

Supplementary Materials

Effects of Steroids and Tocilizumab on the Immune Response Profile of Patients with COVID-19-Associated ARDS Requiring or Not Veno-Venous Extracorporeal Membrane Oxygenation

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Methods

Lymphocyte subpopulations were analysed with AQUIOS CL Flow Cytometry system with AQUIOS Tetra Panels (Tetra-1 and Tetra-2) and Software for automated analysis (Beckman Counter, Inc. Ireland). The fluorochrome-conjugated antibodies used for this study are shown in table below.

Marker	Fluorochrome	AQUIOS Panel
CD3	PC5	Tetra-1
CD4	RD1	Tetra-1
CD8	ECD	Tetra-1
CD45	FITC	Tetra-1
CD19	ECD	Tetra-2
CD56+CD16	RD1	Tetra-2

Software algorithms automatically detect the subpopulations of interest. The lymphocyte gate, based on CD45+, electronic volume and light scatter characteristics, is automatically identified and optimized. All cells were then gated for each subpopulation as follow: CD4+ Th cells (CD3+ CD4+); CD8+ T cytotoxic cells (CD3+ CD8+); B cells (CD3- CD19+); NK cells (CD3- CD56/CD16+), as described in figure below and in AQUIOS Tetra System Guide.

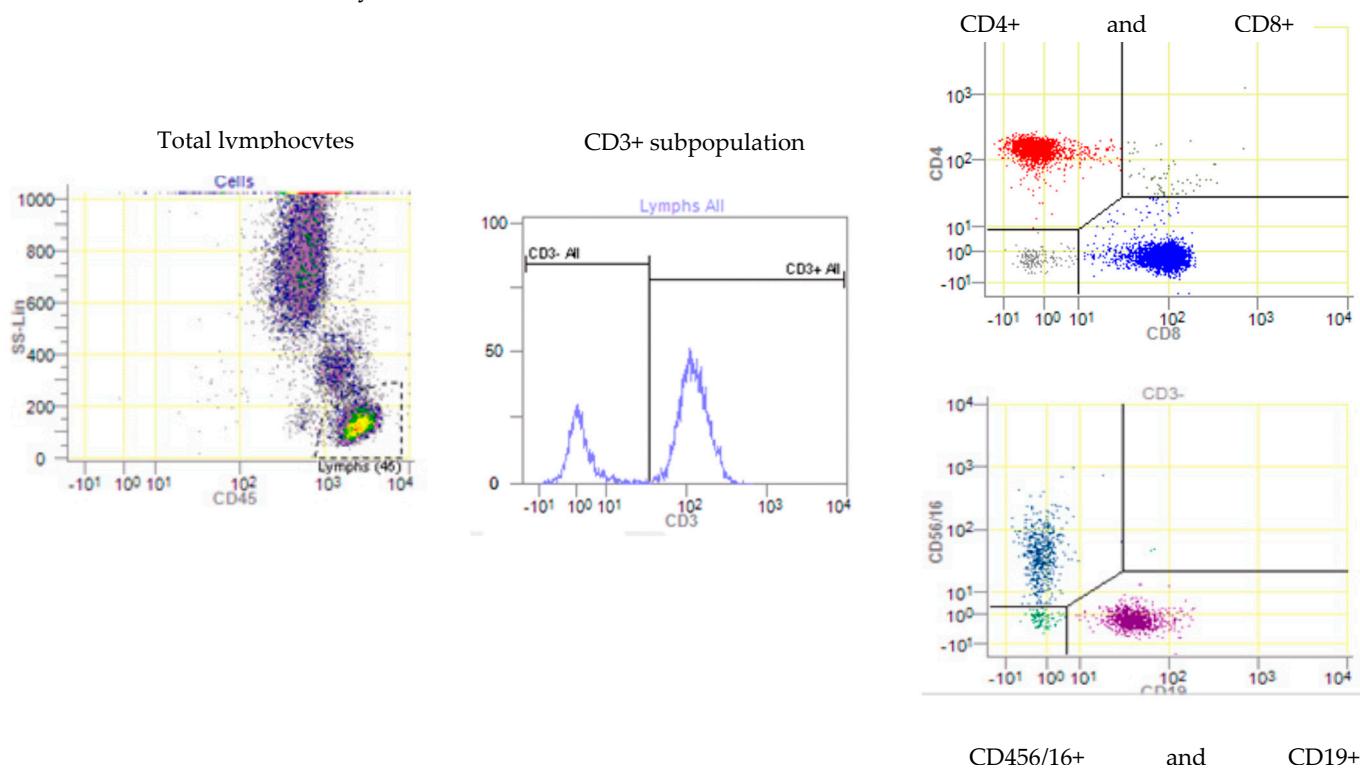


Table S1. Mixed-effects linear regression model involving independent variables related to log-CD3⁺ cell count in both study groups, adjusted for SOFA.

Variables	Coefficient	[95% Confidence Interval]	p	
noECMO group				
Steroids	0.248	-0.603	1.100	0.567
Tocilizumab	0.488	0.041	0.935	0.032
Steroids+Tocilizumab	-0.248	-1.655	-0.621	0.000
ECMO group				
Steroids	-0.082	-0.734	0.569	0.804
Tocilizumab	-0.544	-1.263	0.174	0.138
Steroids+Tocilizumab	-0.911	-1.451	-0.371	0.001

Table S2. Mixed-effects linear regression model involving independent variables related to log-CD19⁺ cell count in both study groups, adjusted for SOFA.

Variables	Coefficient	[95% Confidence Interval]	p
noECMO group			
Steroids	0.758	-0.658 2.175	0.294
Tocilizumab	0.905	0.175 1.635	0.015
Steroids+Tocilizumab	-2.459	-3.595 -1.323	0.000
ECMO group			
Steroids	-0.471	-1.517 0.574	0.377
Tocilizumab	0.393	-0.775 1.562	0.510
Steroids+Tocilizumab	-0.948	-1.840 -0.056	0.037

Table S3. Mixed-effects linear regression model involving independent variables related to PCT in both study groups adjusted for SOFA.

Variables	Coefficient	[95% Confidence Interval]	p
noECMO group			
Steroids	-1.121	-3.785 1.541	0.409
Tocilizumab	-2.105	-3.406 -0.805	0.002
Steroids+Tocilizumab	-3.367	-4.872 -1.862	0.000
ECMO group			
Steroids	-0.145	-2.352 2.062	0.897
Tocilizumab	-0.355	-2.842 2.131	0.779
Steroids+Tocilizumab	-0.265	-1.860 1.329	0.744

Table S4. Mixed-effects linear regression model involving independent variables related to log-CD45⁺ cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.157	-0.713 0.398	0.578
Tocilizumab	0.348	-0.108 0.805	0.135
Steroids+Tocilizumab	-1.252	-1.685 -0.819	0.000

Table S5. Mixed-effects linear regression model involving independent variables related to log-CD3⁺CD4⁺ cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.216	-0.819 0.386	0.482
Tocilizumab	0.318	-0.194 0.832	0.223
Steroids+Tocilizumab	-1.176	-1.644 -0.709	0.000

Table S6. Mixed-effects linear regression model involving independent variables related to log-CD3⁺CD8⁺ cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	0.130	-0.479 0.741	0.674
Tocilizumab	0.200	-0.276 0.677	0.410
Steroids+Tocilizumab	-0.884	-1.377 -0.390	0.000

Table S7. Mixed-effects linear regression model involving independent variables related to log-CD16⁺CD56⁺ cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.204	-0.963 0.555	0.598
Tocilizumab	-0.010	-0.603 0.582	0.973
Steroids+Tocilizumab	-1.462	-2.061 -0.862	0.000

Table S8. Mixed-effects linear regression model involving independent variables related to CD4⁺/CD8⁺ ratio cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.662	-2.737 1.412	0.531
Tocilizumab	0.078	-1.820 1.662	0.929
Steroids+Tocilizumab	-0.198	-1.880 1.484	0.817

Table S9. Mixed-effects linear regression model involving independent variables related to log-neutrophils cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.161	-0.551 0.228	0.417
Tocilizumab	-0.320	-0.733 0.092	0.128
Steroids+Tocilizumab	-0.594	-0.845 0.006	0.054

Table S10. Mixed-effects linear regression model involving independent variables related to log-monocytes cell count adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.150	-0.607 0.306	0.519
Tocilizumab	-0.306	-0.669 0.055	0.097
Steroids+Tocilizumab	-0.702	-0.897 -0.248	0.001

Table S11. Mixed-effects linear regression model involving independent variables related to log-eosinophils cell count in both study groups adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-0.782	-3.430 1.866	0.563
Tocilizumab	1.018	0.003 2.032	0.059
Steroids+Tocilizumab	0.195	-1.594 1.985	0.830

Table S12. Mixed-effects linear regression model involving independent variables related to log-basophils cell count, adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	0.191	-0.443 0.826	0.554
Tocilizumab	0.007	-0.463 0.479	0.974
Steroids+Tocilizumab	-0.653	-1.134 -0.172	0.008

Table S13. Mixed-effects linear regression model involving independent variables related to CRP, adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-28.897	-89.444 31.648	0.350
Tocilizumab	-119.419	-165.062 -73.775	0.000
Steroids+Tocilizumab	-111.183	-159.254 -63.111	0.000

Table S14. Mixed-effects linear regression model involving independent variables related to DDimer, adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-10334.26	-24436.24 3767.72	0.151
Tocilizumab	-3625.475	-11080.05 3829.101	0.340
Steroids+Tocilizumab	-11190.21	-19539.15 -2841.26	0.009

Table S15. Mixed-effects linear regression model involving independent variables related to ferritin concentration, adjusted for SOFA and ECMO.

Variables	Coefficient	[95% Confidence Interval]	p
Steroids	-689.636	-1843.685 464.4123	0.242
Tocilizumab	-1117.214	-2084.336 -150.0922	0.024
Steroids+Tocilizumab	-579.401	-1428.472 269.6692	0.181

Table S16. Proportion of antibiotics prescribed before and at ICU admission.

Antimicrobial Therapy	All Patients (N=31)	ECMO (N=13)	noECMO (N=18)
Pre-ICU			
at home, n (%)	22 (71)	10 (77)	12 (67)
at home and general ward, n (%)	27 (87)	13 (100)	14 (78)
ICU admission, n (%)	31 (100)	13 (100)	18 (100)
Empiric therapy, n (%)	29 (94)	12 (92)	17 (94)
Combination therapy (≥ 2), n (%)	28 (90)	13 (100)	15 (83)

Table S17. Proportion of antibiotic molecules prescribed.

Antibiotic molecules	All Patients (N=31)	ECMO (N=13)	noECMO (N=18)
Beta lactamic	31(100)	13 (100)	18 (100)
Pip/Tazo	10 (32)	7 (54)	3 (17)
Ceftriaxone	9 (29)	0 (0)	9 (50)
Ceftobiprole	6 (19)	2 (15)	4 (22)
Meropenem	4 (13)	3 (23)	1 (5)
Ceft/Avibactam	1 (0)	0 (0)	1 (5)
Cefepime	1 (3)	1 (8)	0 (0)
Vancomycin	6 (19)	5 (38)	1 (5)
Linezolid	7 (23)	6 (46)	1 (5)
Daptomycin	1 (3)	0 (0)	1 (5)
Fosfomycin	1 (3)	0 (0)	1 (5)
Macrolids	2 (6)	0 (0)	2 (10)
Levofloxacin	1 (3)	1 (8)	0 (0)
Antifungal therapy	8 (26)	4 (31)	4 (22)

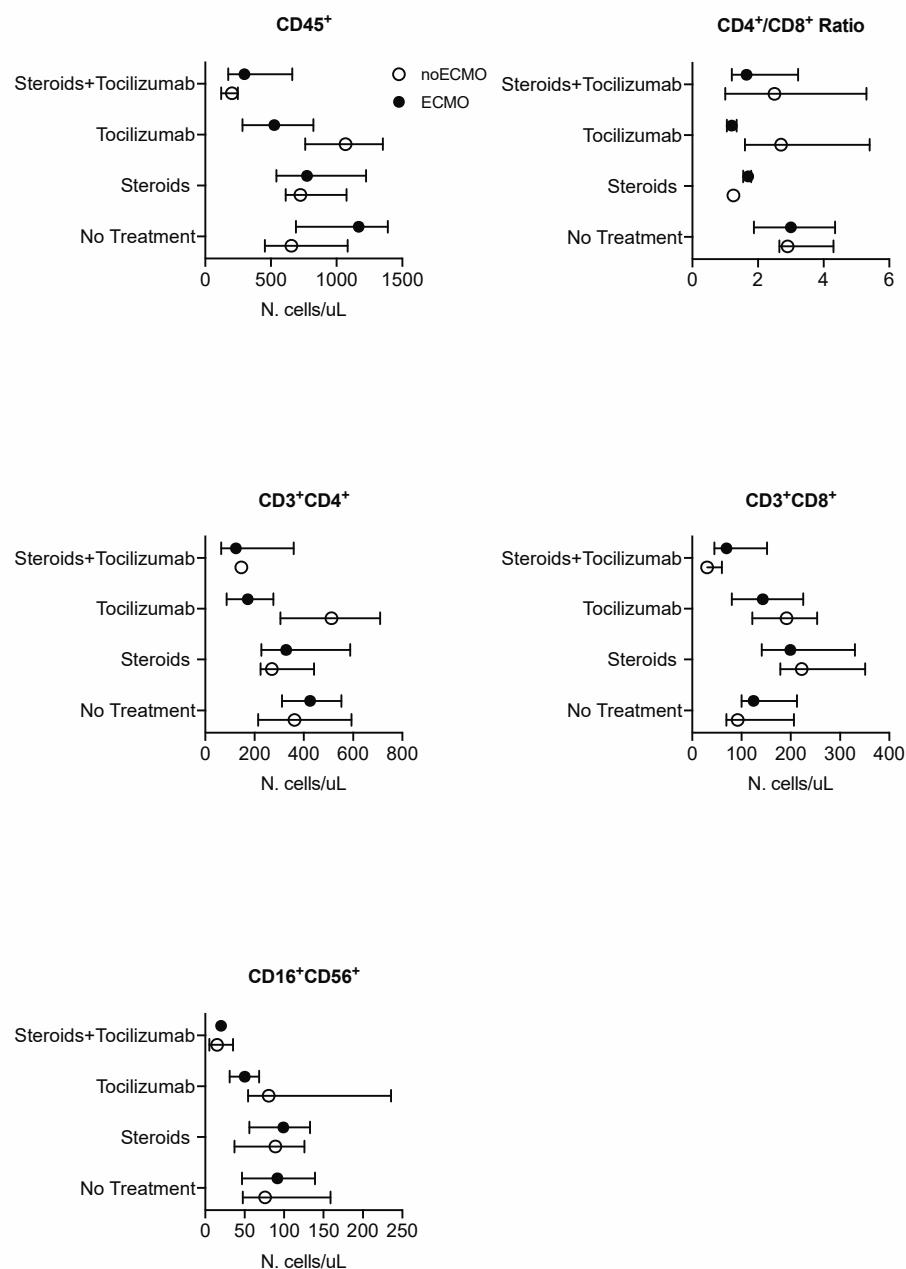


Figure S1. CD45⁺, CD3⁺CD4⁺ (Th cell), CD3⁺CD8⁺, CD4⁺/CD8⁺, CD16⁺CD56⁺ (NK cell) subpopulation counts in presence or not of Steroids and Tocilizumab therapy in COVID-19 patients with ARDS who required or not ECMO support.

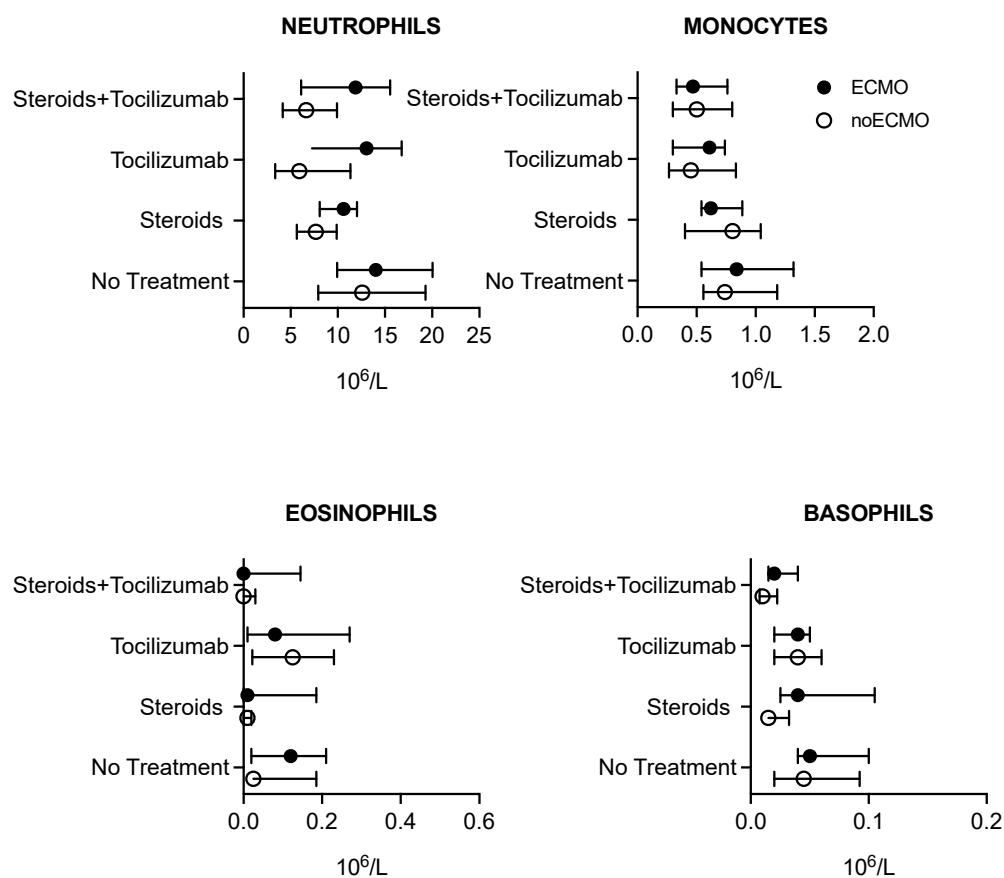


Figure S2. Neutrophils, monocytes, eosinophils and basophils counts in presence or not of Steroids and Tocilizumab therapy in COVID-19 patients with ARDS who required or not ECMO support.

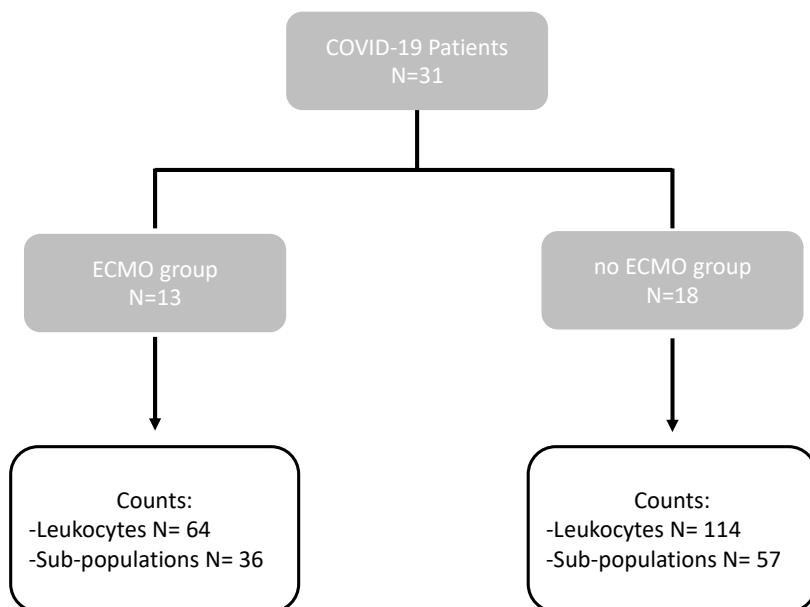


Figure S3. Diagram showing patients number and leukocyte count grouping.