+ | -0.370 | 0.897 | 0.814 | 0.074 |
| CD4+ IL17A+ | -0.769 | 0.244 | -0.090 | 1.000 |
| CD4+ IL6+ | -2.253 | 0.560 | -1.111 | 0.649 |
| CD8 (%) | -1.450 | 0.517 | 0.168 | 0.135 |
| CD8 Memory (%) | -1.540 | 0.120 | 0.576 | 0.319 |
| CD8 Na√Øve (%) | -0.228 | 0.362 | 0.584 | 0.256 |
| CD8 TCM (%) | -4.626 | 0.291 | -0.155 | 0.321 |
| CD8 TEM (%) | 0.529 | 0.031 | 0.203 | 0.609 |
| CD8 TEMRA (%) | -1.018 | 0.679 | 1.294 | 1.000 |
| CD8 TN (%) | 0.040 | 0.763 | -0.769 | 0.030 |
| CD8+ G-CSF+ | 1.960 | 1.000 | 1.933 | 0.013 |
| CD8+ Granzyme B+ | 0.005 | 1.000 | -3.186 | 0.279 |
| CD8+ HLADR+ | 0.459 | 0.462 | -0.024 | 0.865 |
| CD8+ IFNa+ | -2.367 | 0.044 | 0.413 | 0.227 |
| CD8+ IFNg+ | 0.501 | 0.395 | 0.088 | 0.752 |
| CD8+ IL17A+ | -0.157 | 1.000 | 1.175 | 0.080 |
| CD8+ IL6+ | -1.332 | 0.601 | -0.595 | 0.502 |
| mDC | 1.593 | 0.963 | -4.745 | 0.053 |
| pDC | -1.667 | 0.172 | -0.074 | 0.834 |
| TFH (%) | -2.569 | 0.531 | -0.128 | 0.529 |