

Supplementary Material

Impact of sex on circulating leukocytes composition in COPD patients

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Supplementary Table 1. List of fluorochrome-conjugated antibodies used for FACS analysis

Marker	Clone	Fluorochrome	Company
CCR6	REA190	APC	Miltenyi
CD127	HIL-7R-M21	PE-Cy 7	BD
CD14	REA599	VioBlue	Miltenyi
CD141	AD5-14H12	APC	Miltenyi
CD16	3G8	PerCP-Cy 5.5	BioLegend
CD19	LT19	PE-Vio 770	Miltenyi
CD1c	AD5-8E7	PE	Miltenyi
CD25	BC96	APC-Cy 7	BioLegend
CD3	BW264/56	PerCP-Vio 700 PE-Vio 770	Miltenyi
CD303	AC144	FITC	Miltenyi
CD4	M-T466	VioBlue PE	Miltenyi
CD45	HI30	BV510	BD
CD56	MEM-188	FITC	BioLegend
CD8	REA734	APC-Vio 770	Miltenyi
CRTH2	BM-16	PE	Miltenyi
CXCR3	REA232	VioBright FITC	Miltenyi
HLA-DR	L243	APC-Cy 7	BioLegend

Abbreviations: APC, allophycocyanin; APC-Cy7, allophycocyanin-cyanin 7; APC-Vio770, allophycocyanin-Vio770; BV510, brilliant violet 510; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PE-Cy7, phycoerythrin-cyanin 7; PE-Vio770, phycoerythrin-Vio770; PerCP-Cy5.5, peridinin chlorophyll-cyanin 5.5; PerCP-Vio700, peridinin chlorophyll-Vio700.

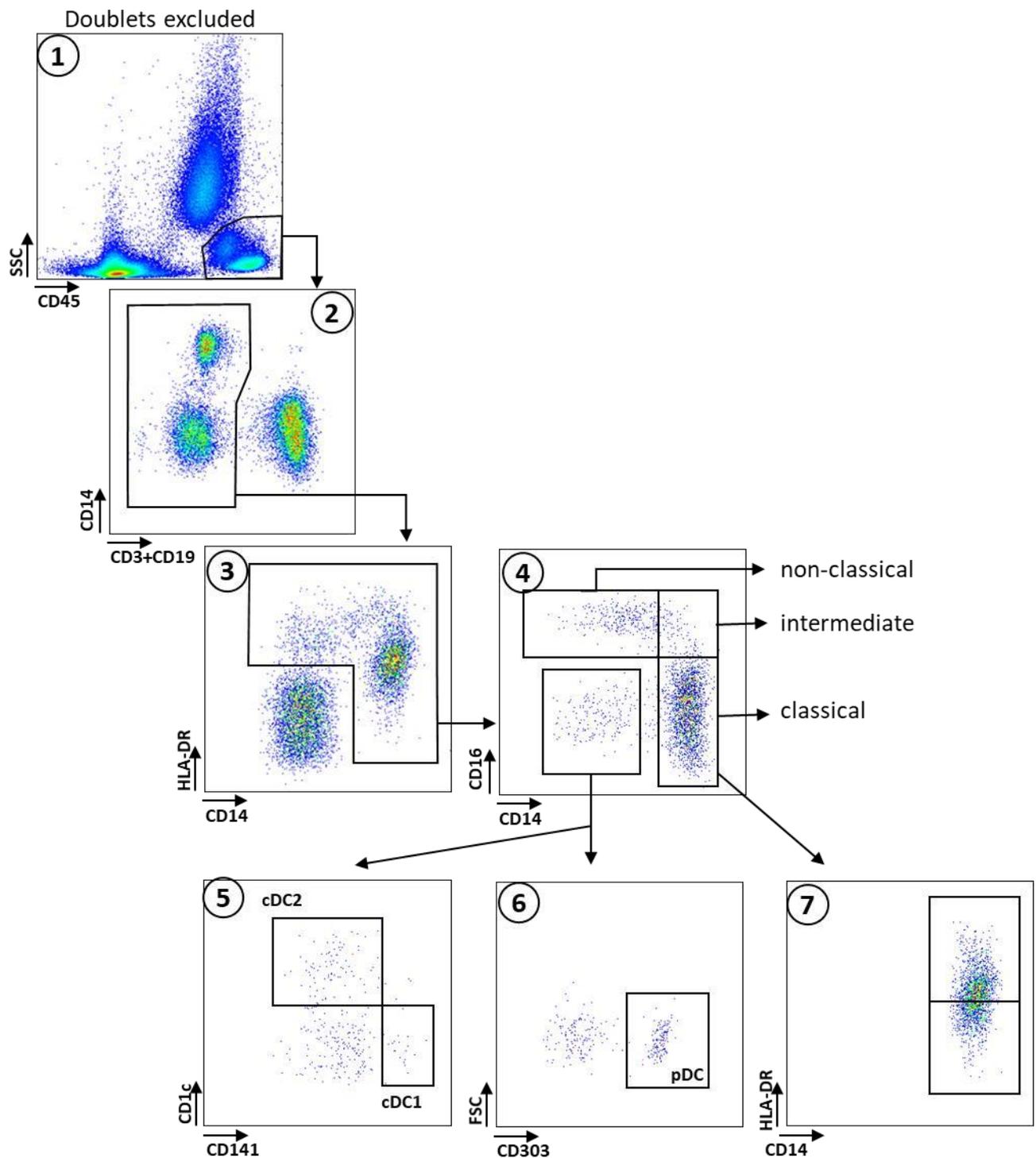
Supplementary Table 2. Concentration and differential counts of leukocyte populations calculated by automated CBC

ALL DONORS	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
WBC (10 ⁶ /mL)	63	6.050 (5.310-6.940)	50	6.515 (5.448-7.450)	0.4767 ^t
NLR	63	2.1 (1.7-2.7)	50	2.400 (1.700-3.625)	0.073 [§]
Neutrophils (10 ⁶ /mL)	63	3.66 (2.96-4.08)	50	3.985 (3.008-5.040)	0.2364 [§]
Lymphocytes(10 ⁶ /mL)	63	1.66 (1.36-2.13)	50	1.58 (1.21-1.86)	0.062[§]
Monocytes (10 ⁶ /mL)	63	0.49 (0.42-0.61)	50	0.49 (0.39-0.63)	0.7833 [§]
Eosinophils (10 ⁶ /mL)	63	0.13 (0.09-0.22)	50	0.14 (0.07-0.20)	0.470 [§]
Basophils (10 ⁶ /mL)	63	0.04 (0.03-0.06)	45	0.04 (0.03-0.05)	0.497 [§]
Neutrophils (%)	63	60.3 (56.0-64.6)	50	61.25 (55.6-70.2)	0.129 [§]
Lymphocytes (%)	63	28.7 (24.2-32.4)	50	25.7 (18.75-32.18)	0.0521 ^t
Monocytes (%)	63	8.2 (6.9-9.7)	50	7.796 (6.300-9.537)	0.219 [§]
Eosinophils (%)	63	2.1 (1.5-3.5)	50	2.0 (1.3-3.4)	0.483 [§]
Basophils (%)	63	0.7 (0.5-0.9)	45	0.6 (0.45-0.85)	0.321 [§]
MALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
WBC (10 ⁶ /mL)	35	5.91 (5.32-6.59)	31	6.89 (5.80-7.62)	0.0017^t
NLR	35	2.2 (1.7-2.7)	31	2.730 (1.964-4.075)	0.0256[§]
Neutrophils (10 ⁶ /mL)	35	3.47 (2.86-3.87)	35	4.48 (3.26-5.04)	0.0021[§]
Lymphocytes(10 ⁶ /mL)	35	1.62 (1.35-1.87)	31	1.66 (1.10-1.87)	0.5937 ^t
Monocytes (10 ⁶ /mL)	35	0.48 (0.44-0.61)	31	0.56 (0.47-0.64)	0.1942 ^t
Eosinophils (10 ⁶ /mL)	35	0.13 (0.08-0.2)	31	0.14 (0.07-0.21)	0.8857 [§]
Basophils (10 ⁶ /mL)	35	0.04 (0.03-0.06)	27	0.04 (0.03-0.05)	0.5216 [§]
Neutrophils (%)	35	59.7 (56-66)	31	62.9 (55.8-71-1)	0.0191^t
Lymphocytes (%)	35	27.8 (23.9-32.6)	31	24.3 (17.2-29.6)	0.0065^t
Monocytes (%)	35	8.4 (7.3-9.7)	31	7.9 (6.700-9.647)	0.4871 ^t
Eosinophils (%)	35	2.4 (1.4-3.5)	31	2.1 (1.3-3.4)	0.4041 [§]
Basophils (%)	35	0.8 (0.5-0.9)	27	0.5 (0.4-0.9)	0.0675 [§]
FEMALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
WBC (10 ⁶ /mL)	28	6.265 (5.288-7.933)	19	5.61 (4.17-6.83)	0.038^t
NLR	28	2.0 (1.625-2.700)	19	1.9 (1.5-3.3)	0.868 [§]
Neutrophils (10 ⁶ /mL)	28	3.82 (3.130-5.068)	19	3.08 (2.36-4.70)	0.1490 ^t
Lymphocytes(10 ⁶ /mL)	28	1.99 (1.560-2.443)	19	1.55 (1.27-1.86)	0.0294^t
Monocytes (10 ⁶ /mL)	28	0.52 (0.3900-0.6775)	19	0.40 (0.34-0.51)	0.0286[§]
Eosinophils (10 ⁶ /mL)	28	0.15 (0.0925-0.2650)	19	0.12 (0.08-0.18)	0.3237 [§]
Basophils (10 ⁶ /mL)	28	0.04 (0.02-0.06)	18	0.04 (0.0275-0.0525)	0.5685 ^t
Neutrophils (%)	28	61 (54.08-64.43)	19	58 (52.6-70.1)	0.9529 [§]
Lymphocytes (%)	28	29.4 (24.25-32.40)	19	30 (21.1-36.3)	0.8834 ^t
Monocytes (%)	28	7.65 (6.825-9.675)	19	7.1 (5.8-9.5)	0.2934 [§]
Eosinophils (%)	28	2.1 (1.500-3.825)	19	2 (1.7-3.3)	0.97 [§]
Basophils (%)	28	0.6 (0.425-0.875)	18	0.7 (0.575-0.825)	0.5781 ^t

Values reported indicate the number (n) of subjects and the median for each parameter (interquartile range). t: Student t-test; \$: Mann Whitney test. Significant differences are in bold.

Abbreviations: COPD, chronic obstructive pulmonary disease; NLR, neutrophil-to-lymphocyte ratio; WBC, white blood cells.

Supplementary Figure 1



Supplementary Figure 1. Gating strategy for myeloid subtypes.

Gating was performed after doublets exclusion. PBMC were selected on CD45⁺ (1). T and B cells were excluded by CD3⁺ and CD19⁺ (2). CD14⁺/HLA-DR⁺ cells were gated (3) and displayed as CD14 vs CD16 (4). Non-classical CD14^{low}CD16⁺ monocytes, intermediate CD14⁺CD16⁺ monocytes and classical CD14⁺CD16⁻ monocytes were defined (4). From CD14⁻CD16⁻ cells, cDC1 were identified as CD141⁺CD1c⁻ cells and cDC2 as CD141⁻CD1c⁺ cells (5), while pDC were identified as CD303⁺ population (6). On classical monocytes monocyte-myeloid-derived suppressor cells (M-MDSC) were identified as CD14⁺HLA-DR^{low} (7).

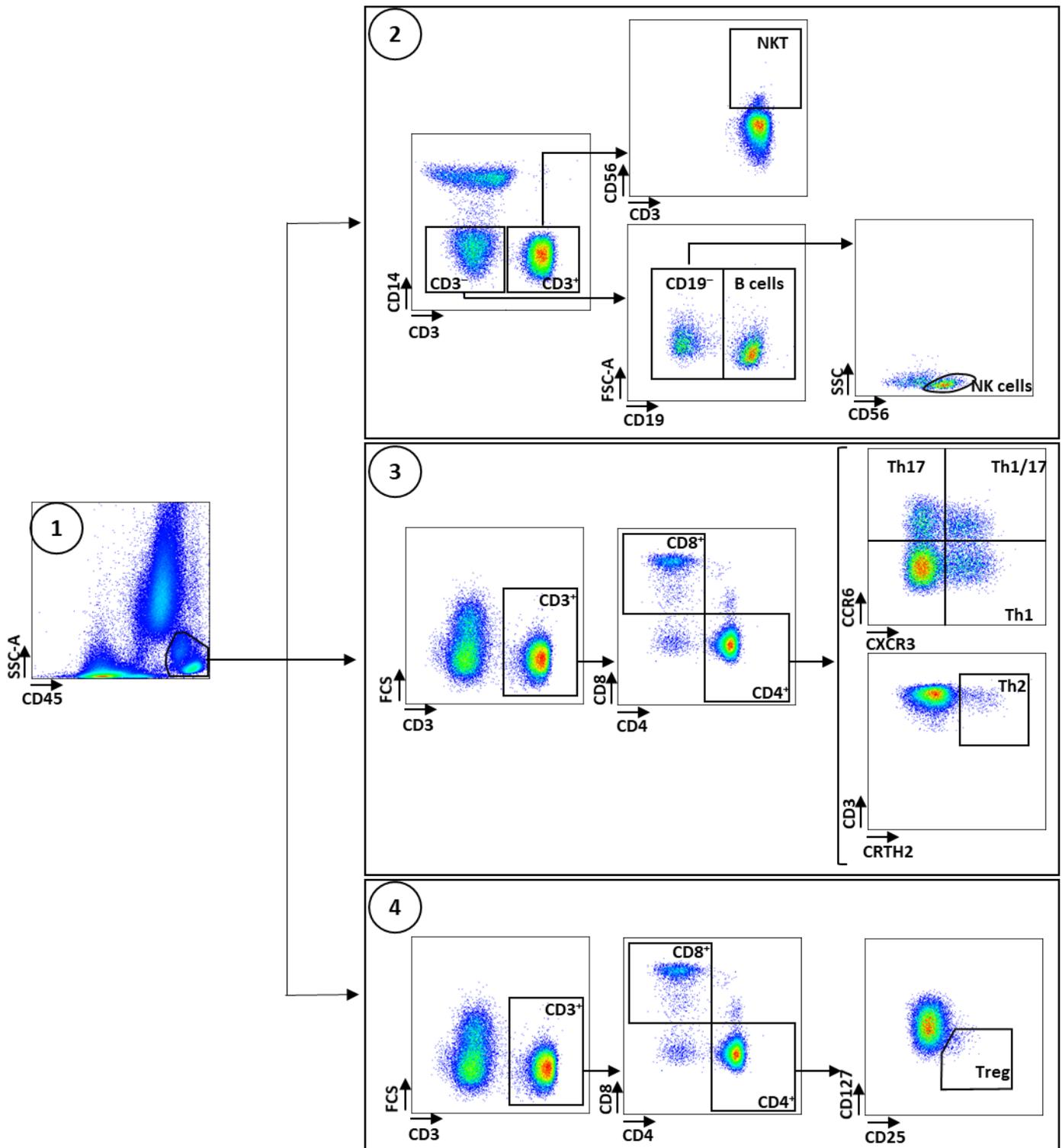
Supplementary Table 3. Concentration and differential counts of the main monocyte and dendritic cell subtypes identified by FACS

MALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
Classical Mo (% of Mo)	35	87.4 (83.0-90.4)	31	88.9 (85.4-91.1)	0.2193 ^t
M-MDSC (% of Mo)	35	12.1 (7.5-19.9)	31	12.0 (4.4-24.8)	0.7089 ^{\$}
Intermediate Mo (% of Mo)	35	3.1 (2.34-3.78)	35	3.73 (2.36-4.42)	0.2480 ^{\$}
Non-classical Mo (% of Mo)	35	10.2 (6.1-12.6)	31	7.0 (5.5-11.1)	0.2130 ^{\$}
DC (10 ⁶ /mL)	35	0.022 (0.016-0.03)	31	0.023 (0.016-0.03)	0.9947 ^t
pDC (% of DC)	35	36.68 (32.71-49.37)	31	40.17 (32.57-50.96)	0.668 ^t
cDC1 (% of DC)	35	5.19 (3.95-7.02)	31	5.07 (3.85-7.34)	0.7666 ^{\$}
cDC2 (% of DC)	35	57.16 (32.57-50.96)	31	54.28 (43.71-62.72)	0.5064 ^t
FEMALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
Classical Mo (% of Mo)	28	87.95 (85.25-90.88)	19	88.1 (78.7-91.2)	0.6099 ^{\$}
M-MDSC (% of Mo)	28	9.25 (3.85-25.68)	19	7.4 (4.3-19.5)	0.61 ^{\$}
Intermediate Mo (% of Mo)	28	2.705 (2.175-4.400)	19	3.37 (2.78-4.48)	0.1343 ^{\$}
Non-classical Mo (% of Mo)	28	9.2 (5.725-11.930)	19	8.2 (5.0-13.5)	0.8524 ^t
DC (10 ⁶ /mL)	28	0.021 (0.0135-0.0308)	19	0.018 (0.014-0.022)	0.2597 ^{\$}
pDC (% of DC)	28	41.09 (31.96-46.13)	19	44.74 (39.90-54.79)	0.1265 ^t
cDC1 (% of DC)	28	4.265 (2.125-5.160)	19	5.24 (3.95-7.29)	0.055 ^t
cDC2 (% of DC)	28	56.05 (47.97-64.26)	19	47.37 (41.10-56.41)	0.0626 ^t

Values reported indicate the number (n) of subjects and the median for each parameter (interquartile range). Mo: monocytes; t: Student t-test; \$: Mann Whitney test. Significant differences are in bold.

Abbreviations: cDC1, type 1 conventional dendritic cells; cDC2, type 2 conventional dendritic cells; COPD, chronic obstructive pulmonary disease; DC, dendritic cells; M-MDSC, monocyte-myeloid derived suppressor cells; Mo, monocytes; pDC, plasmacytoid dendritic cell.

Supplementary Figure 2



Supplementary Figure 2. Gating strategy for lymphocyte subtypes.

Singlets were gated based on FSC-A and FSC-H parameters (not shown) and mononuclear cells were selected as SSC^{low} CD45⁺ (panel 1). The staining for NK cells (panel 2), T helper subtypes (panel 3), and Tregs (panel 4) was performed separately with appropriate antibodies sets. (2) CD14⁺ monocytes were excluded; NK cells were defined as CD3⁻CD19⁻CD56⁺ and B cells as CD3⁻CD19⁺ cells; NKT cells were defined as CD3⁺CD56⁺ cells.

(3) T cells were identified as CD3⁺ cells. Expression of CD8⁺ or CD4⁺ identified CTL or T helper, respectively. T helper subsets were defined by CCR6 and CXCR3 expression: Th1 defined as CXCR3⁺CCR6⁻ cells, Th2 defined as CD3⁺CRTH2⁺, Th17 defined as CXCR3⁻ CCR6⁺ and Th1/Th17 hybrid population defined as CXCR3⁺CCR6⁺. (4) Treg cells were identified from CD4⁺ T cells as CD127⁻CD25⁺ cells.

Supplementary Table 4. Concentration and differential counts of the main lymphocyte subtypes identified by FACS

MALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
T-lymphocytes (10 ⁶ /mL)	35	1.03 (0.82-1.12)	31	0.97 (0.7-1.2)	0.965 [†]
B-lymphocytes (10 ⁶ /mL)	35	0.13 (0.08-0.22)	31	0.17 (0.06-0.22)	0.5458 [§]
NK cells (10 ⁶ /mL)	35	0.3 (0.16-0.48)	31	0.23 (0.17-0.39)	0.5762 [§]
NKT cells (10 ⁶ /mL)	34	0.09 (0.050-0.135)	31	0.06 (0.03-0.13)	0.293 [§]
CD4+Th (% T-lymphocytes)	35	63.3 (53.9-70.1)	31	65.2 (50.2-73.5)	0.9695 [†]
CD8+CTL (% T-lymphocytes)	35	25.5 (20.1-36.8)	31	33.3 (19.7-44.3)	0.0811 [§]
Th1 (% of CD4+)	35	19.7 (16.2-27.8)	31	17.8 (13.3-22.2)	0.1754 [§]
Th2 (% of CD4+)	29	1.71 (1.250-2.915)	13	1.57 (0.920-2.895)	0.5587 [§]
Th17 (% of CD4+)	35	11 (9.3-16.3)	31	11.1 (8.3-16.7)	0.6716 [†]
Th1/17 (% of CD4+)	35	8.1 (5.3-11.9)	31	7 (5.0-9.9)	0.4226 [§]
Treg (% of CD4+)	35	2.28 (1.75-3.09)	31	3.26 (2.53-5.09)	0.0114[§]
FEMALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
T-lymphocytes (10 ⁶ /mL)	28	1.1 (0.99-1.49)	18	0.97 (0.803-1.278)	0.1957 [§]
B-lymphocytes (10 ⁶ /mL)	28	0.24 (0.1375-0.3500)	18	0.15 (0.0975-0.2300)	0.0337[§]
NK cells (10 ⁶ /mL)	28	0.25 (0.1450-0.3875)	19	0.2 (0.15-0.31)	0.1234 [†]
NKT cells (10 ⁶ /mL)	26	0.12 (0.038-0.235)	19	0.07 (0.03-0.13)	0.3 [§]
CD4+ Th (% T-lymphocytes)	26	67.15 (61.43-75.28)	18	62.55 (55.35-71.93)	0.117 [†]
CD8+ CTL (% T-lymphocytes)	26	26.25 (17.3-36.4)	18	31.15 (23.65-38.58)	0.1304 [†]
Th1 (% of CD4+)	28	23 (17.20-27.73)	19	22.3 (19.8-26.4)	0.7031 [§]
Th2 (% of CD4+)	21	1.620 (1.215-2.785)	11	1.6 (1.29-2.10)	0.5237 [†]
Th17 (% of CD4+)	28	11.05 (8.45-13.15)	19	11.6 (6.7-14.5)	0.7760 [§]
Th1/17 (% of CD4+)	28	6.75 (4.90-9.65)	19	8.5 (4.5-11.3)	0.2937 [§]
Treg (% of CD4+)	28	2.443 (1.193-3.860)	19	4.1 (2.75-7.18)	0.0014[†]

Values reported indicate the number (n) of subjects and the median for each parameter (interquartile range). t: Student t-test; \$: Mann Whitney test. Significant differences are in bold.

Abbreviations: COPD, chronic obstructive pulmonary disease; CTL, cytotoxic T lymphocytes; NK, natural killer cells; NKT, natural killer T cells ; Th, T helper cells; Th1, type 1 T helper cells; Th2, type 2 T helper cells; Th17, T helper 17 cells, Th1/17, Th1-like Thelper 17 cells; Treg, regulatory T cells.

Supplementary Table 5. Smoking status and COPD comorbidities in male and female COPD patients and control donors

MALES	Control donors		COPD patients		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
Smoker (never/former/current)		25/10/0		1/22/6	<0.0001 ^x
Smoking (pack/year)	35	0 (0-12)	29	40 (25-58.15)	<0.0001 ^{\$}
Charlson Comorbidity Index	26	1 (0-3)	30	2 (1-3)	0.0697 ^{\$}
CVD (yes/no)		8/22		11/20	0.4572 ^x
CAD (yes/no)		2/33		7/20	0.0336 ^x
Allergy (yes/no)		4/25		1/29	0.1945 ^x
Hypertension (yes/no)		18/15		25/4	0.012 ^x
Dyslipidemia (yes/no)		10/21		11/16	0.5884 ^x
Artrrosis (yes/no)		0/35		15/11	<0.0001 ^x
Thyroid diseases (yes/no)		2/33		2/29	1 ^x
BMI	30	25.57 (24.03-29.48)	22	27.25 (24.16-30.06)	0.6297 ^{\$}
FEMALES	Control donors		COPD		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
Smoker (never/former/current)		17/8/3		4/10/5	0.0258 ^x
Smoking (pack/year)	27	0 (0 52)	19	22.5 (6.5-40)	0.0004 ^{\$}
Charlson Comorbidity Index	19	2 (1-2)	19	1 (0-2)	0.1969 ^{\$}
CVD (yes/no)		3/23		3/16	0.6861 ^x
CAD (yes/no)		1/27		2/17	0.5571 ^x
Allergy (yes/no)		6/20		5/14	1 ^x
Hypertension (yes/no)		16/10		13/6	0.7565 ^x
Dyslipidemia (yes/no)		6/19		6/13	0.735 ^x
Artrrosis (yes/no)		10/17		5/14	0.5329 ^x
Thyroid diseases (yes/no)		5/20		5/13	0.7174 ^x
BMI	23	27.03 (24.82-28.76)	18	27.00 (22.08-34.53)	0.5478 ^{\$}

Values reported for Smoking (pack/year), Charlson Comorbidity index and BMI indicate the number (n) of subjects and the median for each parameter (interquartile range), for the remaining parameters the number of subjects belonging to each sub-class are reported. \$ – Mann-Whitney test; ^x – Fisher's exact test. Significant differences are in bold.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease.

Supplementary Table 6. COPD severity, smoking status and COPD comorbidities in male and female COPD patients

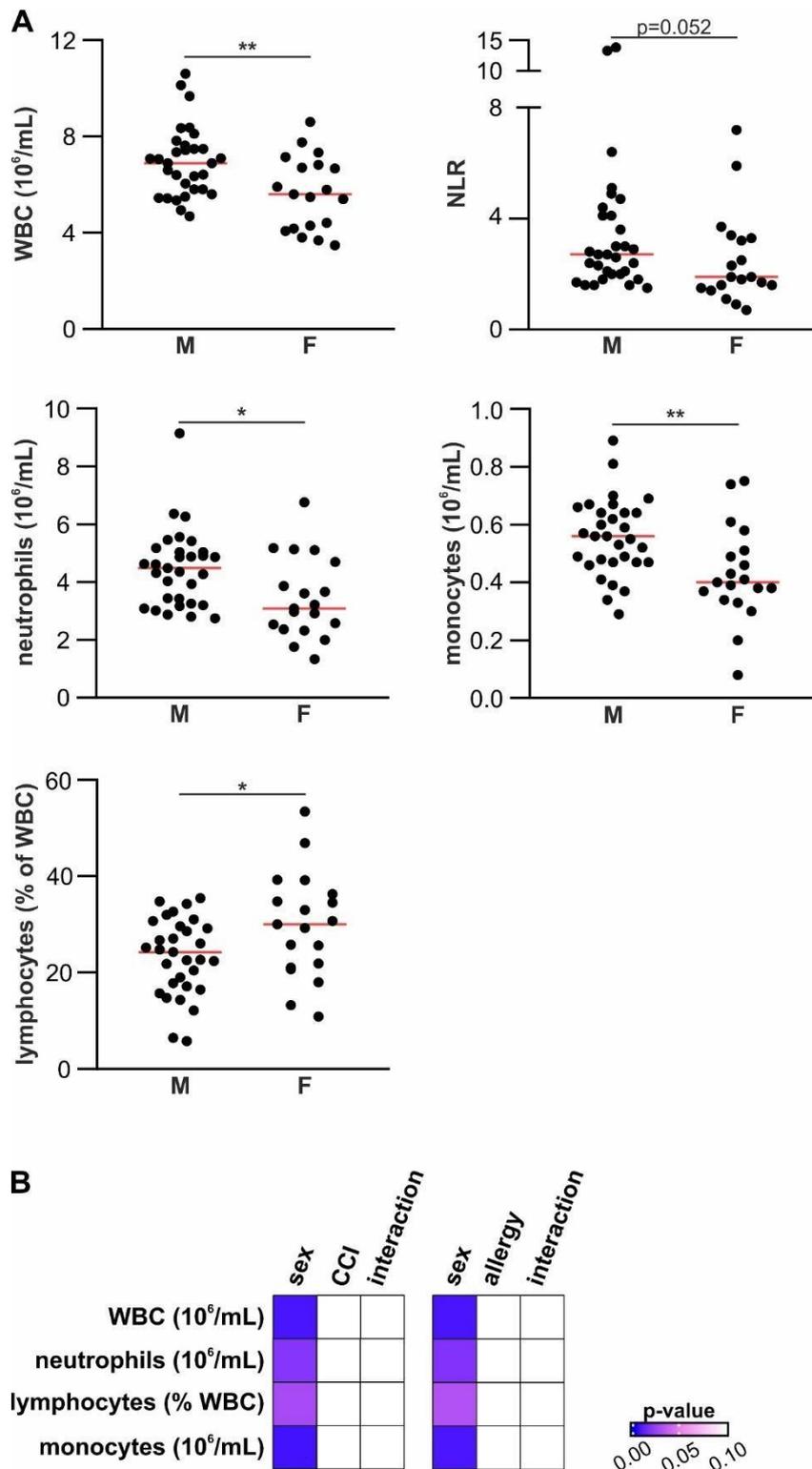
COPD	Male		Female		P value
	n	Median (Q1-Q3)	n	Median (Q1-Q3)	
GOLD (1/2/3/4)		8/11/9/2		4/10/5/0	0.543 ^{x*}
FEV₁ % of predicted	30	59 (43-82)	19	70 (50-76)	0.5295 ^t
FEV₁/FVC	30	0.503 (0.382-0.601)	19	0.504 (0.395-0.554)	0.808 ^t
Smoker (never/former/actual)		1/22/6		4/10/5	0.105 ^x
Charlson Comorbidity Index	30	2 (1-3)	19	1 (0-2)	0.0128 ^{\$}
CVD (yes/no)		11/20		3/16	0.1322 ^x
CAD (yes/no)		7/20		2/17	0.2704 ^x
Allergy (yes/no)		1/29		5/14	0.0269 ^x
Hypertension (yes/no)		25/4		13/6	0.164 ^x
Dyslipidemia (yes/no)		11/16		6/13	0.555 ^x
Artrosis (yes/no)		15/11		5/14	0.067 ^x
Thiroid diseases (yes/no)		2/29		5/13	0.0841 ^x
BMI	22	27.25 (24.16-30.06)	18	27.00 (22.08-34.53)	0.973 ^{\$}

Values reported for FEV₁ % of predicted, FEV₁/FVC, Charlson Comorbidity index and BMI indicate the number (n) of subjects and the median for each parameter (interquartile range), for the remaining parameters the number of subjects belonging to each sub-class are reported. ^t – Student t-test, ^{\$} – Mann-Whitney test; ^x – Fisher’s exact test. Significant differences are in bold.

* GOLD stage 3 and 4 were combined for Fisher’s exact test calculation.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease.

Supplementary Figure 3



Supplementary Figure 3. Sex-specific alteration of circulating leukocytes in COPD patients.

(A) White blood cell total count (WBC), neutrophil-to-lymphocyte ratio (NLR), neutrophil total count, monocyte total count and differential count of lymphocytes (% of WBC) in male and female COPD patients. The red line represents the median. *: $P < 0.05$, **: $P < 0.01$ according to Mann Whitney or Student t-test. (B) Heat map representation of the ANOVA analysis in COPD patients. P -value

obtained from the ANOVA analysis for sex, Charlson Comorbidity index (CCI) or allergy, alone or interacting with sex (interaction) are plotted according to the scale shown on the right.

Supplementary Table 7. Correlation of leukocyte populations with parameters of COPD severity.

	MALES			FEMALES		
	FEV ₁ %	GOLD	FEV ₁ /FVC	FEV ₁ %	GOLD	FEV ₁ /FVC
WBC (10⁶/mL)	0.237 (0.206) [§]	-0.171 (0.367) [§]	0.395 (0.031)[§]	0.024 (0.923) [†]	0.221 (0.364) [§]	-0.026 (0.914) [†]
lymphocytes (10⁶/mL)	0.339 (0.066) [†]	-0.292 (0.117) [§]	0.395 (0.031)[†]	0.352 (0.139) [†]	-0.279 (0.248) [§]	0.265 (0.272) [†]
B cells (10⁶/mL)	0.567 (0.001)[§]	-0.560 (0.001)[§]	0.491 (0.006)[§]	-0.040 (0.874) [§]	-0.108 (0.669) [§]	-0.424 (0.079) [§]
NKT (10⁶/mL)	0.411 (0.024)[§]	-0.449 (0.013)[§]	0.373 (0.043)[§]	0.192 (0.43) [§]	-0.138 (0.573) [§]	0.387 (0.102) [§]
pDC (% of DC)	-0.301 (0.106) [†]	-0.356 (0.054) [§]	0.087 (0.648) [†]	0.498 (0.03)[†]	-0.368 (0.121) [§]	0.408 (0.083) [†]
M-MDSC (% of Mo)	-0.069 (0.714) [§]	0.137 (0.469) [§]	0.057 (0.766) [§]	0.791 (<0.0001)[§]	-0.744 (0.0003)[§]	0.677 (0.001)[§]
eosinophils (10⁶/mL)	-0.139 (0.462) [§]	0.267 (0.153) [§]	-0.094 (0.622) [§]	-0.661 (0.002)[§]	0.723 (0.0005)[§]	-0.269 (0.265) [§]
basophils (10⁶/mL)	0.353 (0.071) [§]	-0.201 (0.315) [§]	0.288 (0.145) [§]	-0.392 (0.107) [†]	0.653 (0.003)[§]	-0.363 (0.139) [†]
cDC2 (% of DC)	-0.301 (0.106) [†]	0.411 (0.024)[§]	-0.095 (0.618) [†]	-0.372 (0.117) [†]	0.254 (0.293) [§]	-0.291 (0.227) [†]
cDC1 (% of DC)	0.196 (0.298) [§]	-0.202 (0.285) [§]	0.032 (0.868) [§]	-0.551 (0.015)[†]	0.479 (0.038)[§]	-0.514 (0.025)[§]
Th1 (% of CD4+)	0.065 (0.732) [§]	-0.026 (0.891) [§]	-0.280 (0.354) [§]	-0.519 (0.023)[†]	0.436 (0.046)[§]	-0.421 (0.073) [†]
T reg (% of CD4+)	-0.251 (0.181) [§]	0.235 (0.211) [§]	-0.249 (0.185) [§]	-0.572 (0.01)[†]	0.547 (0.016)[§]	-0.407 (0.084) [†]

†: Pearson correlation coefficient; §: Spearman correlation coefficient

p-value for the correlation is reported in brackets below the correlation coefficient value. Significant differences are in bold.

Abbreviations: cDC1, type 1 conventional dendritic cells; cDC2, type 2 conventional dendritic cells; DC, dendritic cells; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, The Global Initiative for Obstructive Lung Disease; Mo, monocytes; M-MDSC, monocytemyeloid-derived suppressor cells; NKT, natural killer T cells; pDC, plasmacytoid dendritic cell; Th1, type 1 T helper cells; Treg, regulatory T cells; WBC, white blood cells.