ONLINE SUPPLEMENTARY MATERIAL

Carter M, Fish M, Jennings A et al. Immunophenotyping of Circulating Leukocytes in Multi-system Inflammatory Syndrome of Childhood Temporally Associated with SARS-CoV-2 Infection: Descriptive cohort study.

eMETHODS

1. Enzyme Linked Immunosorbent Assays for antibody to SARS-CoV-2
2. SARS-CoV-2 pseudotyped virus neutralization assay.
3. Flow cytometry
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eTable-2: Description of healthy control children. Healthy control children were recruited from elective surgical lists for minor procedures.

eTable-3: Modified pediatric SOFA Score. Adapted from Matics et al.[1] and Shime et al.[2] Abbreviations: FiO_2, fraction of inspired oxygen; MAP, mean arterial pressure; pSOFA, pediatric Sequential (Sepsis-related) Organ Failure Assessment; SpO_2, peripheral oxygen saturation. * PaO_2 measured in mmHg. SI conversion factors: To convert bilirubin to µmol/L multiply by 17.104; to convert creatinine to µmol/L multiply by 88.4; and platelet count to $\times 10^9$/L, multiply by 1.

eTable-4: Antibody panels for flow cytometry analysis.

eTable-5: Characteristics of the study cohort by serological status to SARS-CoV-2. *2 children with asthma (1 with eczema and 1 with autistic spectrum disorder), 1 child with food allergy, 1 child with hemoglobin C trait, and 1 child with aplastic anemia and immunosuppression (ciclosporin). **Variables adjusted by age group (see eTable-3[1]). ***1 patient treated with anakinra, 4 patients with infliximab and 10 patients with tocilizumab. **** Montreal Z-score[3] >= 2 on echocardiography or computed tomography. Primary end-point pneumonia was defined as per Cherian T et al[4]. Tachycardia was determined using thresholds developed for the UK National Institute of Health and Clinical Excellent *Guidelines for the Recognition and Management of Sepsis*.

eTable-6: Description of previously published cohorts. Published studies with ≥ 5 patients and unselected for presenting features, 30th June 2020.

eFIGURES

eFigure-1: Flow diagram of recruitment to the study cohort.

eFigure-2: Whole blood sampling time points relative to fever onset (A) and presentation to hospital (B) for flow cytometry in the study cohort. Red bars refer to T1 (acute phase), orange bars refer to T2 (resolution phase) and green bars refer to T3 (convalescent phase). Samples for cytokine analysis were all taken prior to the administration of intravenous immunoglobulin.

eFigure-3: Pseudoviral neutralization assay by serology to SARS-CoV-2. This was undertaken on 24/25 children with MIS-C and who had serum samples available for analysis. The samples with some neutralization activity but seronegative status is most likely secondary to presence of IgM/IgA.

eFigure-4: Clinical biomarkers in patients with MIS-C. Serum ferritin concentrations (A) decreased from acute and resolution phases of illness (T1 and T2) into convalescence (normal range shown as dashed lines, 22–275 µg/L). Serum troponin T concentrations (B) were raised in the majority of children in the acute phase of illness and normalized by convalescence (upper threshold for acute myocardial infarction in adults shown as dashed line, 30 ng/L). Blood platelet count (C) increased significantly across time points from a relative thrombocytopenia in the acute phase to thrombocytosis by convalescence (normal range shown as dashed lines, 150–450 x 10^9/L). Serum D-dimer concentrations (D) were elevated in the acute and resolution phases and returned towards normal (but still raised) in convalescence (upper threshold of normal shown as dashed line, 0.55 mg/L FEU).

e-Figure 5: Additional information on Innate immune cell alterations on T1, T2 and T3.
Innate alterations persist in PIMS-TS. Monocyte CD14 (TLR4 coreceptor) expression was significantly reduced compared to controls (A). Monocyte CD64 expression was increased at T1 (B). Classical monocyte proportions remained unchanged (C). Classical monocyte had significantly reduced CD86 expression (D). For boxplots the lowest whisker indicates the 1.5 times the interquartile range from the hinge; bottom border of the box, 25th percentile; line bisecting the shaded region of each box, median; top border of the box, 75th percentile; and highest whisker, 1.5 times the interquartile range from the hinge. Statistical analysis for boxplots was performed by Wilcoxon signed rank test. For correlations Spearman's rank was performed. Dots are coloured by serology to SARS-CoV-2 result; positive (red), negative (blue), hospital controls with no serology measure (black).

eFigure-6: Additional information on T cell alterations on T1, T2 and T3. Specific subsets of T cells are preferentially activated. The CD8+ T cell compartment was not activated. HLA-DR expression was measured on total CD8+ T cells (A), CD8+ CCR7- T cells (B), and CD8+ CCR7+ T cells (C). Proportion of CCR7+ subsets in CD8+ T cells (D) was raised in the acute phase. HLA-DR proportion of CD8+ CCR7+ T cells was approximately stable across cohorts / phases of disease (E). HLA-DR expression was measured on CD4+ CCR7- T cells (F) and did not change across cohorts / phases of disease. The proportion of CCR7+ CD4+ T cells (G), and the proportion of HLA-DR+ regulatory T cells (H) did not change across cohorts / phases of disease. For boxplots the lowest whisker indicates the 1.5 times the interquartile range from the hinge; bottom border of the box, 25th percentile; line bisecting the shaded region of each box, median; top border of the box, 75th percentile; and highest whisker, 1.5 times the interquartile range from the hinge. Statistical analysis for boxplots was performed by Wilcoxon signed rank test. For correlations Spearman's rank was performed. Dots are colored by serology to SARS-CoV-2 result; positive (red), negative (blue), hospital controls with no serology measure (black).

eFigure-7: Epidemiology of SARS-Cov-2 infection in England prior to, and during the study period. Cumulative (laboratory-confirmed) cases of infection with SARS-CoV-2 in regions of England during the study period, and in the period from 23rd March where severe UK-wide movement restrictions ("lockdown") were in place. Evelina London Children's Hospital is in central London and also provides tertiary immunology, infectious diseases, cardiology and intensive care to children in south-east England in collaboration with other tertiary centers (referred to "approximate catchment"). Data from the UK Government (https://coronavirus.data.gov.uk/) accessed on 22nd June 2020. "Index case" denotes the first case of PIMS-TS identified at Evelina London Children's Hospital, admitted at 14th April 2020[5].

eFigure-8: Innate immune cell panel and gating approach[6].
Gating strategy used to examine absolute innate cell counts in Cytodelics-preserved human whole blood by flow cytometry. A time gate was used to check data acquisition. A singlet gate was used to remove doublets. The green laser (FITC) was used to check the Precision Count beads (Biolegend). Cells were re-gated based on size (FSC vs SSC) to identify immune cells. CD14 expression was used to differentiate total monocytes (CD14+) from other immune cells (CD14-). Total monocytes were

further gated against CD16 to identify classical monocytes (CD14+/CD16-) and non-classical monocytes (CD14+/CD16+). From the CD14- cells, CD56 was plotted against HLA-DR to identify total dendritic cells (DCs) (HLA-DR+/CD56-) and natural killer (NK) cells (HLADR-/CD56+). Total DCs were further gated to identify plasmacytoid dendritic cells (pDCs) (HLA-DR+/CD123+) and myeloid dendritic cells (mDCs) (HLA-DR+/ CD11c+). From the CD14- cells, cells were gated on CD15+ cells. CD15+ cells were further gated to identify CD16+ total neutrophils (CD14+/CD15+/CD16+). The MFI of CD64, CD10 and HLA-DR was used to assess neutrophil activation. The MFI of HLA-DR and CD86 was used to assess monocyte and DC activation.

eFigure-9: T cell panel and gating approach[6]

Gating strategy used to examine absolute T cell counts in Cytodelics-preserved human whole blood by flow cytometry. A time gate was used to check data acquisition. A singlet gate was used to remove doublets. The green laser (FITC) was used to check the Precision Count beads (Biolegend). Cells were re-gated based on size (FSC vs SSC) to identify lymphocytes. CD3 was used to identify T cells (CD3+). CD3 was plotted against the gamma delta TCR to distinguish gamma delta T cells from alpha beta T cells. Alpha beta T cells were further gated on CD4 and CD8 to identify CD4+ T cells (CD3+/CD4+) and CD8+ T cells (CD3+/CD8+). CD4+ T cells were further gated on CD25 and CD127 to identify total regulatory T cells (Tregs) (CD4+/CD25+/CD127-). CD4+ T cells were gated on CCR7 to identify CD4+/CCR7+ and CD4+/CCR7- T cells. The MFI of HLA-DR was used to assess activation of gamma delta T cells, total Tregs, CD4+/CCR7+ and CD4+/CCR7- cells.

eFigure-10: B cell panel and gating approach[6]

Gating strategy used to examine absolute B cell counts in Cytodelics-preserved human whole blood by flow cytometry. A time gate was used to check data acquisition. A singlet gate was used to remove doublets. The green laser (FITC) was used to check the Precision Count beads (Biolegend). CD19 was used to differentiate CD19+ total B cells from other immune cells. From total B cells, CD5 was used to identify CD5+ B cells (CD19+/CD5+). From total B cells CD24 was plotted against CD38 to identify transitional B cells (CD19+/CD24+/CD38+). From the non-transitional cells, CD27 was plotted against CD38 to identify plasmablasts (CD19+/CD27+/CD38+). From the non-plasmablasts, CD27 was plotted against IgM to identify naïve B cells (CD19+/IgM+/CD27-) and class-switched memory B cells (CD19+/IgM-/CD27+). From the IgM+/CD27+ cells, expression of IgD (MFI) was used to identify natural effector B cells (CD19+/IgM+/CD27+/IgD+). The MFI of HLA-DR was used to assess activation of total B cells, CD5+ B cells, naïve and natural effector B cells.

eMethods

1. Enzyme Linked Immunosorbent Assays for antibody to SARS-CoV-2

Protein expression

N protein was obtained from the Leo James and Jakub Luptak at LMB, Cambridge. The N protein used is a truncated construct of the SARS-CoV-2 N protein comprising residues 48-365 (both ordered domains with the native linker) with an N terminal uncleavable hexahistidine tag. N was expressed in *E. Coli* using autoinducing media for 7h at 37°C and purified using immobilised metal affinity chromatography (IMAC), size exclusion and heparin chromatography.

S protein consists of a pre-fusion S ectodomain residues 1-1138 with proline substitutions at amino acid positions 986 and 987, a GGGG substitution at the furin cleavage site (amino acids 682-685) and an N terminal T4 trimerisation domain followed by a Strep-tag II [18]. The plasmid was obtained from Philip Brouwer, Marit van Gils and Rogier Sanders at The University of Amsterdam. The protein was expressed in 1 L HEK-293F cells (Invitrogen) grown in suspension at a density of 1.5 million cells/mL. The culture was transfected with 325 µg of DNA using PEI-Max (1 mg/mL, Polysciences) at a 1:3 ratio. Supernatant was harvested after 7 days and purified using StrepTactinXT Superflow high capacity 50% suspension according to the manufacturer's protocol by gravity flow (IBA Life Sciences).

The RBD plasmid was obtained from Florian Krammer at Mount Sinai University [19]. Here the natural N-terminal signal peptide of S is fused to the RBD sequence (319 to 541) and joined to a C-terminal hexahistidine tag. This protein was expressed in 500 mL HEK-293F cells (Invitrogen) at a density of 1.5 million cells/mL. The culture was transfected with 1000 µg of DNA using PEI-Max (1 mg/mL, Polysciences) at a 1:3 ratio. Supernatant was harvested after 7 days and purified using Ni-NTA agarose beads.

Serology Analysis

All sera were heat-inactivated at 56°C for 30 mins before use in the in-house ELISA. High-binding ELISA plates (Corning, 3690) were coated with antigen (N, S or RBD) at 3 µg/mL (25 µL per well) in PBS, either overnight at 4°C or 2 hr at 37°C. Wells were washed with PBS-T (PBS with 0.05% Tween-20) and then blocked with 100 µL 5% milk in PBS-T for 1 hr at room temperature. Wells were emptied and sera and plasma diluted at 1:50 and 1:25 respectively in milk were added and incubated for 2 hours at room temperature. Control reagents included CR3009 (2 µg/mL), CR3022 (0.2 µg/mL), negative control plasma (1:25 dilution), positive control plasma (1:50) and blank wells. Wells were washed with PBS-T. Secondary antibody was added and incubated for 1 hr at room temperature. IgM was detected using Goat-anti-human-IgM-HRP (1:1,000) (Sigma: A6907) and IgG was detected using Goat-anti-human-Fc-AP (1:1,000) (Jackson: 109-055-043-JIR). Wells were washed with PBS-T and either AP substrate (Sigma) was added and read at 405 nm (AP) or 1-step TMB substrate (Thermo Scientific) was added and quenched with 0.5 M H_2S0_4 before reading at 450 nm (HRP).

2. SARS-CoV-2 pseudotyped virus neutralization assay.

Virus preparation

Pseudotyped HIV virus incorporating the SARS-Cov2 spike protein was expressed in a 10 cm dish seeded the day prior with 3.5 x 10^6 HEK293T/17 cells in 10 ml of complete Dulbecco's Modified Eagle's Medium (DMEM-C) containing 10% (vol/vol) foetal bovine serum (FBS), 100 IU/ml penicillin and 100 µg/ml streptomycin. Cells were transfected using 35 µg of PEI-Max (1 mg/mL, Polysciences) with: 1500 ng of HIV-luciferase plasmid, 1000 ng of HIV 8.91 gag/pol plasmid and 900 ng of SARS-2 spike protein plasmid (provided by Nigel Temperton).[7] The media was changed 18 hours post-transfection and supernatant was harvested 48 hours post-transfection. Virus particle was filtered through a 0.45µm filter and stored at -80°C.

Neutralization assays
Serial dilutions of serum samples (heat inactivated at 56°C for 30mins) were prepared with DMEM media and incubated with pseudovirus for 1-hour at 37°C in 96-well plates. Next, Hela cells stably expressing the ACE2 receptor (provided by Dr James Voss, The Scripps Research Institute) were added and the plates were left for 72 hours. Infection level was assessed in lysed cells with the Bright-Glo luciferase kit (Promega), using a Victor™ X3 multilabel reader (Perkin Elmer). As seropositivity is based on IgG to N or S.

3. Flow cytometry
Whole blood stabilization
Whole blood was collected on the Paediatric intensive care unit. 0.5ml was immediately mixed with 0.5ml Cytodelics Stabilizer buffer (1:1 ratio), incubated at room temperature for 10 minutes and stored at -80ºC.

Flow Cytometry and Acquisition
Whole blood sample staining was done on using Cytodelics processing kit (Cytodelics AB, Stockholm, Sweden). Frozen blood samples were thawed for 1 minute at 37ºC water bath by gently swirling. 200µl of blood was added for each panel (Innate, T cell, B cell) to a 96-well V-bottom plate and centrifuged at 2000rpm for 2mins (Centrifugation settings remain the same throughout). Cells were resuspended in 100µl of respective antibody cocktail and incubated at room temperature for 20 mins. All flow cytometry antibodies and concentrations used for whole blood surface staining can be found in Supplementary Table-4. 100ul of PBS (Gibco) was added to each well and the plate was centrifuged. Supernatant was removed and 200µl of fixative buffer (Cytodelics)(1:1 dilution of Fix Concentrate in Fix Diluent) was added. The plate was incubated at room temperature for 15mins, centrifuged and supernatant removed. Cells were resuspended in 200µl of lysis buffer (Cytodelics)(1:4 dilution of lysis buffer in distilled H_20) and incubated at room temperature for 15 mins. The plate was centrifuged, and supernatant removed. Cells were resuspended in 200µl wash buffer (Cytodelics)(1:5 dilution of wash buffer concentrate in distilled H_20) and centrifuged. The supernatant was removed, and cells were resuspended in 175 µl of sterile PBS. Prior to acquisition, 25µl of Precision Count Beads™ (Biolegend) were added to each sample. All samples were analyzed on a five laser BD Fortessa flow cytometer equipped with a BD High Throughput Sampler (HTS) with a flow rate of 1µl/second. Flow cytometer was set up using application settings by using cytometer set up and tracking beads (Becton Dickson).

Flow Cytometry Data Analysis
FCS files acquired using BD FACSDiva™ and analysed using FlowJo (10.6.2, Treestar). Gating strategies for all panels are outlined in eFigure2-4. Event counts and median fluorescent intensities were calculated using FlowJo for relevant markers on specific populations. Absolute cell counts using Biolegend Precision Count Beads™ were calculated using the following equation:

$$\boldsymbol{Absolutecellcount}(\boldsymbol{cells}/\mu l) = \frac{(\text{Cellcount} \times \text{Precision Count } BeadsTM\ \ volume)}{(\text{Precision Count } BeadsTM\ count \times whole\ blood\ volume)} \times Bead\ concentration$$

4. Cytokine assays from acute serum

Acute serum samples (pre-intravenous immunoglobulin administration) from 15/25 children recruited had cytokines measured. The following cytokines were measured: interleukin (IL) -2 receptor agonist, interferon-gamma, IL-10, IL-17, IL-1beta, IL-6, IL-8, tumor necrosis factor (TNF) alpha. Serum was initially diluted 1 : 2 in distilled water. We used the Ella 600-100 (R&D Systems) automated ELISA platform within ViaPath laboratories at King's College Hospital, London, UK. This performs a sandwich ELISA using a microfluidics Simple Plex cartridge. This immunoassay works by routing the sample through a microfluidic channel that binds the protein of interest. The unbound analyte is removed by washing, before a detection antibody is added. Because each channel has three Glass Nano Reactors (GNRs) coated with a capture antibody, a triplicate set of results are produced for each sample. Results were then generated using the manufacturer-calibrated standard curve.

eTABLES

eTable-1: Comparison of definitions for the syndrome of hyperinflammation in children temporally associated with SARS-CoV-2.

Paediatric Inflammatory Multisite Syndrome Temporarily Associated with SARS-Cov-2 (PIMS-TS; RCPCH 2020)
A child presenting with persistent fever, inflammation (neutrophilia, elevated CRP and lymphopenia) and evidence of single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder). This may include children meeting full or partial criteria for Kawasaki disease. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus (waiting for results of these investigations should not delay seeking expert advice). SARS-CoV-2 PCR testing may be positive or negative
Multisystem Inflammatory Syndrome in Children (MIS-C; CDC 2020)
An individual aged <21 years presenting with fever[i], laboratory evidence of inflammation[ii], and evidence of clinically severe illness requiring hospitalization, with multisystem (>2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND No alternative plausible diagnoses; AND Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms i. Fever >38.0°C for ≥24 hours, or report of subjective fever lasting ≥24 hours ii. Including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin Additional comments Some individuals may fulfil full or partial criteria for Kawasaki disease but should be reported if they meet the case definition for MIS-C Consider MIS-C in any pediatric death with evidence of SARS-CoV-2 infection
Multisystem inflammatory syndrome in children and adolescents temporally related to COVID-19 (WHO 2020)
Children and adolescents 0–19 years of age with fever > 3 days AND two of the following: • Rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs (oral, hands or feet). • Hypotension or shock. • Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including ECHO findings or elevated Troponin/NT-proBNP), • Evidence of coagulopathy (by PT, PTT, elevated d-Dimers). • Acute gastrointestinal problems (diarrhea, vomiting, or abdominal pain). AND Elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin. AND No other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes. AND Evidence of COVID-19 (RT-PCR, antigen test or serology positive), or likely contact with patients with COVID-19.

eTable-2: Description of healthy control children.

Characteristic	N = 7 children
Age (Years, Median; IQR; Range)	11.7 (5.3–13.9; 4.9–14.9)
Girls (N; %)	2 (29%)
Self-reported ethnicity	
Asian (N; %)	0
Black (N; %)	3 (43%)
White (N; %)	4 (57%)
Other (N; %)	0

eTable-3: Modified paediatric SOFA Score.

Variables	Score				
	0	**1**	**2**	**3**	**4**
Respiratory					
PaO2 : FiO2 or	≥400	300-399	200-299	100-199 With respiratory support	<100 With respiratory support
SpO2 : FiO2	≥292	264-291	221-264	148-220 With respiratory support	<148 With respiratory support respiratory support
Coagulation					
Platelet count ($\times 10^3$ /μL)	≥150	100-149	50-99	20-49	<20
Hepatic					
Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12
Cardiovascular					
MAP by age group or vasoactive infusion (mm Hg or μg/kg/min)					
Inotropes	-	Milrinone (any)	Dopamine ≤5 or dobutamine (any)	Dopamine ≥5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine ≥15 or epinephrine >0.1 or norepinephrine >0.1
<1 month	≥46	<46			
1-11 months	≥55	<55			
12-23 months	≥60	<60			
24-59 months	≥62	<62			
60-143 months	≥65	<65			
144-216 months	≥67	<67			
>216 months	≥70	<70			
Neurologic					
Glasgow Coma Score	15	13-14	10-12	6-9	<6
Renal					
Creatinine by age group (mg/dL)					
<1 month	<0.8	0.8-0.9	1.0-1.1	1.2-1.5	≥1.6
1-11 months	<0.3	0.3-0.4	0.5-0.7	0.8-1.1	≥1.2
12-23 months	<0.4	0.4-0.5	0.6-1.0	1.1-1.4	≥1.5
24-59 months	<0.6	0.6-0.8	0.9-1.5	1.6-2.2	≥2.3
60-143 months	<0.7	0.7-1.0	1.1-1.7	1.8-2.5	≥2.6
144-216 months	<1.0	1.0-1.6	1.7-2.8	2.9-4.1	≥4.2
>216 months	<1.2	1.2-1.9	2.0-3.4	3.5-4.9	≥5

eTable-4: Antibody panels for flow cytometry analysis

Panel	Target	Fluorophore	Clone	Manufacturer	Cat. number	Volume/ 100ul
Innate	CD15	BV786	HI98	BD	563838	1.00
Innate	CD14	BV421	M5E2	BD	565283	1.00
Innate	CD56	BB515	B159	BD	564489	2.00
Innate	CD86	BUV737	2331	BD	612784	1.00
Innate	CD68	PE-CF594	Y1/82A	BD	564944	1.00
Innate	HLA-DR	BV510	G46-6	BD	563083	1.00
Innate	CD11c	BV650	B-ly6	BD	563404	1.00
Innate	CD123	PerCP-Cy5.5	7G3	BD	560904	1.00
Innate	CD16	Pe-Cy7	3G8	BD	560918	1.00
Innate	CD64	AF700	10.1	BD	561188	2.00
Innate	CD161	PE		BD	556081	1.00
Innate	CD10	BUV395	HI10a	BD	563871	3.00
Innate	CD19	APC-Cy7		BD	557791	1.00
Innate	CD3	APC-Cy7	SK7	BD	560176	1.00
T cell	CD3	BUV395	SK7	BD	564001	2.00
T cell	CD4	BV786	SK3	BD	563877	2.00
T cell	CD8	BV605	SK1	BD	564116	2.00
T cell	$\gamma\delta$ TCR	PE-Cy7	11F2	BD	655410	5.00
T cell	CCR7 (CD197)	BV421	2-L1-A	BD	566743	2.00
T cell	CCR4 (CD194)	PE-CF594	1G1	BD	565391	2.00
T cell	CCR6 (CD196)	BB515	11Ag	BD	564479	2.00
T cell	CD45RO	PE		BD	555493	5.00
T cell	CD45RA	APC		BD	550855	5.00
T cell	CXCR3 (CD183)	BB700	1C6	BD	566532	2.00
T cell	CD25	BV510	M-A251	BD	563352	2.00
T cell	CD25	BV510	2A3	BD	740198	2.00
T cell	HLA-DR	APC-R700	G46-6	BD	565127	2.00
T cell	CD127	BUV737	HIL-7R-M21	BD	612794	2.00
B cell	CD19	BV711	SJ25C1	BD	563038	2.00
B cell	CD27	BV786	L128	BD	563327	2.00
B cell	CD43	BV421	1G10	BD	562916	2.00
B cell	CD24	BUV395	ML5	BD	563818	2.00
B cell	IgG	APC	G18-145	BD	550931	5.00
B cell	IgD	BUV737	IA6-2	BD	612798	2.00
B cell	IgM	BB515	G20-127	BD	564622	2.00
B cell	CD38	PE	HIT-2	BD	555460	10.00
B cell	CD5	PE-Cy7	L17F12	BD	348810	2.00
B cell	CD25	APC-R700	2A3	BD	565106	2.00
B cell	HLA-DR	BV510	G46-6	BD	563083	2.00

eTable-5: Demographic and clinical characteristics of the MIS-C cohort by serology to SARS-Cov-2.

Characteristics	N = 25 patients	SARS-Cov2 serology	
		Negative (N = 8)	Positive (N = 17)
Age (Years, Median; IQR; Range)	12.5 (7.7–14.4; 1.1–16.8)	10.1 (3.9–12.9; 1.1–15.5)	13.0 (11.5–15.6; 3.5–16.8)
Female (N; %)	10 (40%)	6 (75%)	4 (24%)
Self-reported ethnicity			
Asian (N; %)	5 (20%)	1 (13%)	4 (24%)
Black (N; %)	9 (36%)	1 (13%)	8 (47%)
White (N; %)	10 (40%)	5 (63%)	5 (29%)
Other (N; %)	1 (4%)	1 (13%)	0
Comorbidity (N; %)*	5 (20%)	3 (38%)	2 (12%)
Clinical features at presentation			
Duration of fever prior to hospitalisation (Median; IQR; Range)	5 (4–5; 0–9)	5 (3.3–5.3; 0–7)	5 (4–5, 2–9)
Patients meeting classical Kawasaki disease criteria (N; %)	3 (12%)	2 (25%)	1 (6%)
Oral mucocutaneous involvement (N; %)	6 (24%)	4 (50%)	2 (12%)
Bilateral non-purulent conjunctivitis (N; %)	10 (40%)	3 (38%)	7 (41%)
Polymorphous non-blanching rash (N; %)	12 (48%)	6 (75%)	6 (35%)
Erythema or oedema of hands or feet (N; %)	5 (20%)	3 (38%)	2 (12%)
Periungual desquamation (N; %)	1 (4%)	0	0
Cervical lymphadenopathy (N; %)	4 (16%)	1 (13%)	3 (18%)
Respiratory distress (N; %)	4 (16%)	1 (13%)	3 (18%)
Tachycardia (N; %)	6 (24%)	2 (25%)	4 (24%)
Gastrointestinal symptoms (N; %)	18 (72%)	4 (50%)	14 (82%)
SOFA score at presentation (Median; IQR; Range)**	2 (1–3, 0–6)	1 (1–2.3; 1–6)	2 (2–3; 0–6)
Worst SOFA score (Median; IQR, Range)**	2 (1–5, 1–8)	1 (1–2.3, 1–8)	3 (2–5, 1–6)
Additional findings during admission			
Primary endpoint pneumonia (N; %)	7 (28%)	1 (13%)	6 (35%)
Worst coronary artery Z-score (Median; IQR; Range)	1.6 (0.7–2.2; -2.0–4.6)	1.3 (0.9–1.5; -2.0–1.8)	1.7 (0.6–3.0; -0.8–4.6)

Worst left ventricular fractional shortening (Median; IQR; Range)	27 (23–36; 15–47)	39 (36–41; 33–47)	25 (21–27; 15–44)
Treatments during admission			
PICU admission (N; %)	19 (82%)	6 (75%)	15 (88%)
Mechanical ventilation (N; %)	2 (8%)	1 (13%)	1 (6%)
Vasoactive infusion (N; %)	14 (56%)	2 (25%)	12 (71%)
High dose corticosteroids (N; %)	21 (84%)	5 (63%)	16 (94%)
Intravenous immunoglobulin (N; %)	23 (92%)	7 (88%)	16 (94%)
Biologic immunomodulation (N; %)***	15 (60%)	2 (25%)	13 (76%)
Outcome			
PICU length of stay (Days; Median; IQR; Range)	3 (1–5; 0–19)	1.5 (0.8–2.8; 0–19)	4 (2.0–5.0; 0–10)
Hospital length of stay (Days; Median; IQR; Range)	8 (6–10, 2–25)	6 (5–7; 2–25)	8 (8–11; 5–14)
Presence of coronary artery aneurysms (N; %)	7 (28%)	0	7 (41%)
Myocardial infarction (N; %)	1 (4%)	0	1 (6%)
Pulmonary embolus or other significant thrombus (N; %)	2 (7%)	1 (13%)	1 (6%)
Significant acute mental health diagnosis (N; %)	1 (4%)	1 (13%)	0
SARS-Cov-2 PCR positive (N; %)	1 (4%)	0	1 (6%)
SARS-Cov-2 IgG antibody positive (N; %)	17 (68%)	–	–

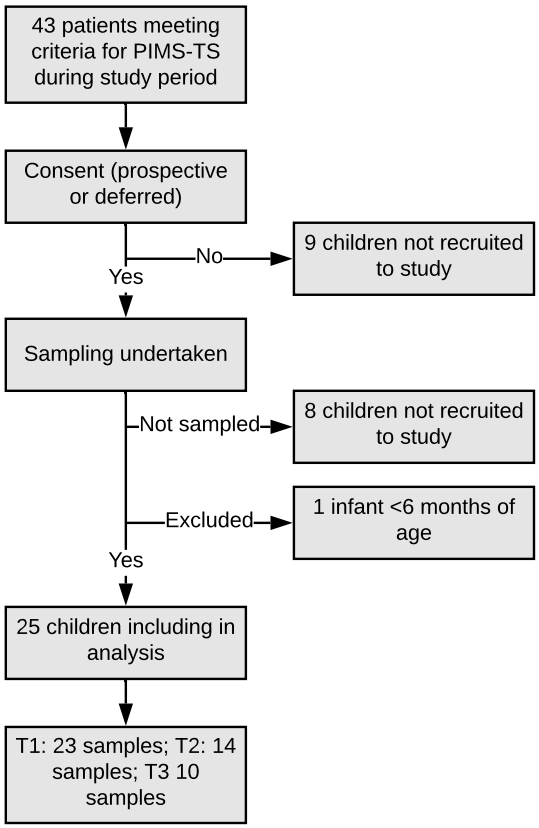
Reference	Riphagen et al.[5]	Verdoni et al.[8]	Belhadjer et al.[9]	Toubiana et al.[10]	Whittaker et al.[11]	Kaushik et al.[12]	Chiotis et al.	Capone et al.[13]	Dufort et al.[14]	Feldstein et al.[15]
Number of patients	8	10	35	21	58	33	6	33	99	186
Setting	PICU	Hospital	PICUs	University Hospital	University Hospitals	University Hospitals	University Hospital	University Hospital	General hospitals	Health Centers
Location	London, UK	Bergamo, Italy	France and Switzerland	Paris, France	UK	New York City, USA	Philadelphia, USA	New York City, USA	New York State, USA	26 states, USA
Age (Median; IQR)	8 (6–12.3)	7.3 (2.9–8.1)	10	7.9 (3.7–16.6)	9 (5.7–14)	10 (6–13)	7.5 (5.3–11.3)	8.6 (5.5–12.6)	68% aged 6–20 years	74% aged 5–20 years
Female sex	3 (38%)	3 (30%)	17 (49%)	12 (57%)	33 (57%)	13 (39%)	5 (83%)	13 (39%)	46 (46%)	71 (38%)
Ethnicity*										
Asian (N; %)	2 (25%)	–	–	6 (15%)	18 (31%)	1 (3%)	0	3 (9%)	4/78 (5%)	–
Black (N; %)	6 (75%)	–	–	24 (57%)	22 (38%)	13 (39%)	2 (33%)	8 (24%)	31/78 (40%)	46 (25%)
White (N; %)	0	–	–	12 (29%)	12 (21%)	3 (9%)	2 (33%)	3 (9%)	29/78 (37%)	92 (49%)
Other (N; %)	0	–	–	–	6 (10%)	16 (48%)	2 (33%)	19 (57%)	14/78 (18%)	50 (27%)
Clinical features at presentation		***						****		
Duration of prior fever (Median; IQR; Range)	4 (4–5)	–	–	5 (0–12)		4.5 (3–6)	–	4 (3–5)	4 (3–6)	6 (5–8)
Abdominal pain (N; %)	6 (75%)	–	–	–	31 (53%)	21 (63%)	5 (83%)	–	60 (61%)	–
Diarrhea (N;%)	7 (88%)	–	–	–	30 (52%)	16 (48%)	4 (67%)	–	49 (49%))	–
Vomiting (N; %)	3 (38%)	–	–	–	26 (45%)	23 (69%)	–	–	57 (58%)	–
Gastrointestinal symptoms	–	–	29 (83%)	21 (100%)	–	–	–	32 (97%)	79 (80%)	~92%****
Oral mucocutaneous involvement (N; %)	–	–	–	16 (76%)	17 (29%)	7 (21%)	3 (50%)	–	27 (27%)	78 (42%)
Bilateral non-purulent conjunctivitis (N; %)	5 (63%)	–	–	17 (81%)	26 (45%)	12 (36%)	2 (33%)	–	55 (56%)	103 (55%)
Polymorphous non-blanching rash (N; %)	4 (50%)	–	20 (57%)	16 (76%)	30 (52%)	14 (42%)	2 (33%)	–	59 (60%)	110 (59%)
Erythema or oedema of hands or feet (N; %)	–	–	–	10 (48%)	9 (16%)	–	2 (33%)	–	9 (9%)	69 (37%)
Periungual desquamation (N; %)	–	–	–	–	–	–	–	–	–	–
Cervical lymphadenopathy (N; %)	–	–	21 (60%)	12 (57%)	9 (16%)	–	0	–	6 (6%)	18 (10%)
Respiratory symptoms (N; %)	1 (13%)	5 (50%)	23 (65%)	–	12 (21%)	11 (33%)	4 (67%)	17 (52%)	40 (40%)	~44%*****
Treatments during admission										
Mechanical ventilation	5 (63%)	0	22 (62%)	11 (52%)	25 (43%)	5 (15%)	3 (50%)	6 (18%)	10 (10%)	–
Vasoactive infusion	8 (100%)	2 (20%)	28 (80%)	15 (71%)	27 (47%)	17 (51%)	5 (83%)	25 (76%)	61 (62%)	–
High dose corticosteroids	5 (63%)	8 (80%)	12 (34%)	10 (48%)	37 (64%)	17 (51%)	5 (83%)	23 (70%)	63 (64%)	91 (49%)
Intravenous immunoglobulin	8 (100%)	10(100%)	25 (71%)	21 (100%)	41 (71%)	18 (54%)	6 (100%)	33 (100%)	69 (70%)	144 (77%)
Biologic immunomodulation	1 (13%)	0	3 (8%)	0	11 (19%)	16 (48%)	0	8 (24%)	–	38 (20%)
Additional findings and outcomes										
Cardiac dysfunction/myocarditis	8 (100%)	6 (60%)	28 (80%)	16 (76%)	29 (50%)	21 (65%)	4 (67%)	19 (58%)	52 (53%)	150 (80%)
Coronary art. aneurysms (Z-score >2/qualitative)	1 (13%)	2 (20%)	6 (17%)	5 (24%)	8 (14%)	2 (6%)	1 (13%)	5 (15%)	9 (9%)	15 (8%)
Death	1 (13%)	0	0	0	1 (2%)	0	0	0	2 (2%)	4 (2%)
SARS-CoV-2 PCR positive	2 (25%)	2 (20%)	12 (35%)	0	15 (26%)	11 (33%)	3 (50%)	9 (27%)	50/98 (51%)	73 (56%)
SARS-CoV-2 serology positive	–	8 (80%)	30 (86%)	19 (91%)	40/46 (81%)**	27 (81%)	5 (83%)	30 (91%)	76/77 (99%)	58 (44%)

*Varying definitions of ethnicity. **Serology not done in all patients; in total 45 (78%) patients had PCR or serological evidence of SARS-CoV-2 infection. ***5 (50%) patients with classic Kawasaki disease, 5 (50%) with incomplete disease. ****21 (64%) patients with classic Kawasaki disease. ***** Proportions presented graphically.

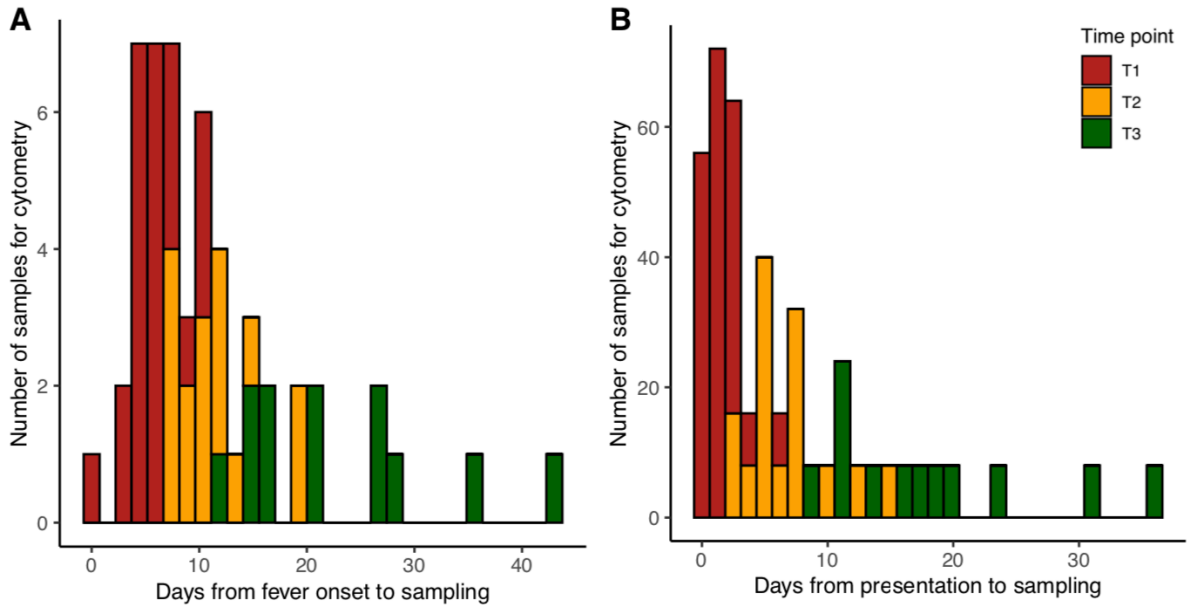
eTable-6: Description of previously published cohorts.

eFIGURES

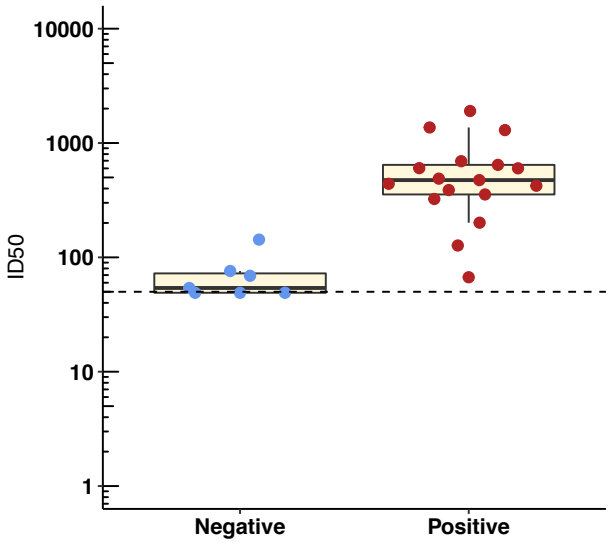
eFigure-1: Flow diagram of recruitment to the study cohort.



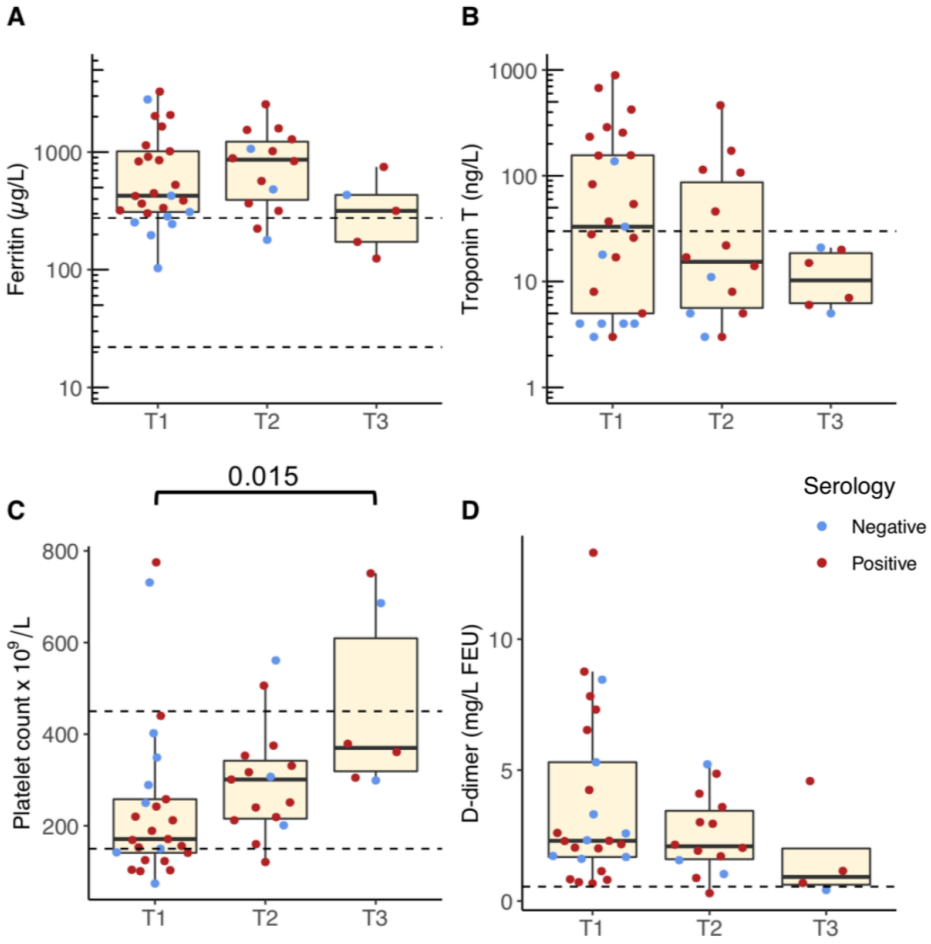
eFigure-2: Sampling time points relative to fever onset (A) and presentation to hospital (B).



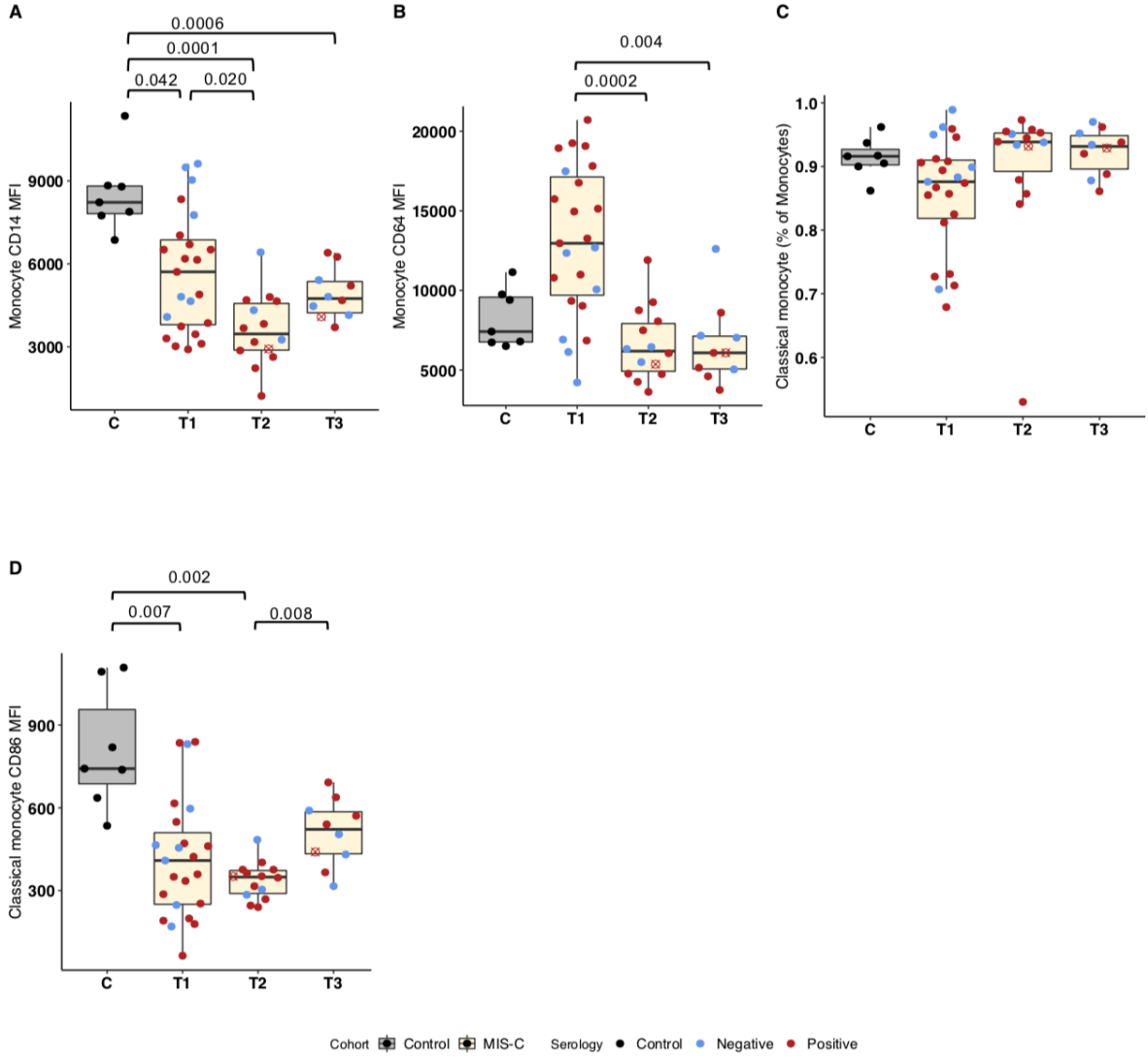
eFigure-3: Pseudoviral neutralization assay results by serology status to SARS-CoV-2.



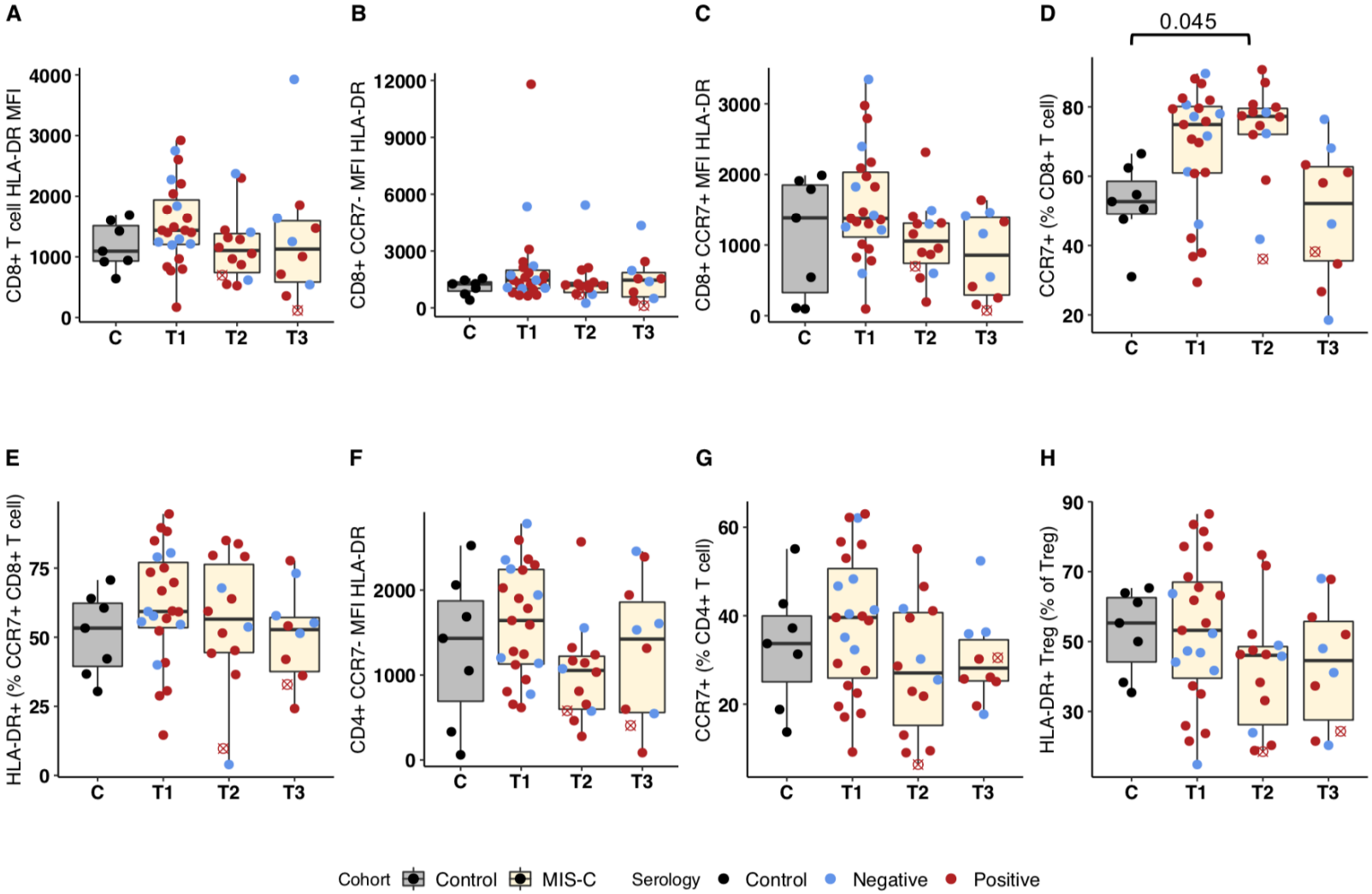
e-Figure 4: Additional clinical biomarkers in patients with MIS-C.



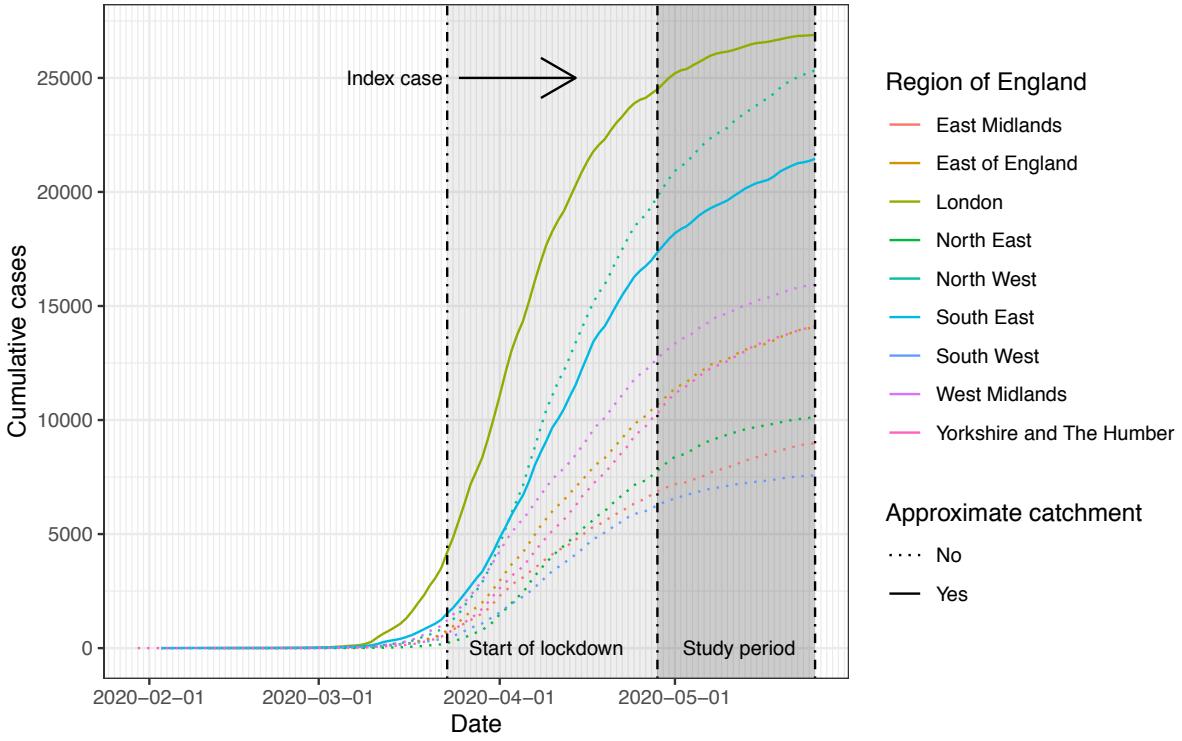
e-Figure 5: Additional information on innate immune cell alterations in T1, T2 and T3.



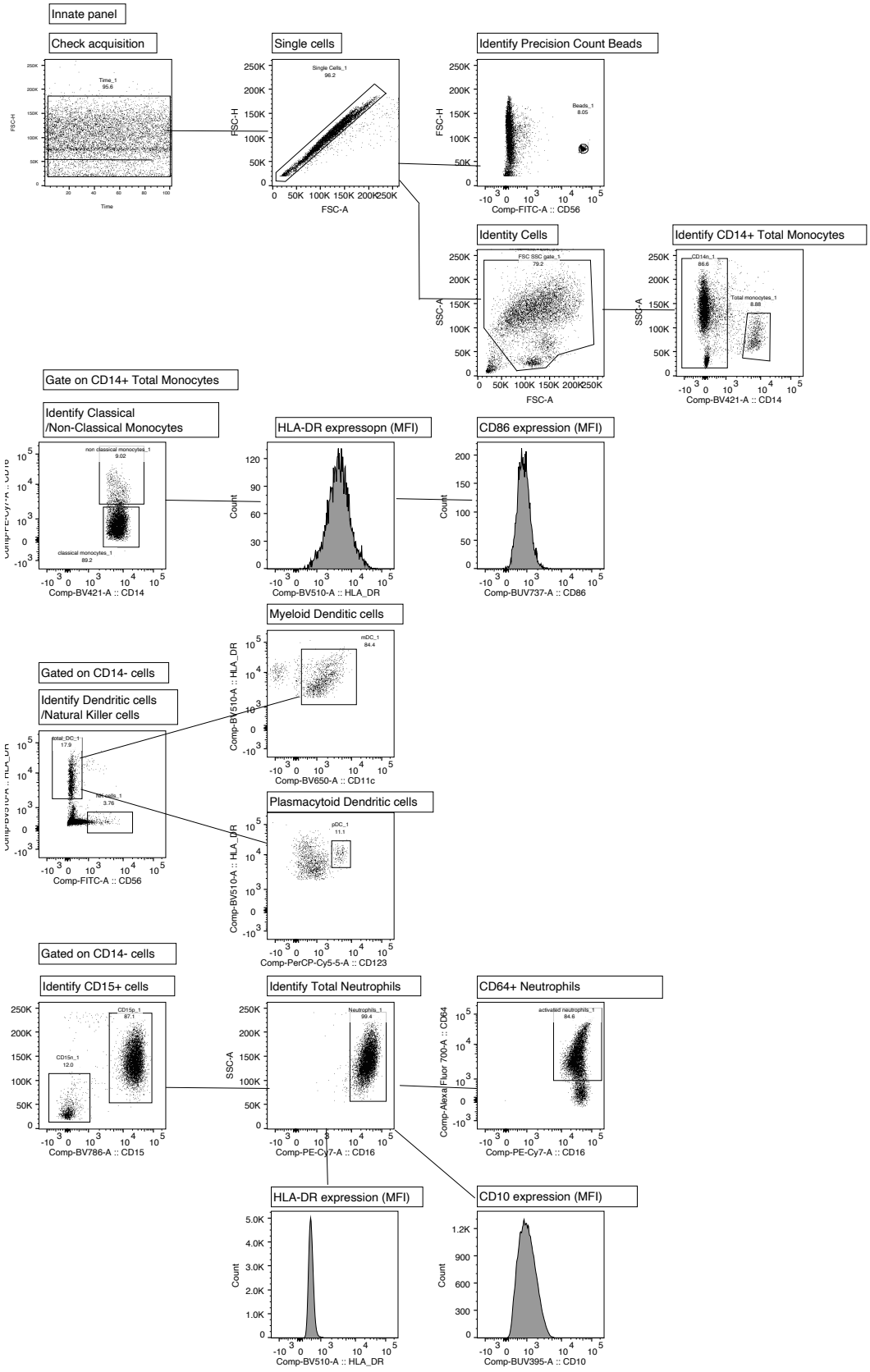
eFigure-6: Additional information on T cell alterations on T1, T2 and T3.



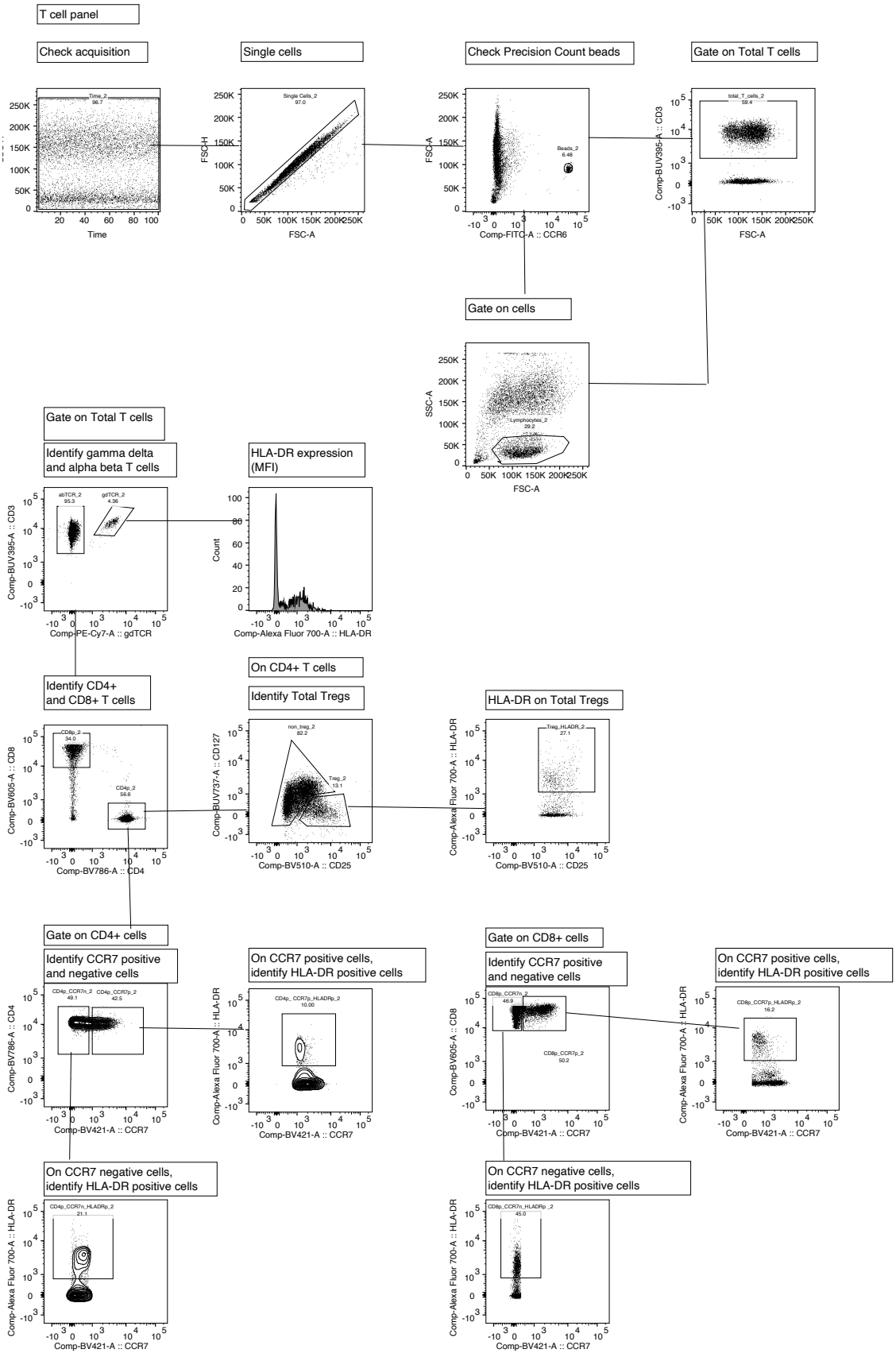
eFigure-7: Epidemiology of SARS-Cov-2 infection in England prior to, and during the study period.



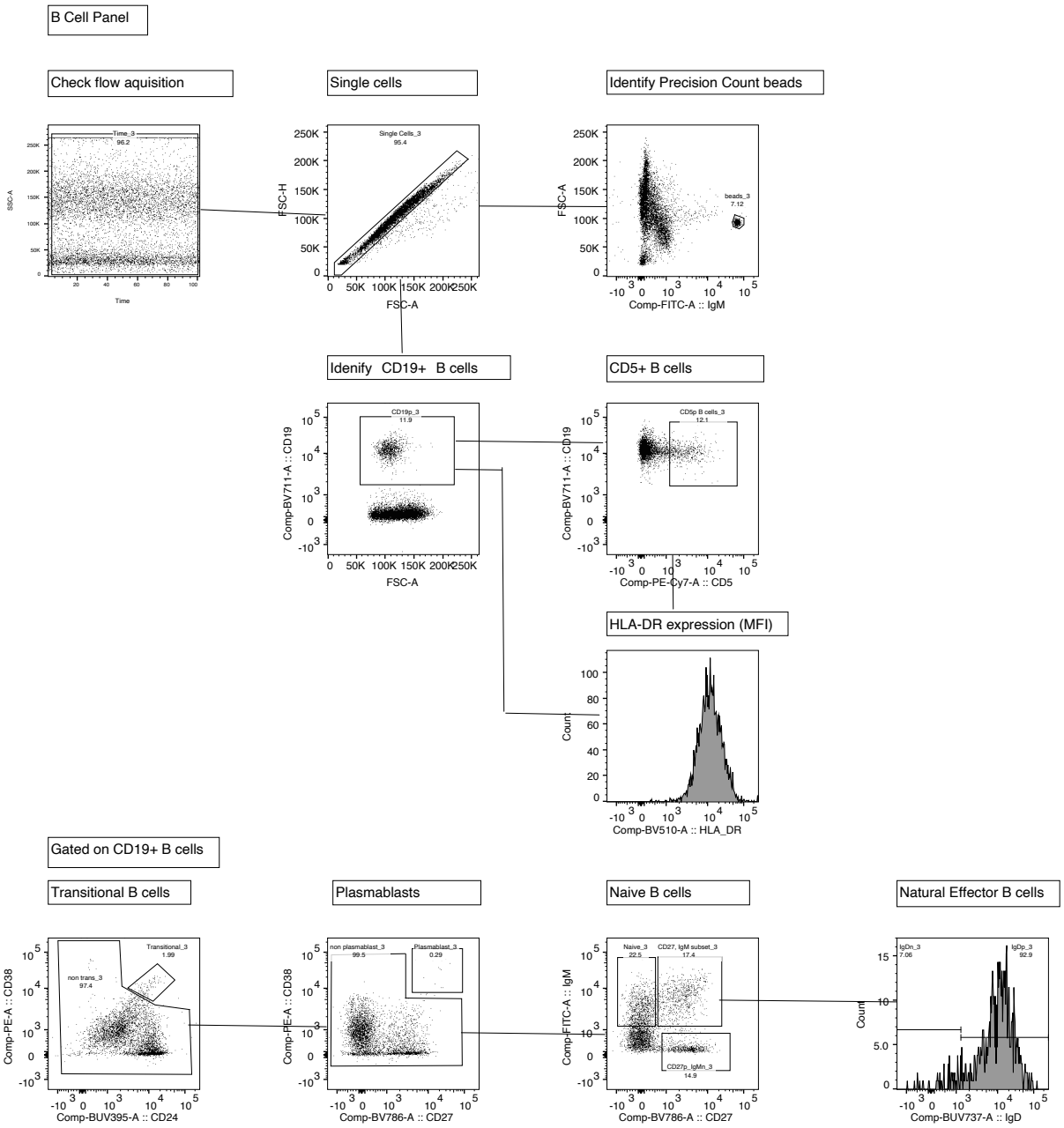
eFigure-8: Innate immune cell panel and gating approach[6]. *Gating strategy used to examine absolute innate cell counts in Cytodelics-preserved human whole blood by flow cytometry.*



eFigure-9: T cell panel and gating approach[6] *Gating strategy used to examine absolute T cell counts in Cytodelics-preserved human whole blood by flow cytometry.*



eFigure-10: B cell panel and gating approach[6]. *Gating strategy used to examine absolute B cell counts in Cytodelics-preserved human whole blood by flow cytometry.*



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