Supplemental Materials for: Kahn B, et al. Multisystem Inflammation and Organ Dysfunction after BNT162b2 mRNA Covid-19 Vaccination

Appendices.

Supplemental Tables

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Appendices

Appendix A. Diagnostic studies and discussion

A1. Initial laboratory evaluation
His initial laboratory evaluation was notable for white blood cell count 21.9 x 10^3/uL (ref: 4.5-13.5 x 10^3/uL) with 0.61 x 10^3/uL absolute lymphocyte count (ref: 1.0-5.0 x 10^3/uL) and 19.8 x 10^3/uL absolute neutrophil count (ref: 1.8-7.5 x 10^3/uL), bicarbonate 24 mmol/L (ref: 22-32 mmol/L), creatinine 3.81 mg/dL (ref: 0.64-1.27 mg/dL), blood urea nitrogen 45 mg/dL (ref: 8-20 mg/dL), anion gap 16 (ref: 10-20), pH 7.33 (ref: 7.35-7.45), pCO2 51 mmHg (ref: 34-48 mmHg), lactic acid 2.6 mmol/L (ref: 0.5-1.26 mmol/L), albumin 5.0 g/dL (ref: 3.5-5.1 g/dL), and total bilirubin 1.7 mg/dL (ref: 0.3-1.2 mg/dL). Urinalysis showed packed red blood cells (ref: 0-2/high powered field) and protein >4000 mg/dL (ref: 0 mg/dL).

A2. Inflammatory markers
Expanded laboratory diagnostic evaluation was notable for a systemic dysregulated inflammatory process including: elevated high-sensitivity C-reactive protein (CRP) 138.3 mg/L (ref: ≤ 7.4 mg/L), non-cardiac CRP 2.50 mg/dL (ref: ≤ 0.80 mg/dL), erythrocyte sedimentation rate (ESR) 29 mm/h (ref: 0-20 mm/h), D-dimer 15.86 ug/mL (ref: 0-0.5), and lactate dehydrogenase (LDH) 204 U/L (ref: 98-192 U/L). Analysis of peripheral blood on hospital day (HD) 2 was notable for lymphopenia: CD3+ count 381/uL (ref: 900-3245/uL), CD4+ count 91/L (ref: 560-1840/L), CD8+ count 258/uL (ref. 260-1230/uL), and CD56+/16+/3- NK cells 112/uL (ref: 159-432/uL). An expanded cytokine panel on HD 8 demonstrated elevated soluble IL-2 receptor 2321.2 pg/mL (ref: 175.3-858.2 pg/mL), IL-10 11.2 pg/mL (ref: ≤ 2.8 pg/mL), IL-13 3.1 pg/mL (ref: ≤ 2.3 pg/mL), and IL-6 18.2 pg/mL (ref: ≤ 2.0 pg/mL). He had no elevation in IL-2, IL-4, IL-5, IL-12, IL-13, or IFN-gamma. He had elevated kappa free light chains of 42.8 mg/L (ref: 3.3-19.4 mg/L), but lambda free light chains, serum protein electrophoresis, and urine protein gel electrophoresis showed no evidence for paraprotein. He had a mildly elevated creatinine kinase of 584 U/L (ref: 49-397 U/L) that peaked at 833 U/L. Ferritin and free cortisol were normal. Triglycerides were elevated at 587 mg/dL (ref: 24-150 mg/dL).

A3. Autoimmune serologies, malignancy evaluation, and clotting cascade testing
An extensive serologic evaluation for autoimmune disease was negative including for anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA) including PR-3 and MPO, C3, C4, anti-glomerular basement membrane antibodies, and anti-Scl-70 antibodies. Quantitative immunoglobulins were within normal limits.

Malignancy evaluation included peripheral flow cytometry notable only for lymphopenia, unremarkable pleural fluid cytology and flow cytometry, and computed tomography (CT) scan of the abdomen and pelvis without suspicious lesions.

He had mildly prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT), decreases in clotting factors II, VII, X, and XI, and an elevated dilute Russell's viper venom time of 50.5 seconds (ref: 28-42 seconds) which normalized later in the admission. All other hypercoagulable evaluation was unremarkable including beta-2-glycoprotein, cardiolipin antibodies, Factor V Leiden, ADAMTS13, anti–platelet factor 4 (PF4) antibodies (for heparin-

induced thrombocytopenia), lupus anticoagulant, antiphospholipid antibodies, fibrinogen, thrombin time, reptilase time, flow cytometry for paroxysmal nocturnal hemoglobinuria, and direct antiglobulin test (DAT).

MR angiogram of the head and neck on HD 8 was unremarkable and did not suggest central nervous system vasculitis as the source of ischemic foci.

A4. Infectious disease testing

Negative infectious disease testing included: blood and urine cultures; SARS-CoV-2 nucleic acid PCR three times and SARS-CoV-2 Nucleocapsid IgG; non-pandemic respiratory viruses; pleural fluid cultures; qualitative and quantitative human immunodeficiency virus (HIV); cerebrospinal fluid serologies and cultures; sexually transmitted infections; enteric pathogens; and systemic viral infections. An oral swab revealed rare Methicillin-Resistant Staphylococcus aureus consistent with colonization. Pericardial fluid culture grew Cutibacterium (Propionibacterium) acnes from broth only which was thought to be a skin contaminant. A skin punch biopsy on the upper chest revealed acute suppurative folliculitis with colonies of cocci and few focal yeast forms consistent with Pityrosporum species and consistent with an acneiform process. Skin culture was negative. Of note, the rash resolved quickly after beginning high-dose corticosteroids. The complete absence of fever and repeatedly negative testing for acute and prior SARS-CoV-2 infection was inconsistent with Multisystem Inflammatory Syndrome in Children (MIS-C) and in Adults (MIS-A).

A5. Immune cell profiling

On HD 3, the patient's whole blood was analyzed in a non-clinical, research-based cytometry by time of flight (CyTOF) assay. This analysis demonstrated a significantly expanded granulocytic population with concomitant relative lymphopenia more severe for CD4+ cells, with an inverted CD4:CD8 ratio (**eFigures 2A, 2B, 2D**). A follow-up sample obtained on HD 7 revealed sustained lymphopenia but correction of the CD4:CD8 ratio led by expansion of a regulatory T-cell–like CD4+ CD25+ CD127– cell population (**eFigures 2A, 2B, 2C**). CyTOF analysis at both time points was notable for a high frequency of immature neutrophils (CD45+ CD66b^{lo} CD11c+ CD14+), most likely reflecting demarginating granulocytes in the setting of high-dose glucocorticoids, and low B-cell expression of CD38 and HLA-DR (**eFigures 2D, 2E**).

A6. Whole exome sequencing

The patient's family history was notable for autoimmune hepatitis in his mother and rheumatoid arthritis in a maternal aunt. Rapid whole exome sequencing (XomeDxXpress, GeneDx) using search terms relevant to his clinical syndrome was initially non-diagnostic as no pathogenic or likely pathogenic variants were identified. Full exome analysis, however, revealed the presence of potentially relevant homozygous variants of uncertain significance in two genes: homozygous variants (c.-1G>T) in *C1S* and homozygous variants (c.1523 G>A; p.R508Q) in *SCNN1A*. Of note, his CH50 was present but low at 19.6 U/mL (ref: 38.7-89.9 U/mL) and notably drawn after the patient had undergone plasmapheresis. Mitochondrial DNA sequencing and deletion/duplication analysis was negative for pathogenic variants.

In summary, his whole exome sequencing revealed the presence of homozygous variants of uncertain significance in both the *C1S* and *SCNN1A* genes. C1S deficiency was considered due

to the reported phenotypes of autoimmune hepatitis (a diagnosis present in the patient's mother) and virus-associated hemophagocytic syndrome, but a non-zero CH50 level effectively ruled this out.[1,2] Hemophagocytic lymphohistiocytosis (HLH) was considered, but effectively ruled out by a normal serum ferritin. Comprehensive testing of the complement cascade revealed normal plasma levels of C1S, normal activity for both the classical and alternative pathways, and slightly decreased C4 (albeit only after plasma exchange), C5, and C8. A *SCNN1A*-related disorder was also considered. This can be associated with severe pseudohypoaldosteronism presenting early in life.[3] The patient had no known pre-existing kidney disease or electrolyte mishandling; in the hospital after vaccination he did have hyperkalemia that was difficult to control even with kidney replacement therapy. While other channelopathies are associated with hearing loss, *SCNN1A* gene variants have not been reported to be.[4] Of note, preliminary reports of a signal for hearing loss after Covid-19 vaccination have not been confirmed in large observational studies.[5]

A7. Renal evaluation including kidney biopsy
On admission, bladder ultrasound was notable only for mobile heterogeneous dependent material consistent with blood products. CT scan of the abdomen and pelvis without contrast demonstrated attenuation of the renal pelvises and ureters, consistent with the presence of blood products and hemorrhage, and no nephrolithiasis or hydroureteronephrosis. A 24-hour urine collection started on HD 4 contained 4.7 grams of protein.

Kidney biopsy: Two cores were obtained with an 18-gauge needle. Nine glomeruli were present. Light microscopy showed packed red blood cells in the tubules, but essentially normal-appearing glomeruli and only mild tubular injury. There was no significant interstitial fibrosis. Electron microscopy (EM) showed mild-moderate glomerular injury, with segmental foot process effacement involving approximately 30-40% of the capillary surface area. Endothelial cells showed frequent swelling and fenestration loss. No immune deposits were present on EM. Consistent with this, immunofluorescence was negative for IgG, IgA, IgM, C3, C1q, kappa, lambda, and fibrinogen. Immunofluorescence for COL4A5 to evaluate for collagen IV nephropathy/Alport syndrome showed a normal glomerular staining pattern.

A8. Sensorineural hearing loss
Otoscopic examination was unremarkable. CT scan of the head on HD 3 was unremarkable. Non-contrast magnetic resonance imaging (MRI) of the head on HD 5 revealed several punctate strokes in multifocal vascular territories of the bilateral hemispheres and the left cerebellum (**eFigure 3**), a distribution that did not explain the patient's hearing loss, and no labyrinthitis or evidence of prior labyrinthine insult or masses in the internal auditory canals. Lumbar puncture was notable for an opening pressure of 7 cmH_2O (ref: 6-25 cmH_2O) and cerebral spinal fluid glucose 85 mg/dL (ref: 40-70 mg/dL), otherwise unremarkable including extensive infectious studies. Since the patient was receiving treatment with glucocorticoids for his systemic inflammatory syndrome, no additional empiric treatment was used for hearing loss. Audiometry examination showed no response to pure tone or speech stimuli except for vibro-tactile responses consistent with a profound bilateral sensorineural hearing loss.

A9. Ischemic strokes
MR angiogram of the head and neck on HD 8 was unremarkable and did not suggest central nervous system vasculitis as the source of the ischemic foci. His pro-thrombotic evaluation was

unremarkable (**Appendix A3**) and he had no thrombosis found on four-extremity vascular ultrasonography. Follow-up MRI on HDs 10 and 21 showed no new infarcts and expected evolution of the previously noted punctate infarcts. His clinical neurological deficits remained limited to hearing loss.

Supplemental Tables

eTable 1. Laboratory results

Component	Hospital Day 1	Reference range
Basic metabolic panel		
Glucose	105 mg/dL	70 - 99 mg/dL
Sodium	135 mmol/L	136 - 144 mmol/L
Potassium	4.8 mmol/L	3.6 - 5.1 mmol/L
Chloride	95 mmol/L	101 - 111 mmol/L
Bicarbonate	24 mmol/L	22 - 32 mmol/L
Anion gap	16	3 - 12
Blood urea nitrogen	45 mg/dL	8 - 20 mg/dL
Creatinine	3.81 mg/dL	0.64 - 1.27 mg/dL
Calcium	10.2 mg/dL	8.9 - 10.3 mg/dL
Liver function panel		
Total protein	8.4 g/dL	6.1 - 7.9 g/dL
Albumin	5 g/dL	3.5 - 5.1 g/dL
Alanine aminotransferase (ALT)	48 U/L	17 - 63 U/L
Aspartate aminotransferase (AST)	38 U/L	15 - 41 U/L
Alkaline phosphatase	85 U/L	38 - 126 U/L
Total bilirubin	1.7mg/dL	0.3 - 1.2 mg/dL
Direct bilirubin	0.4mg/dL	0.1 - 0.5 mg/dL
Unconjugated bilirubin	1.3 mg/dL	0.2 - 0.7 mg/dL
Coagulation profile		
Prothrombin time (PT)	14.1 second(s)	9.4 - 12.5 second(s)
International normalized ratio (INR)	1.2	0.8 - 1.1
Partial thromboplastin time (PTT)	32.6 second(s)	25.1 - 36.5 second(s)
Complete blood count		
White blood cells	21.9 x 10^3/uL	4.5 - 13.5 x 10^3/uL
Red blood cells	5.75 x 10^6/uL	4.30 - 5.80 x 10^6/uLL
Hemoglobin	16.6 g/dL	13.5 - 17.5 g/dL
Hematocrit	49%	40 - 52 %
RDW	14%	11.5 - 14.5 %
MCH	29 pg	27 - 33 pg
MCHC	34 g/dL	31 - 36 g/dL
MCV	86 fL	80 - 100 fL
Platelets	326 x 10^3/uL	150 - 400 x 10^3/uL
Acute phase reactants		
Lactate dehydrogenase (LDH)	204 U/L	98 - 192 U/L
Haptoglobin	132 mg/dL	36 - 195 mg/dL
Erythrocyte sedimentation rate (ESR)	29 mm/h	0 - 20 mm/h

eTable 1. Laboratory results (cont.)

Acute phase reactants	Hospital Day 2	Hospital Day 10	Hospital Day 11	Hospital Day 12	Hospital Day 13	Hospital Day 15	Hospital Day 18	Hospital Day 21	Hospital Day 39
High-sensitivity C-reactive protein (CRP) Ref: <7.4 mg/L	138.3 (H)	-	-		-	-	15.3 (H)	-	13.1 (H)
Erythrocyte sedimentation rate (ESR) Ref: 0 - 20 mm/h	29 (H)	29 (H)	-	1	-	-	-	-	-
Ferritin Ref: 23.9 - 336.2 ng/mL	-	70.5	-	240	-	-	132.3	-	-
Non-cardiac CRP Ref: ≤0.80 mg/dL	-	2.50 (H)	-	1.70 (H)	-	-	-	0.8	-
D-dimer Ref: 0.00 - 0.50 ug/mL	-	15.86 (H)	-	5.44 (H)	-	-	2.65 (H)	-	-
Lactate dehydrogenase (LDH) Ref: 98 - 192 U/L	204 (H)	-	823 (H)	777 (H)	351 (H)	196 (H)	-	-	-

Hematologic and clotting assays	Hospital Day 6	Hospital Day 17
Anticardiolipin IgG (GPL) Ref: 0.0 - 11.0 unit(s)	0.5	-
Anticardiolipin IgM (MPL) Ref: 0.0 - 12.0 unit(s)	3	-
Anti-beta-2 glycoprotein IgG Ref: 0.0 - 7.9 unit(s)	0.5	-
Anti-beta-2 glycoprotein IgM Ref: 0.0 - 12.9 unit(s)	0.5	-
Tissue thromboplastin inhibitor Ref: 0.0 - 1.4	1.4	-
Dilute Russell's viper venom (DRVV) ratio Ref: 0.0 - 1.1	1.4 (H)	-
Dilute Russell's viper venom (DRVV) time Ref: 28.0 - 42.0 second(s)	50.5 (H)	36.8
ADAMTS13 Ref: ≥60 %	61	-
Factor VIII Ref: 50 - 200 %	-	170
Factor IX Ref: 75 - 125 %	-	101
Factor XI Ref: 60 - 140 %	-	39 (L)

eTable 1. Laboratory results (cont.)

Serum protein electrophoresis (SPEP)	Hospital Day 2
Albumin Ref: 3.5 - 5.8 g/dL	3.5
Alpha1-Globulin Ref: 0.2 - 0.4 g/dL	0.4
Alpha2-Globulin Ref: 0.5 - 0.8 g/dL	0.7
Beta-Globulin Ref: 0.6 - 1.0 g/dL	0.9
Gamma-Globulin Ref: 0.7 - 1.2 g/dL	0.8

C-X-C Motif Chemokine Ligand 9 (CXCL9)	Hospital Day 23	Hospital Day 29	Hospital Day 48	Hospital Day 56	Hospital Day 63
CXCL9 Ref: ≤647 pg/mL	2553 (H)	6227 (H)	3574 (H)	1728 (H)	1263 (H)

eTable 1. Laboratory results (cont.)

Connective tissue disease serologies and cytology	Value
Anti-glomerular basement membrane Ref: 0 - 19 AU/mL	0
Anti-nuclear antibodies (ANA) Ref: negative	Negative
Rheumatoid factor Ref: ≤12.4 IU/mL	<7.0
Anti-double stranded DNA Ref: 0 - 99 IU/mL	<10
Anti-Scl-70 Ref: 0.0 - 0.9	0
Anti-serine protease 3 Ref: 0 - 20 unit(s)	3
Anti-myeloperoxidase Ref: 0 - 20 unit(s)	1
Kappa free light chains Ref: 3.3 - 19.4 mg/L	42.8 (H)
Lambda free light chains Ref: 5.7 - 26.3 mg/L	25.4
Kappa:lambda ratio Ref: 0.260 - 1.650	1.685 (H)
IgA Ref: 50 - 500 mg/dL	332
IgG Ref: 650 - 2,000 mg/dL	863
IgM Ref: 40 - 270 mg/dL	51
CD3 lymphocyte % Ref: 62 - 84 %	47 (L)
CD3 lymphocyte count Ref: 900 - 3,245 /uL	381 (L)
CD4 lymphocyte % Ref: 32 - 56 %	11 (L)
CD4 lymphocyte count Ref: 560 - 1,840 /uL	91 (L)
CD8 lymphocyte % Ref: 15 - 40 %	32
CD8 lymphocyte count Ref: 260 - 1,230 /uL	258 (L)
CD4/CD8 ratio Ref: 0.9 - 3.4	0.4 (L)
Natural killer cell % Ref: 6 - 30 %	8
Natural killer cell count Ref: 159 - 432 /uL	112 (L)

eTable 1. Laboratory results (cont.)

Cytokine panel #1	Hospital Day 8
IL-2 Ref: ≤6.5 pg/mL	<6.5
IL-4 Ref: ≤1.5 pg/mL	<1.5
IL-6 Ref: ≤3.5 pg/mL	26.4 (H)
IL-8 Ref: ≤10 pg/mL	32.4 (H)
IL-10 Ref: ≤2.0 pg/mL	<2.0
IL-12 Ref: ≤2.5 pg/mL	<2.5
IL-13 Ref: ≤2.3 pg/mL	<2.5
Interferon Gamma Ref: ≤6.5 pg/mL	<6.5
TNF-alpha Ref: ≤3.5 pg/mL	12.4 (H)

eTable 1. Laboratory results (cont.)

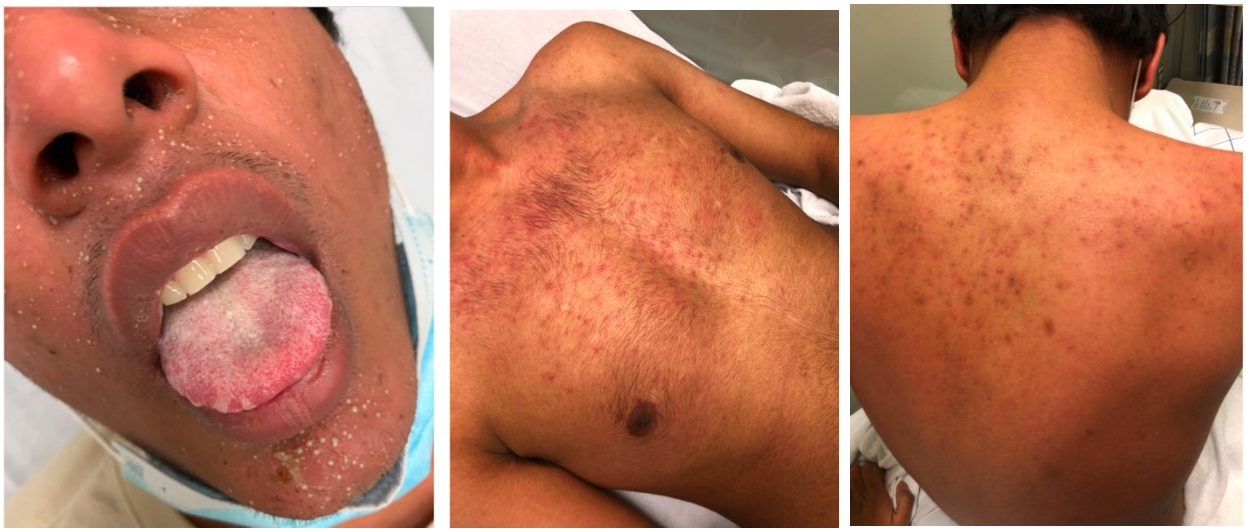
Cytokine panel #2	Hospital Day 9	Hospital Day 12
IL-2 Ref: ≤2.1 pg/mL	<2.1	-
IL-2 Receptor Ref: 175.3 - 858.2 pg/mL	2431.0 (H)	-
IL-4 Ref: ≤2.2 pg/mL	<2.2	-
IL-5 Ref: ≤2.1 pg/mL	<2.1	-
IL-6 Ref: ≤2.0 pg/mL	-	4.4 (H)
IL-10 Ref: ≤2.8 pg/mL	14.3 (H)	-
IL-12 Ref: ≤1.9 pg/mL	<1.9	-
IL-13 Ref: ≤2.3 pg/mL	3.6 (H)	-
Interferon Gamma Ref: ≤4.2 pg/mL	<4.2	-

eTable 1. Laboratory results (cont.)

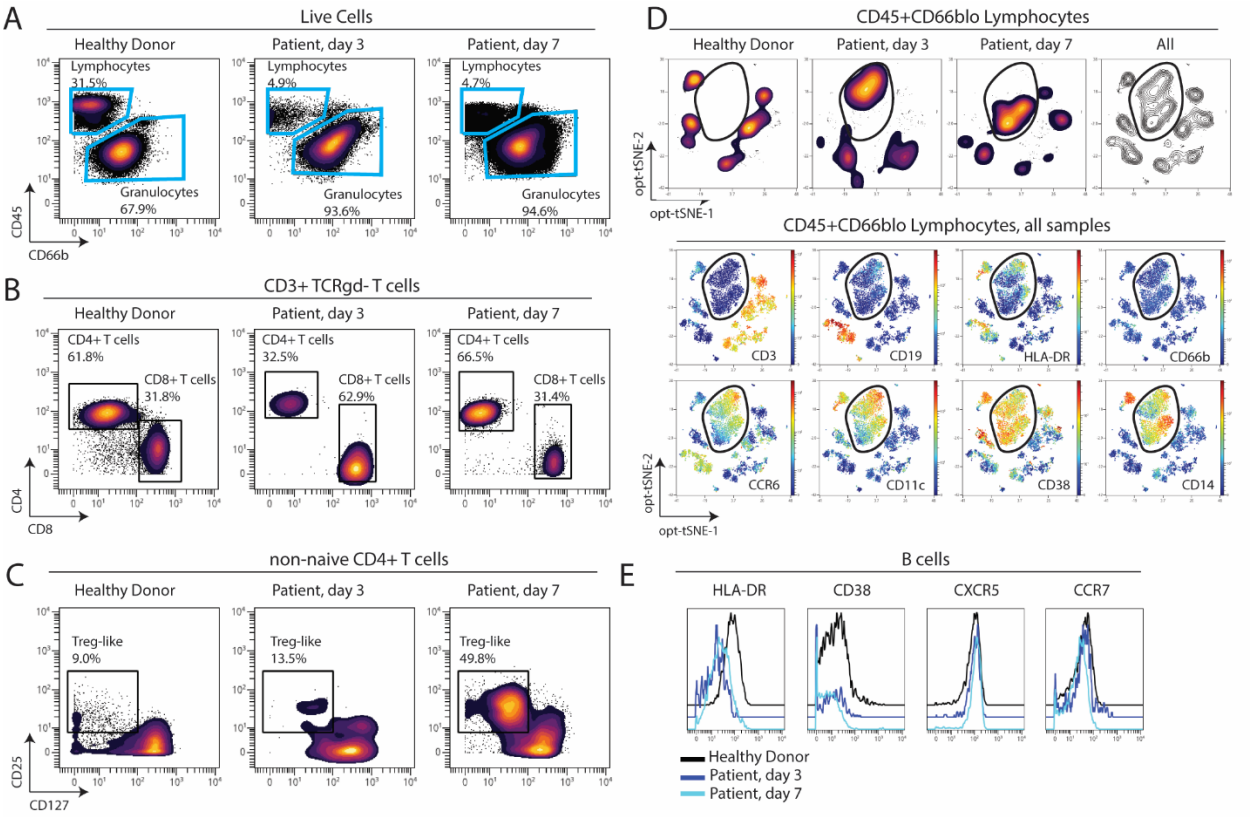
Complement factors	Hospital Day 2	Hospital Day 13 (after plasma exchange)	Hospital Day 16 (after plasma exchange)	Hospital Day 27 (after plasma exchange)
C3 Ref: 88 - 201 mg/dL	106	27 (L)	47 (L)	-
C4 Ref: 16 - 47 mg/dL	25	6 (L)	12 (L)	-
Alternative Pathway (AH50) Ref: 77 - 159 units/mL	-	-	-	87
Classical Pathway Ref: 176 - 362 units/mL	-	-	-	234
C1 Esterase Inhibitor Function Ref: 74-174 % of normal	-	-	-	181 (H)
C1 Esterase Inhibitor Level Ref: 20-37 mg/dL	-	-	-	39 (H)
C1 Function Ref: 116373-264072 units/mL	-	-	-	168,346
C1R level Ref: 61-162 % of STD	-	-	-	90
C1S level Ref: 59-297 % of STD	-	-	-	123
C1Q level Ref: 83-125 mcg/mL	-	-	-	90
C2 level Ref: 22.2-39.8 mcg/mL	-	-	-	28.5
C5 level Ref: 55-113 mcg/mL	-	-	-	44 (L)
C6 level Ref: 28-69 mcg/mL	-	-	-	38
C7 level Ref: 35-96 mcg/mL	-	-	-	56
C8 level Ref: 49-106 mcg/mL	-	-	-	43 (L)
C9 level Ref: 33-95 mcg/mL	-	-	-	83
SC5b-9 (sMAC) level Ref: 72-244 ng/dL	-	-	-	264.4 (H)
C1Q Autoantibody test Ref: 0.0-7.0 % of STD	-	-	-	0.4

Supplemental Figures

eFigure 1. Suppurative folliculitis rash on face, chest, and back

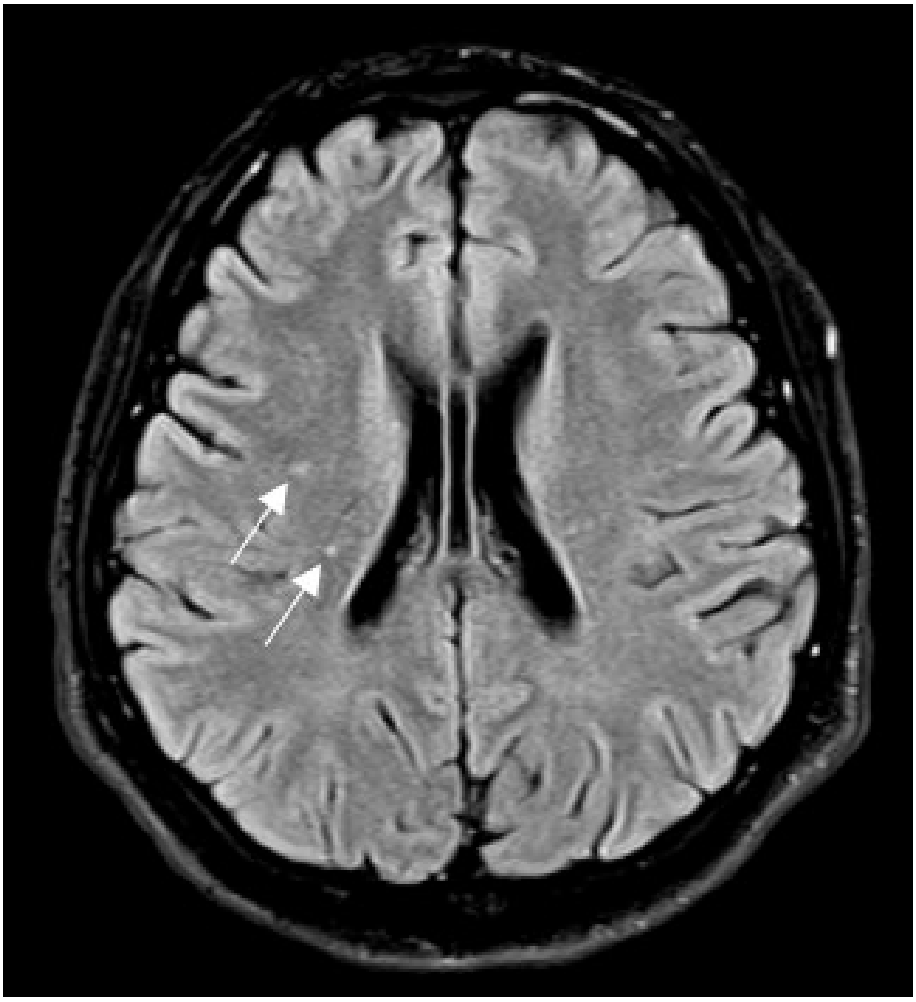


eFigure 2. Immune cell profiling



Immune cell profiling by cytometry by time of flight (CyTOF) demonstrated a significantly expanded granulocytic population with concomitant relative lymphopenia more severe for CD4+ cells, with an inverted CD4:CD8 ratio (**Figures 1A, 1B, 1D**). A follow-up sample obtained on hospital day 7 revealed sustained lymphopenia but correction of the CD4:CD8 ratio led by expansion of a regulatory T cell-like CD4+ CD25+ CD127– cell population (**Figures 1A, 1B, 1C).** CyTOF analysis at both time points was notable for a high frequency of immature neutrophils (CD45+ CD66b^{lo} CD11c+ CD14+), most likely reflecting demarginating granulocytes in the setting of high-dose glucocorticoids, and low B-cell expression of CD38 and HLA-DR (**Figures 1D, 1E).**

eFigure 3. Brain MRI with multifocal ischemic strokes



Supplemental References

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