
Supplementary information

**Extrafollicular B cell responses correlate
with neutralizing antibodies and morbidity
in COVID-19**

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Supplementary Table 1 – Spectral flow B cell panel design

B cell core	B cell extended	Activation	Homing
Viability	CD1c	CD40	CD62L
CD3/CD14	CD10	CD69	CXCR3
CD19	CD11c	CD86	CXCR4
CD45	CD21	HLA-DR	CXCR5
IgD	CD24	PD-1	
IgM	CD27	CD23	
IgG	CD38		
	CD138		

Standardized panel design for the broad characterization and study of peripheral blood B cells

Supplementary Table 2 – Baseline demographic characteristics of research subjects

Characteristic	All Subjects (n = 56)		
	Healthy Controls (n = 37)	COVID-19 Outpatient (n = 7)	COVID-19 ICU (n = 12)
Mean age (range) – yr	35±11 (22-61)	33±6 (26-44)	61±14 (34-81)
Gender – no. (%)			
Male	18 (49)	5 (71)	6 (50)
Female	19 (51)	2 (29)	6 (50)
Race – no. (%)			
Caucasian	25 (68)	6 (86)	1 (8)
African-American	1 (3)	0 (0)	10(83)
Asian	9 (24)	1 (14)	1 (8)
Other	2 (5)	0 (0)	0 (0)
Smoking History – no. (%)			
Never smoked	35 (95)	6 (86)	11 (92)
Former smoker	2 (5)	1 (14)	1 (8)
Current smoker	0 (0)	0 (0)	0 (0)
Symptoms – no. (%)			
Any	0 (0)	7 (100)	12 (100)
Fever	0 (0)	4 (57)	9 (75)
Nasal congestion	0 (0)	5 (71)	1 (8)
Anosmia	0 (0)	2 (29)	0
Headache	0 (0)	4 (50)	4 (33)
Cough	0 (0)	6 (86)	11(92)
Sore Throat	0 (0)	4 (57)	1 (8)
Shortness of breath	0 (0)	1 (14)	10 (83)
Nausea or vomiting	0 (0)	0 (0)	0 (0)
Diarrhea	0 (0)	1 (14)	2 (17)
Myalgia or arthralgia	0 (0)	4 (57)	3 (25)
Chills	0 (0)	4 (57)	5 (42)
Comorbidities – no. (%)			
COPD	0 (0)	0 (0)	2 (17)
Diabetes	2 (5)	0 (0)	5 (42)
Hypertension	4 (11)	0 (0)	10 (83)
Coronary artery disease	0 (0)	0 (0)	2 (17)
Cerebrovascular disease	0 (0)	0 (0)	0 (0)
Cancer	0 (0)	0 (0)	0 (0)
Chronic Kidney Disease	0 (0)	0 (0)	2 (17)
Liver Disease	0 (0)	0 (0)	0 (0)
Tmax during hospitalization – °C			39±1

Supplementary Table 2 – Baseline demographic characteristics of research subjects (continued)

Laboratory Findings (Normal Range)			
White blood cell count – cells x10 ³ /microliter (4.2-9.1)			12.7±5.1
Absolute lymphocyte count [§] – cells x10 ³ /microliter (0.72-3.29)			1.2±0.5
Absolute neutrophil count [§] – cells x10 ³ /microliter (0.67-6.41)			9.2±4.2
Hemoglobin – g/dL (12.9-16.1)			9.7±1.9
Platelet count – cells x10 ³ /microliter (150-400)			268±105
Creatinine – mg/dL (0.7-1.3)			3.5±3.3
Aspartate aminotransferase – units/L (13-39)			76±73
Alanine aminotransferase – units/L (7-52)			55±51
C-reactive protein [%] – mg/L (≤ 10)			185±121
Lactate dehydrogenase [#] – units/L (140-271)			386±170
D-dimer – ng/mL (≤ 574)			9118±12482
Radiographic Abnormalities – no. (%)			
Interstitial abnormalities			11 (92)
Consolidative opacities			3 (25)
Measures of Illness Severity			
Mean PaO ₂ /FiO ₂ Ratio ^{&}			162±96
Mean SOFA Score ^{**&}			10.2±4.6
Complications – no. (%)			
Shock		0 (0)	11 (92)
Acute respiratory distress syndrome		0 (0)	12 (100)
Acute kidney Injury		0 (0)	8 (67)
Treatments – no. (%)			
Antibacterial antibiotics		0 (0)	11 (92)
Antiviral antibiotics		0 (0)	5 (42)
Antifungal antibiotics		0 (0)	0 (0)
Systemic glucocorticoids		0 (0)	1 (8)
Hydroxychloroquine		0 (0)	6 (50)
Azithromycin		0 (0)	5 (42)
Mechanical ventilation		0 (0)	12 (100)
Vasopressors		0 (0)	11 (92)
Extracorporeal membrane oxygenation		0 (0)	2 (17)
Renal-replacement therapy		0 (0)	3 (25)
Intravenous immune globulin		0 (0)	0 (0)
Outcomes – no. (%)			
Death		0 (0)	4 (33)
Recovery		7 (100)	7 (58)
Ongoing critical illness		0 (0)	1 (8)

* Plus-minus values are means±SD. Percentages may not total 100 because of rounding

** SOFA stands for Sequential Organ Failure Assessment

§ Data for absolute lymphocyte count and absolute neutrophil count were missing from four subjects

% Data for CRP and troponin I were missing from two subjects

Data for lactate dehydrogenase were missing or partially missing from three subjects

& Data for PaO₂/FiO₂ ratio were missing for three subjects upon extubation, therefore SOFA scores could not be calculated and therefore this average is higher than if these values were included

Supplementary Table 3 – Antibody catalogue and panel design

Target	Fluorophore	Panel*	Clone	Vendor	Cat#	Dilution (µl/100ul)
CD62L	BV480	v1, v2	DREG-56	BD	566174	5 µl
CD86	PerCP-Cy5.5	v1, v2	IT2.2	BioLegend	305419	5 µl
CD27	BV750	v1, v2, ICS	O323	BioLegend	302849	2.5 µl
CD19	BV570	v1, v2, ICS	HIB19	BioLegend	302235	2.5 µl
CD45	Spark NIR 685	v2	2D1	BioLegend	368552	1.25 µl
CD1c	BV510	v2	L161	BioLegend	331534	1.25 µl
IgM	BV711	v1, v2, ICS	MHM-88	BioLegend	314539	1.25 µl
CXCR3	A647	v1, v2, ICS	G025H7	BioLegend	353711	1.25 µl
CXCR4	PerCP-e710	v1, v2	12G5	eBioscience	46-9999-41	1.25 µl
CCR7	A488	v1	G043H7	BioLegend	353205	1.25 µl
CD24	PerCP	v1, v2, ICS	ML5	BioLegend	311113	1.25 µl
CD3	BUV 805	v1, v2, ICS	UCHT1	BD	612896	0.6 µl
CD11c	APC-Fire750	v1, v2, ICS	S-HCL-3	BioLegend	371509	0.6 µl
CD138	APC-R700	v1, v2	MI15	BD	566051	0.6 µl
HLA-DR	BV650	v1, v2	L243	BioLegend	307649	0.6 µl
CD95	BV785	v1, v2	DX2	BioLegend	305645	0.6 µl
CD14	BUV805	v1, v2	M5E2	BD	612902	0.6 µl
CD23	APC	v2	EBVCS-5	BioLegend	338514	0.3 µl
CD69	BUV 737	v1, v2	FN50	BD	612817	0.3 µl
IgD	BV605	v1, v2, ICS	IA6-2	BioLegend	348231	0.3 µl
CD21	PE-Dazzle594	v1, v2, ICS	Bu32	BioLegend	354921	0.3 µl
CD38	BB515	v1, v2, ICS	HIT2	BD	564499	0.3 µl
CXCR5	PE	v1, v2, ICS	J252D4	BioLegend	356903	0.3 µl
CD40	A532	v1, v2	5C3	Novus	NBP1-43416AF52	0.3 µl
PD-1	PE-Cy7	v1, v2	EH12.2H7	BioLegend	239917	0.3 µl
IgG	BV421	v1, v2	M1310G05	BioLegend	410703	0.15 µl
CD10	PE-Cy5	v1, v2	HI10a	BioLegend	312205	0.15 µl
CD25	e450	v1	BC96	eBioscience	48-0259-41	5 µl
CD1d	BV510	v1	51.1	BioLegend	350313	2.5 µl
ICOS-L	APC	v1	2D3	BioLegend	309407	5 µl
B220	Spark NIR 685	v1	RA3-6B2	BioLegend	103268	2.5 µl
T-bet	APC	ICS	4B10	BioLegend	644814	1.25 µl
Viability	Zombie NIR	v1,2	NA	BioLegend	423106	0.2 µl

*Panel designs include two iterations of surface marker panels (V1, V2), and an additional panel for intracellular staining of T-bet