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Supplemental information

Characterization model of the post COVID-19 condition based on immunological, biochemical, and cytokine markers

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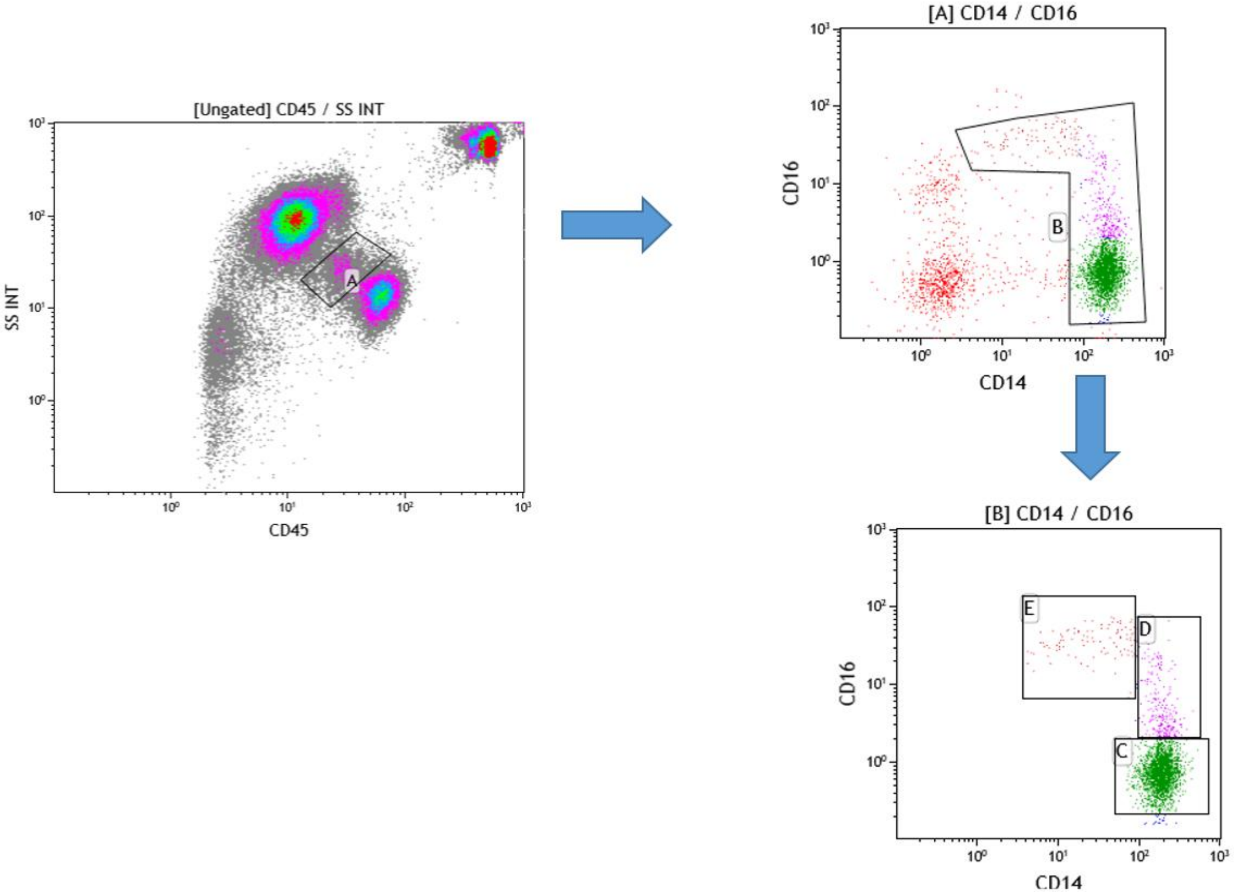
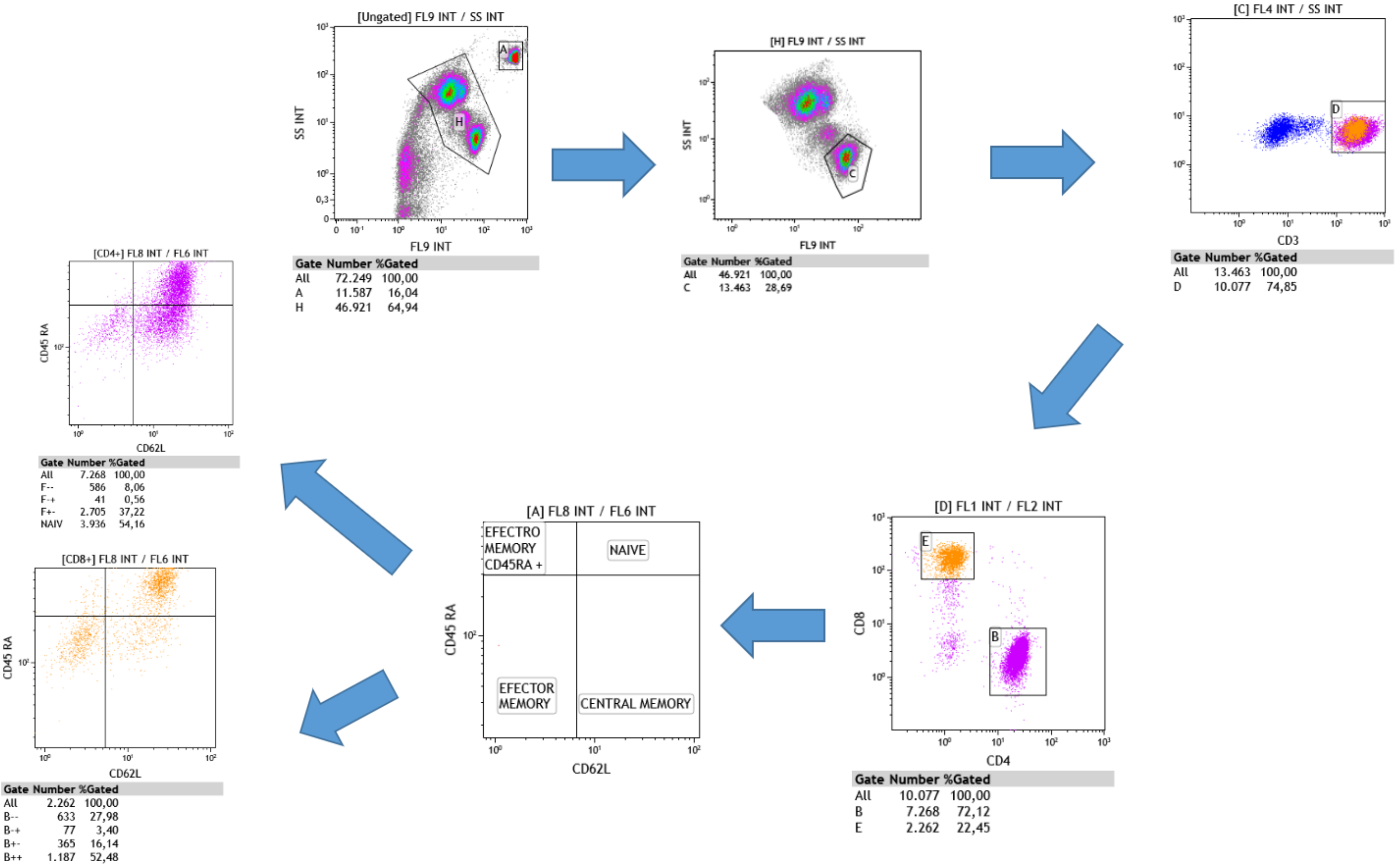


Supplementary Figure S1. Gating strategy employed for monocyte.

Legend:The monocyte fraction (A) was selected based on CD45 and side scatter (SSC). Lymphocytes and Granulocytes were eliminated based on CD14 and CD16 levels (B). Based on the CD14/CD16 profile, monocytes were subdivided into 3 populations Classical (C), Intermediate (D) and Atypical (E).



Gate	Number	%Gated
All	72.249	100,00
A	11.587	16,04
H	46.921	64,94

Gate	Number	%Gated
All	46.921	100,00
C	13.463	28,69

Gate	Number	%Gated
All	13.463	100,00
D	10.077	74,85

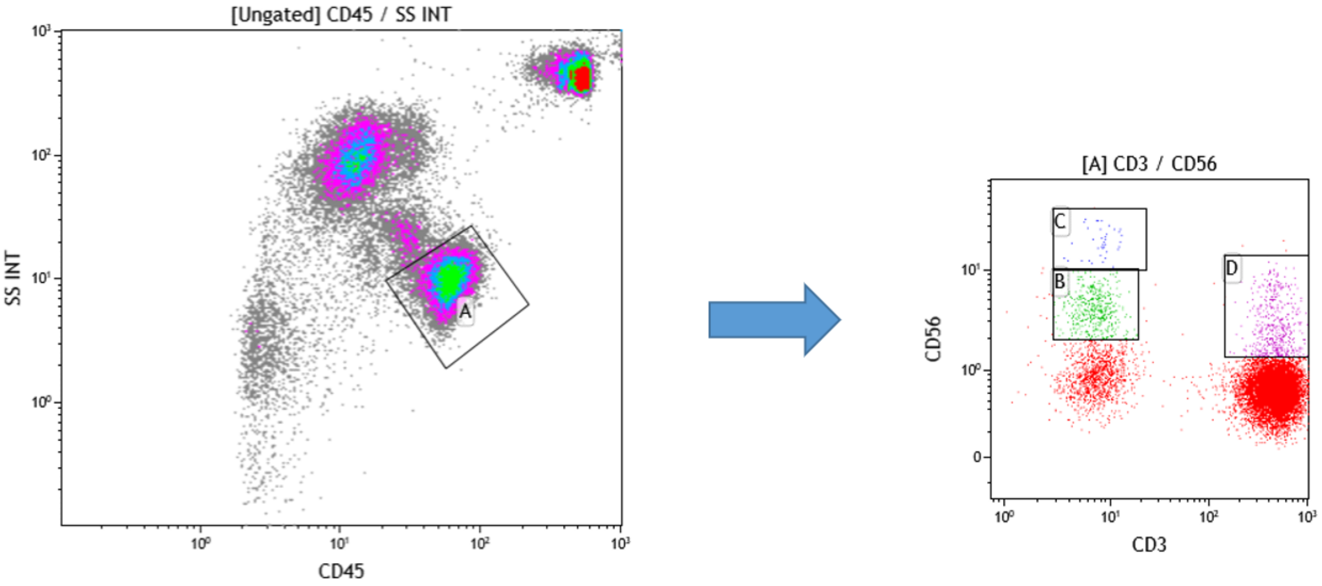
Gate	Number	%Gated
All	10.077	100,00
B	7.268	72,12
E	2.262	22,45

Gate	Number	%Gated
All	7.268	100,00
F--	586	8,06
F-+	41	0,56
F+-	2.705	37,22
NAIV	3.936	54,16

Gate	Number	%Gated
All	2.262	100,00
B--	633	27,98
B-+	77	3,40
B+-	365	16,14
B++	1.187	52,48

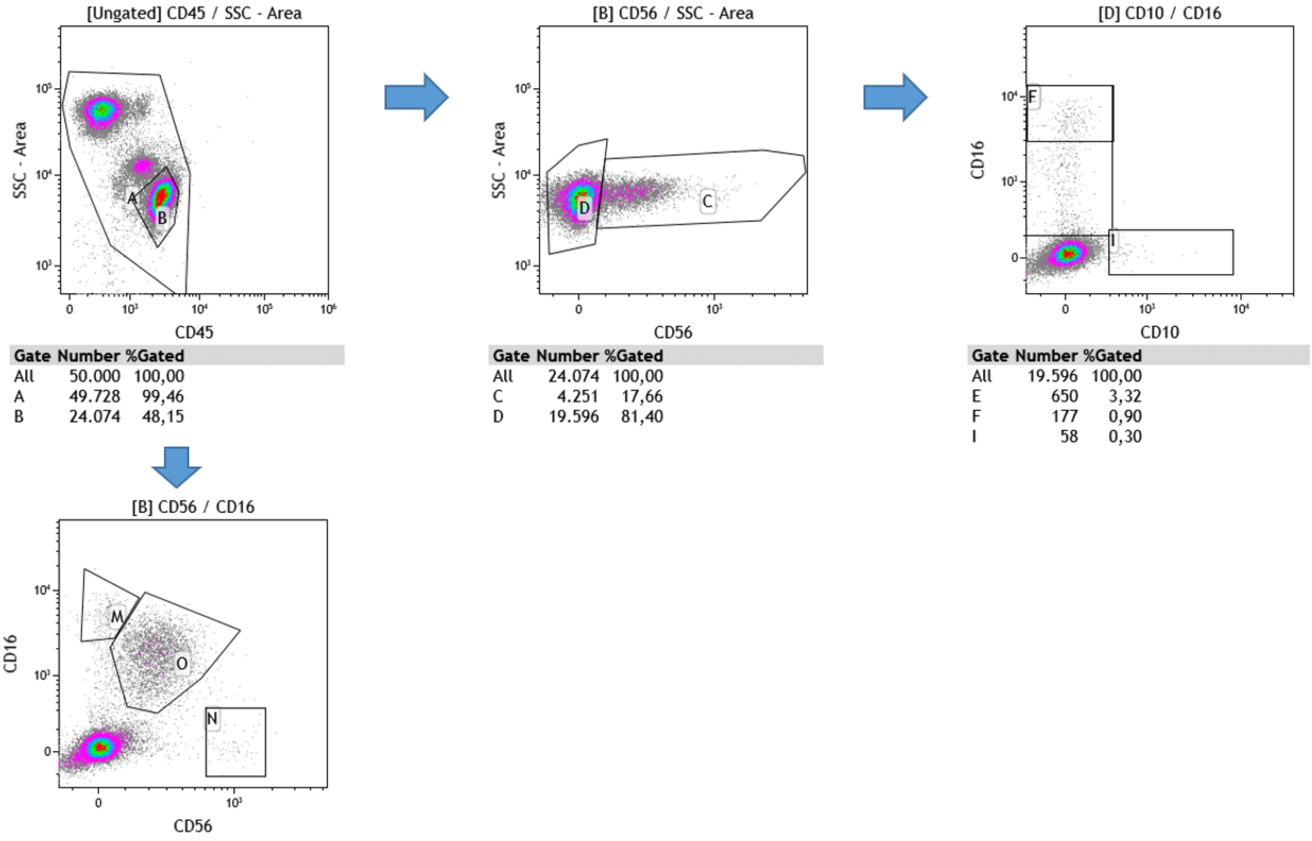
Supplementary Figure S2. Gating strategy employed for T Lymphocyte populations.

Legend: Based on CD45 (FL9) and side scatter (SSC) first total leukocytes and subsequently lymphocytes are selected. Next, we select the T lymphocytes (CD3+), and CD4 and CD8 are used as markers of Th lymphocyte and Tc lymphocyte populations, respectively. Finally in each of these populations we study the expression of CD62L and CD45RA that define the degree of maturation to identify and count naive, central memory, effector memory and CD45RA+ effector memory cells



Supplementary Figure S3. Gating strategy employed for natural Killers.

Legend: First, the lymphocyte population was selected based on CD45 and side scatter (SSC) as indicated. CD3 and CD56 markers were then used to define subpopulations: CD56 dim (B); CD56 Bright (C) and NKTs (D).



Gate	Number	%Gated
All	50.000	100,00
A	49.728	99,46
B	24.074	48,15

Gate	Number	%Gated
All	24.074	100,00
C	4.251	17,66
D	19.596	81,40

Gate	Number	%Gated
All	19.596	100,00
E	650	3,32
F	177	0,90
I	58	0,30

Supplementary Figure S4. Gating strategy employed for NK subpopulations.

Legend: As before, the lymphocyte population was selected based on CD45 and side scatter (SSC) as indicated. Further analysis within this population was based on the expression of CD56 and CD16/CD10. Selecting the CD56neg cells, we selected in that population those without expresión of CD10 that do show expression of CD16 (population F which is CD16+, CD56 -). On the other hand, within the lymphocyte population we look at the co-expression of CD56 and CD16 defining the M (16+, 56-), O (16 dim, 56 dim) and N (56 bright, 16 -) populations.

Supplementary Table S1. Comparison of biochemical profiles, liver and thyroid function tests, and electrolyte statistics measurements not statistically significant between recovered individuals and PCC.

Variables	Reference range	Recovered (n=85)	PCC (n=85)	p–value
Myoglobin, ng/mL	17.4-105.7	31.73 ± 49.43	22.75 ± 15.16	0.116
NT-proBNP, pg/mL	300-900	53.38 ± 41.24	64.25 ± 49.55	0.123
TSH, µUI/mL	0.38-5.33	2.44 ± 1.39	2.86 ± 4.31	0.468
Fe, µg/dL	70-180	89.65 ± 33.52	98.52 ± 37.39	0.106
Ferritin, ng/mL	30-300	126.70 ± 125.19	103.42 ± 96.97	0.177
Transferrin, mg/mL	200-360	261.90 (51.20)	278.50 (56.80)	0.009
Transferrin saturation, %	20-50	25.02 ± 10.20	25.345 ± 9.92	0.836
Haptoglobin, mg/mL	30-200	113.24 ± 48.16	123.76 ± 59.54	0.209
Folic acid, ng/mL	3.1-19.9	9.32 ± 4.77	9.42 ± 5.52	0.907
Vitamin B12, Pg/mL	145-914	331.67 ± 162.16	302.96 ± 167.67	0.260
25-hydroxyvitamin D, nmol/L	75-250	58.20 (30.10)	68.05 (28.80)	0.002
C-Reactive protein, mg/dL	<0.5	0.28 ± 0.32	0.35 ± 0.46	0.265
Glucose, mg/dL	74-106	90.82 ± 12.18	88.54 ± 11.31	0.207
Uric Acid, mg/dL	3.5-7.2	4.76 ± 1.28	4.73 ± 1.34	0.886
Mg, mg/dL	1.8-2.6	2.00 ± 0.14	2.01 ± 0.14	0.666
Triglycerides, mg/dL	30-175	89.38 ± 41.74	104.79 ± 59.67	0.053
Cholesterol HDL, mg/dL	>40	64.00 ± 15.50	64.10 ± 13.00	0.083
Cholesterol LDL, mg/dL	<150	131.13 ± 37.44	140.56 ± 33.02	0.083
Bilirubin, mg/dL	0.3-1.2	0.64 ± 0.28	0.61± 0.27	0.488
Total proteins, g/dL	6.6-8.3	7.06 ± 0.43	7.07 ± 0.47	0.847
Aldolase, U/L	<7.6	3.71 ± 2.58	4.02 ± 5.46	0.647
Alkaline Phosphatase, U/L	30-120	71.31 ± 22.21	79.87 ± 110.61	0.485
GGT, U/L	<55	25.48 ± 19.14	23.69 ± 13.85	0.486
AST, U/L	<50	23.07 ± 6.68	22.54 ± 5.96	0.587
ALT, U/L	<50	21.05 ± 9.48	20.34 ± 8.99	0.619
LDH, U/L	<248	176.88 ± 30.88	178.39 ± 26.79	0.735

P-value from Mann-Whitney's U-test or Student's t-test. NT-proBNP: N-terminal pro b-type natriuretic peptide; TSH: Thyroid-stimulating hormone; Fe: Iron; Mg: Magnesium; GGT: Gamma-glutamyl Transferase; AST: aspartate aminotransferase; ALT: alanine transaminase; LDH: Lactate Dehydrogenase. Values are presented as mean ± standard deviation, median ± interquartile range). The reference range includes the upper and lower limits of each biomarker based on a group of healthy people, indicated by the Miguel Servet University Hospital (Zaragoza, Spain).

Supplementary Table S2. Comparison of antibody levels not statistically significant between recovered individuals and PCC.

Variables	Reference range	Recovered (n=85)	PCC (n=85)	p–value
ASLO, UI/mL	<200	182.41 ± 217.78	153.30 ± 112.68	0.275
Rheumatoid factor, UI/mL	<14	11.20 ± 4.05	10.96 ± 4.79	0.732
Intrinsic factor antibody, AU/mL	<1.53	3.54 ± 17.06	1.17 ± 1.19	0.212
CCP antibodies, UI/mL	<20	4.89 ± 1.28	4.61 ± 0.18	0.048
Alpha-1 Antitrypsin, mg/dL	88-174	138.17 ± 21.11	140.13 ± 27.54	0.602
Beta 2 Microglobulin, mg/dL	0.08-0.24	0.18 ± 0.04	0.18 ± 0.05	0.746
IgA, mg/dL	40-350	239.36 ± 92.39	217.86 ± 81.82	0.11
IgM, mg/dL	50-300	124.95 ± 63.70	124.04 ± 55.08	0.92
IgD, mg/dL	<15	5.15 ± 11.48	3.36 ± 4.74	0.187
IgG2	241.8-7003	382.82 ± 120.22	375.20 ± 129.89	0.693
IgG3	21.8-176.1	62.09 ± 31.99	55.53 ± 31.21	0.179
IgG4	3.9-86.4	34.39 ± 25.13	35.27 ± 28.78	0.832
Complement C1 Inhibidor, mg/dL	15-35	18.60 ± 2.55	19.25 ± 2.53	0.099
C-Peptide, ng/mL	0.78-5.19	1.97 ± 0.83	2.11 ± 0.91	0.298
tTG-IgA, U/mL	<20	1.97 ± 0.36	2.06 ± 1.18	0.504
Anti-dsDNA, UI/mL	<27	11.75 ± 26.77	8.21 ± 3.51	0.228
cANCA, u	<20	2.37 ± 0.90	2.36 ± 1.73	0.968
IgG anticardiolipin antibodies, U/mL	<20	4.35 ± 3.24	15.27 ± 101.22	0.322
IgM anticardiolipin antibodies, U/mL	<20	2.81 ± 3.28	3.60 ± 4.70	0.206

P-value from Mann-Whitney’s U-test or Student's t-test. ASLO: Anti-streptolysin O; CCP: cyclic citrullinated peptide; Ig: immunoglobulins; tTG-IgA: Blood Test: Tissue Transglutaminase IgA; Anti-dsDNA: Anti-double stranded DNA antibodies; cANCA: proteinase 3 antineutrophil cytoplasmic antibody; Values are presented as mean ± standard deviation, median ± interquartile range). The reference range includes the upper and lower limits of each biomarker based on a group of healthy people, indicated by the Miguel Servet University Hospital (Zaragoza, Spain).

Supplementary Table S3. Comparison of blood count and coagulation test statistics not statistically significant between recovered individuals and PCC.

Variables	Reference range	Recovered (n=85)	PCC (n=85)	p–value
Leukocytes, 10^3/µL	3.9-10.2	6.36 ± 1.63	6.57 ± 2.39	0.509
Neutrophils, 10^3/µL	1.5-7.7	3.47 ± 1.21	3.69 ± 2.00	0.378
Eosinophil, 10^3/µL	<0.5	0.16 ± 0.13	0.17 ± 0.15	0.674
Basophils, 10^3/µL	<0.2	0.03 ± 0.04	0.04 ± 0.06	0.701
Monocytes, 10^3/µL	0.1-0.9	0.48 ± 0.15	0.48 ± 0.17	0.876
Lymphocytes, 10^3/µL	1.1-4.5	2.19 ± 0.67	2.18 ± 0.72	0.876
Erythrocyte, 10^6/µL	4.3-5.75	4.66 ± 0.46	4.65 ± 0.35	0.842
Hemoglobin, g/dL	13.5-17.5	13.78 ± 1.10	13.94 ± 1.06	0.353
Hematocrit, %	41-53	41.00 ± 3.08	41.83 ± 3.02	0.076
MCH, pg	26-34	29.67 ± 2.35	29.98 ± 1.56	0.319
RDW, %	11.5-14	13.53 ± 1.01	13.53 ± 1.04	0.982
Platelets, 10^3/µL	140-370	249.84 ± 60.39	259.05 ± 64.93	0.34
MPV, fL	7.5-11	9.24 ± 1.06	10.48 ± 13.16	0.391
Prothrombin activity, %	80-120	120.95 ± 12.12	120.57 ± 16.31	0.865
D-Dimer, µg/L	<500	306.38 ± 168.87	391.37 ± 628.46	0.233

P-value from Mann-Whitney’s U-test or Student's t-test. MCH:Mean corpuscular hemoglobin; RDW: red cell distribution width; MPV: mean platelet volume. Values are presented as mean ± standard deviation, median ± interquartile range). The reference range includes the upper and lower limits of each biomarker based on a group of healthy people, indicated by the Miguel Servet University Hospital (Zaragoza, Spain).

Supplementary Table S4. Comparison of blood count and coagulation test statistics not statistically significant between recovered individuals and PCC.

Variables	Recovered (n=85)	PCC (n=85)	p–value
CD19 B-lymphocytes, 10^3/mm^3	0.22 ± 0.11	0.21 ± 0.10	0.446
CD3+ T-lymphocyte, 10^3/mm^3	1.50 ± 0.48	1.48 ± 0.54	0.792
CD56 NK cells, 10^3/mm^3	0.29 ± 0.16	0.25 ± 0.15	0.187
CD4 + T-lymphocytes, 10^3/mm^3	0.96 ± 0.33	0.92 ± 0.31	0.423
CD8 + T-lymphocytes, 10^3/mm^3	0.49 ± 0.22	0.51 ± 0.35	0.745
Classical monocyte (CD14++CD16-), %	88.44 ± 4.61	87.80 ± 5.13	0.430
Intermediate monocyte (CD14++CD16+), %	5.993 ± 3.11	6.48 ± 3.29	0.348
Nonclassical monocyte (CD14+CD16++), %	5.278 ± 2.51	5.38 ± 3.09	0.821
CD16+ NK cells among total lymphocytes, %	10.12 ± 4.72	9.77 ± 4.69	0.648
CD16+ NKT cells among total lymphocytes, %	4.61 ± 2.43	4.91 ± 5.20	0.654
CD56bright NK cells among total lymphocytes, %	0.53 ± 0.33	0.45 ± 0.26	0.105
CD16+ NK cells among CD56+, %	63.67 ± 14.20	64.26 ± 20.07	0.834
CD16+NKT cells among CD56+, %	29.33 ± 14.32	29.99 ± 20.00	0.816
CD56 bright NK cells among CD56+, %	3.43 ± 1.97	3.43 ± 2.56	0.994
CD56- cells among total lymphocytes, %	82.97 ± 6.21	84.08 ± 6.31	0.278
CD56-CD16+ among CD56- cells, %	6.77 ± 3.28	6.38 ± 2.87	0.442
Naïve CD4+ cells % among CD4+ cells, %	34.07 ± 11.92	37.95 ± 12.87	0.056
Central memory CD4+ among CD4+ cells, %	38.57 ± 9.01	38.68 ± 9.74	0.944
Effector memory CD4+ among CD4+ cells, %	24.33 ± 9.18	21.88 ± 8.55	0.089
Central memory CD8+ among CD8+ cells, %	13.66 ± 5.68	15.83 ± 7.99	0.059
Effector memory CD8+ among CD8+ cells, %	41.06 ± 13.32	38.12 ± 13.91	0.186
CD4CD8 ratio	2.11 ± 0.69	2.30 ± 1.20	0.215
CD4-CD8- double negative cells, %	3.82 ± 3.71	3.47 ± 2.87	0.530
CD4+CD8 low cells among T-lymphocytes %	0.80 ± 0.79	0.92 ± 0.80	0.368
CD8+/CD4 low cells among T-limphocytes %	0.53± 0.56	0.62 ± 0.43	0.297

P-value from Mann-Whitney’s U-test or Student's t-test. CD19: CD19 B-lymphocytes; CD3: CD3 T-lymphocytes; CD56: CD56: natural killer cells; CD4: CD4 T-lymphocytes or "helper T cells; CD8: CD8 T-lymphocytes or " cytotoxic T cells. Naive cells are defined as CD45RA+/CD62L +; Central memory as CD45RA-/CD62L + and Effector memory as CD45RA-/CD62L-. Values are presented as mean ± standard deviation, median ± interquartile range.

Supplementary Table S5. Comparison of Pro-inflammatory cytokines analysis between recovered individuals and PCC.

Variables	Recovered (n=85)	PCC (n=85)	p–value
TNF-α, pg/mL	1.14 (0.67)	1.28 (0.85)	0.036*
IL-6, pg/mL	0.42 (0.50)	0.48 (0.26)	0.012*
IL-8, pg/mL	3.93 (3.20)	4.07 (2.71)	0.028*
IL-10, pg/mL	0.74 (0.64)	3.36 (1.30)	<0.001***
IFN γ, pg/mL	2.05 (1.02)	2.91 (2.91)	<0.001***
IL-4, pg/mL	1.07 (0.74)	1.94 (0.64)	<0.001***
IL-2, pg/mL	0.90 (0.60)	1.20 (0.79)	0.005**
IL-28, pg/mL	3.01 (1.00)	2.04 (1.66)	0.011*
CXCL10, pg/mL	10.00 (10.56)	10.74 (11.58)	0.002**

P-value from Mann-Whitney's U-test, α:alpha; γ:gamma; Values are presented as median (interquartile range). The statistical significance of differences from Mann-Whitney's U-test between the two groups is indicated as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Supplementary Table S6. Descriptive biomarkers statistics of recovered PCC individuals and false positive and false negative patients for the different models.

Variable	PCC (n=85)	False Negative				Recovered (n=85)	False Positive			
		MODEL A		MODEL B			MODEL A		MODEL B	
		MLR (n=12)	RF (n=13)	MLR (n=13)	RF (n=12)		MLR (n=7)	RF (n=11)	MLR (n=6)	RF (n=9)
αGAD, median (IQR)	6.03 (1.09)	5.99 (1.03)	5.78 (0.70)	6.06 (0.98)	5.88 (1.44)	5.33 (0.93)	5.77 (0.93)	6.09 (0.77)	6.04 (1.31)	5.84 (0.95)
IL-10, median (IQR)	3.36 (1.30)	1.02 (0.68)	0.93 (0.49)	0.93 (0.65)	0.79 (0.65)	0.74 (0.64)	2.04 (2.65)	2.74 (2.19)	3.61 (1.12)	3.36 (1.12)
Antithrombin III, mean ± SD	104.83 ± 15.86	98.25 ± 11.56	95.31 ± 11.96	100.08 ± 11.18	99.08 ± 11.07	97.03 ± 10.17	101.86 ± 12.13	101.82 ± 10.77	97.33 ± 11.17	99.00 ± 9.94
Potassium, median (IQR)	4.20 (0.40)	4.30 (0.25)	4.20 (0.25)	4.30 (0.30)	4.30 (0.3)	4.40 (0.40)	4.30 (0.30)	4.40 (0.30)	4.25 (0.50)	4.40 (0.50)
CD56-CD16bright cells, median (IQR)	1.60 (1.17)	1.68 (1.49)	1.68 (1.66)	1.90 (0.69)	1.81 (1.28)	2.21 (2.22)	2.06 (3.49)	2.73 (3.77)	2.48 (1.87)	2.73 (4.49)
pANCA, mean ± SD	2.95 ± 0.52	3.11 ± 0.00	3.11 ± 0.00	3.11 ± 0.00	3.11 ± 0.00	3.31 ± 1.32	3.11 ± 0.00	3.11 ± 0.00	3.11 ± 0.00	3.11 ± 0.00
Prothrombin activity, mean ± SD	120.57 ± 16.31	119.58 ± 14.66	122.69 ± 13.97	123.15 ± 13.22	125.25 ± 12.54	120.95 ± 12.12	117.10 ± 8.25	118.36 ± 6.56	117.50 ± 4.46	120.00± 11.24
Aldolase, mean ± SD	4.02 ± 5.46	2.88 ± 1.41	3.02 ± 1.63	2.36 ± 1.17	2.28 ± 1.24	3.71 ± 2.58	2.44 ± 1.28	2.24 ± 1.11	3.24 ± 2.79	3.05 ± 2.30
Effector CD8+ T cells, median (IQR)	13.15 (12.18)	14.00 (12.00)	14.00 (11.20)	11.90 (6.50)	13.70 (7.50)	16.60 (14.80)	16.70 (19.40)	21.15 (21.50)	23.83 (7.80)	26.20 (7.80)
MCHC, median (IQR)	30.2 (2.1)	33.35 (1.30)	33.50 (1.20)	33.00 (0.90)	33.25 (1.40)	29.90 (2.2)	33.10 (1.50)	33.50 (1.10)	33.50 (1.10)	33.80 (0.90)
Sodium, median (IQR)	139 (3)	139.50 (3)	138 (2)	138 (3)	138 (2)	140 (2)	139 (1)	140 (2)	140.5 (1)	140 (1)
IGFBP3, mean ± SD	6.33 ± 1.24	5.35 ± 1.11	5.50 ± 0.73	5.49 ± 1.12	5.88 ± 1.11	5.64 ± 1.10	5.84 ± 1.35	6.35 ± 1.05	6.45 ± 1.22	6.20 ± 1.13
CD16+ NKT-like cells, mean ± SD	4.91 ± 5.20	3.24 ± 2.38	4.02 ± 2.67	3.31 ± 1.92	4.32 ± 3.48	4.61 ± 2.43	4.80 ± 2.25	3.95 ± 1.77	3.73 ± 1.69	4.10 ± 1.65
CD16+ NK cells, mean ± SD	9.77 ± 4.69	8.42 ± 3.76	8.47 ± 4.13	10.23 ± 4.51	10.17 ± 4.72	10.12 ± 4.72	11.90 ± 2.80	12.85 ± 6.33	11.15 ± 7.57	11.96 ± 6.66
NT-proBNP, mean ± SD	64.25 ± 49.55	42.94 ± 28.71	47.51 ± 30.26	43.30 ± 23.29	46.184 ± 23.48	53.38 ± 41.24	60.09 ± 83.74	44.79 ± 34.20	34.53 ± 45.39	33.69 ± 37.02
Total of persistent symptoms, mean ± SD	5.32 ± 2.28	6.33 ± 2.10	5.31 ± 2.29	5.08 ± 2.47	4.92 ± 2.50	0	0	0	0	0

Recovered: individuals who completely recovered within 3 months after acute COVID-19; PCC: individuals diagnosed with Post Covid-19 Condition; RF:Random Forest; MLR multivariate logistic regression; αGAD: GAD antibody; IGFBP3: Insulin-like growth factor-binding protein 3 CD16: CD16 natural killer cells; CD56: CD56 natural killer cells; NT-proBNP: N-terminal pro b-type natriuretic peptide; CD8: CD8 T-lymphocytes or " cytotoxic T cells; pANCA: perinuclear anti-neutrophil cytoplasmic antibodies. Variables included Model A: αGAD + IL-10 + Antithrombin III + Potassium + CD56-CD16bright + pANCA + Prothrombin activity + Aldolase + Effector CD8+ T cells MCHC + Sodium + IGFBP3 + CD16+NKT cells + CD16+ NK cells + NT-proBNP. Variables included Model B: αGAD + IL-10+ Potassium + CD56-CD16bright.

Supplementary Table S7. Biomarkers routinely used in patient's care obtained from primary health care.

Biochemical profile:	Glucose (mg/dL), glycosylated hemoglobin (HbA1c) (%), creatinine (mg/dL), uric acid (mg/dL), bilirubin (mg/dL), glomerular filtration rate (GFR) (mL/min*1.73 m2), total cholesterol (mg/dL), high-density lipoprotein cholesterol (HDL) (mg/dL), low-density lipoprotein cholesterol (LDL) (mg/dL), non-HDL cholesterol (mg/dL), triglycerides (mg/dL), iron (µg/dL), ferritin (ng/mL), transferrin (mg/mL), transferrin saturation (%), haptoglobin (mg/mL); ions: sodium (mEq/L), potassium (mEq/L), magnesium (mg/dL); folic acid (ng/mL), vitamin B12 (pg/mL), 25-hydroxyvitamin, gamma-glutamyl transferase (GGT) (U/L), aspartate aminotransferase (AST) (U/L), alanine transaminase (ALT) (U/L), thyroid function: thyrotropin (TSH) (µIU/mL); lactate dehydrogenase (LDH) (U/L), creatine kinase (CK) (ng /mL), C-Reactive protein (mg/dL). As several patients reported taking supplements such as 25-hydroxyvitamin, B12 and iron since the onset of their persistent symptoms, these markers could not be studied for the sake of diagnostic value.
Complete blood count	Red blood cells (10^6/µL), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (MCV) (fL), mean corpuscular hemoglobin (MCH) (pg) , mean corpuscular hemoglobin (MCHC) concentration (g/dL), red cell distribution width (RDW) (%) erythrocyte sedimentation rate (ESR) (mm/hour); leukocytes, neutrophils, eosinophils, basophils, monocytes, total lymphocytes (10^3/µL); CD19 B-lymphocytes, CD3+ T-lymphocyte, CD56 NK cells, CD4 T-lymphocytes, CD8 T-lymphocytes (10^3/mm^3); platelets (10^3/µL), mean platelet volume (MPV) (fL);
Coagulation test	International normalized ratio (INR), Partial thromboplastin time (sec), activated partial thromboplastin time ratio (APTT ratio), prothrombin time (sec), prothrombin activity (%), D-dimer (µg/L), fibrinogen (g/L), antithrombin (%)
Protein study	Myoglobin (ng/mL), aldolase (U/L), phosphatase (U/L), B-type natriuretic peptide (pg/mL), total protein (g/dL), albumin (g/dL), Immunoproteins (IgG) (G1, G2, G3, G4) (mg/dL), alpha-1 antitrypsin (mg/dL), beta-2 microglobulin (mg/dL), Immunoglobulins IgE (IU/mL), IgM, IgD IgG, IgA (mg/dL), complement inhibitor C1, C3, C4 (mg/dL), C-peptide (ng/mL), Insulin-like growth factor-binding protein (IGFBP-3) (µg/mL).
Serology	Anti-intrinsic factor antibodies (AU/mL), Anti-double stranded DNA antibodies (UI/mL), proteinase 3 antineutrophil cytoplasmic antibody (u), perinuclear anti-neutrophil cytoplasmic antibodies (u), anticardiolipin IgG (U/mL), anticardiolipin IgM (U/mL), SARS CoV 2 Nucleocapsid IgG positive, SARS-CoV 2 Spike S IgG, anti-streptolysin-O (IU/mL), rheumatic factor (IU/mL), anti-cyclic citrullinated peptide antibodies (IU/ mL), anti-glutamic acid decarboxylase antibodies (U/mL), anti-tissue transglutaminase IgA antibodies (U/mL).

Supplementary Table S8. Volumes needed for each antibody cocktail for cytometric staining.

Antibody cocktails	Volume
Anti CD45 CF-Blue	1,25 µl
Anti CD45 RA	5 µl
Anti CD62L	5 µl
Anti CD3	2 µl
Anti CD56	2,5 µl
Anti CD14	10 µl
Anti CD16	5 µl
Anti CD10	5 µl
CD4-FITC/CD8-PE/CD3-PC5 Antibody Cocktail, 50 Tests	10 µL

Supplementary Table S9. Parameter tuning of the RF, and MLR classifiers.

Classifier	**Parameters**
RF-A	"{'bootstrap': True, 'ccp_alpha': 0, 'criterion': 'gini', 'max_depth': 3, 'max_features': 'sqrt', 'max_leaf_nodes': 4, 'max_samples': 10, 'min_impurity_decrease': 0, 'min_samples_leaf': 5, 'min_samples_split': 5, 'n_estimators': 1000, 'n_jobs': -1, 'random_state': 0}"
RF-B	{'bootstrap': True, 'ccp_alpha': 0, 'criterion': 'gini', 'max_depth': 3, 'max_features': 'sqrt', 'max_leaf_nodes': 4, 'max_samples': 50, 'min_impurity_decrease': 0, 'min_samples_leaf': 5, 'min_samples_split': 5, 'n_estimators': 10, 'n_jobs': -1, 'random_state': 0}
MLR-A	{'C': 1, 'l1_ratio': 0, 'max_iter': 10000, 'multi_class': 'multinomial', 'n_jobs': -1, 'penalty': 'l1', 'random_state': 0, 'solver': 'saga', 'tol': 0.001}
MLR-B	"{'C': 100, 'max_iter': 10000, 'multi_class': 'ovr', 'n_jobs': -1, 'penalty': 'l2', 'random_state': 0, 'solver': 'saga', 'tol': 0.0001}"