

Supplementary

Table S1 Comparison of background characteristics and biomarker levels of healthy non-smokers and smokers with COPD and without AO [mean (SD), median (IQR) or proportion (%) of the main characteristics]

Characteristics	Healthy non-smokers (n=35)	P (between healthy non-smokers and smokers with COPD)	P (between healthy non-smokers and smokers without AO)
Demographics			
Age, mean ± SD	53.37±9.76	NS	NS
Male, n (%)	8 (22.9)	<0.0001 ^{***}	<0.0001 ^{***}
Physiology			
BMI, kg/m ² , mean ± SD	26.44±4.25	NS	NS
FEV1, liters, mean ± SD	2.93±0.72	<0.013 ^{*#}	NS
FVC, liters, mean ± SD	3.52±0.87	NS	NS
FEV1/FVC, %, mean ± SD	83.27±5.541	<0.0001 ^{*#}	<0.0001 ^{*#}
Cytokines			
IL-4, pg/mL	1.32 (1.01; 1.60)	NS	NS
IL-6, pg/mL	0.99 (0.54; 1.86)	0.016 ^{****}	NS
IL-8, pg/mL	0.98 (0.78; 1.40)	0.019 ^{****}	NS
IL-10, pg/mL	4.28 (3.34; 5.24)	0.002 ^{****}	0.001 ^{****}
hs-CRP, mg/L	0.13 (0.06; 1.21)	<0.0001 ^{****}	<0.0001 ^{****}
TNF-α, pg/mL	1.48 (1.12; 2.04)	NS	<0.0001 ^{****}
Enzyme			
MMP9, ng/mL	6.99 (5.38; 12.35)	0.042 ^{****}	NS
Pneumoproteins			
SP-D, ng/mL	287 (185.00; 337.00)	<0.0001 ^{****}	<0.0001 ^{****}
CC16, ng/mL	5.51 (4.76; 8.53)	NS	NS
Other biomarkers			
TIMP, ng/mL	1,312.00 (1,147.00; 1,535.00)	NS	NS
Vitamin D	18.97 (13.36; 24.80)	0.040 ^{****}	NS

^{*}, statistically significant factors; [#], independent samples *t*-test, ^{**}, Pearson Chi-square test, ^{***}, non-parametric: Mann-Whitney U test for distribution. COPD, chronic obstructive pulmonary disease; AO, airflow obstruction; SD, standard deviation; IQR, interquartile range; BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; MMP9, matrix metalloproteinase 9; CC16, Clara cell secretory protein 16; SP-D, surfactant protein D; TIMP, tissue inhibitor of metalloproteinases; NS, non-significant.

Table S2 Selected biomarkers and their association with COPD or exposure to tobacco smoke

Biomarkers	Comments
COPD biomarkers from cohorts/prospective studies and systematic reviews/meta-analysis (Cazzola M <i>et al.</i> , 2019)	
(I) Single biomarkers	
CC16, SP-D, vitamin D, WBC	Associated with disease progression
SP-D, NTproBNP, MMP-9, hs-CRP	Associated with exacerbation risk
NTproBNP	Associated with mortality
(II) Combination of biomarkers	
CC16 + sRAGE + fibrinogen + hs-CRP + SP-D; hs-CRP + WBC + IL-6 + IL-8 + fibrinogen; hs-CRP + IL-6 + TNF- α	Associated with disease progression
WBC + fibrinogen + hs-CRP + IL-6 + IL-8 + TNF- α	Associated with exacerbation risk
WBC + fibrinogen + hs-CRP + IL-6 + IL-8 + TNF- α ; CC16 + sRAGE + fibrinogen + hs-CRP + SP-D; SP-D + hs-CRP + fibrinogen	Associated with mortality
Interpretation of single COPD biomarkers (Stockley RA <i>et al.</i> , 2019)	
CC16	Stable, anti-inflammatory
SP-D	Stable, anti-inflammatory, antioxidant
hs-CRP	Unstable, nonspecific acute-phase response
IL-6	Unstable, pro- and anti-inflammatory, acute-phase response, chemokine signaling but complete, overlap in COPD
MMPs (with TIMP)	Contribute to tissue damage, activate of growth factors, through those lead to small airway disease
α 1-antitrypsin	The major serum inhibitor of serine proteinases (especially neutrophil elastase)
A model of assessing COPD by using multiple classes of proteins of blood and sputum (Moon JY <i>et al.</i> , 2018)	
Acute phase proteins (incl. hs-CRP)	Systemic inflammation (predictive, diagnostic, prognostic biomarker)
Signaling proteins (interleukins, TNF- α)	Systemic inflammation (predictive, diagnostic, prognostic biomarker)
Pneumoproteins (SP-D, CC16)	Impaired lung homeostasis (diagnostic, prognostic, responsive biomarker)
Natriuretic peptide hormone (incl. NTproBNP)	Comorbid cardiovascular disease (diagnostic, prognostic biomarker)
Protease enzymes (incl. MMPs)	Oxidant injury (diagnostic, prognostic biomarker)
A network model for cigarette smoking-induced immune response (Pan Z <i>et al.</i> , 2016)	
IL-6, TNF- α	Proinflammatory pathways
IL-4, IL-10	Anti-inflammatory/regulatory pathways
IL-6, IL-10	Molecular mediators, through those the inflammatory and anti-inflammatory/regulatory pathways interlink with each other
Metalloproteases (MMPs)	Cause of tissue damage
COPD, chronic obstructive pulmonary disease; CC16, Clara cell secretory protein 16; SP-D, surfactant protein D; NTproBNP, NTpro-brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; WBC, White blood cell; MMP9, matrix metalloproteinase 9; sRAGE, soluble receptor for advanced glycation end products; IL, interleukin; TNF- α , tumor necrosis factor- α ; MMPs, matrix metalloproteinases; TIMP, tissue inhibitor of metalloproteinases.	

Table S3 Comparison of demographic, clinical and physiological characteristics of heavy smokers (>40 pack-years) and other smokers [mean (SD), proportion (%) or median (IQR) of the main characteristics]

Characteristics	Smoking status		P
	Heavy smokers (>40 pack-years) (n=22)	Other smokers (<40 pack-years) (n=54)	
Demographic			
Age, years, mean ± SD	57.82±6.61	55.15±7.87	NS
Male, n (%)	20 (90.9)	34 (63.0)	0.015 ^{*##}
Smoking status			
Current smoker, n (%)	19 (86.4)	37 (68.5)	NS
Duration of smoking, years, mean ± SD	38.32±8.79	30.72±8.76	0.001 ^{*#}
Cigarettes per day, mean ± SD	29.05±8.87	16.91±5.79	0.000 ^{*#}
Pack-years, mean ± SD	52.80±10.22	25.18±8.14	0.000 ^{*#}
Occupational exposure to dust/gas/fumes, n (%)			
Dusty job, ≥10 years	5 (22.7)	15 (28.3)	NS
Gas/fumes exposed, ≥10 years	10 (45.5)	13 (24.5)	NS
Indoor exposure to biomass fuels, n (%)			
Wood/coal stove higher use, >7 times/week	4 (18.2)	6 (11.1)	NS
Clinical data, n (%)			
COPD	13 (59.1)	25 (46.3)	NS
Cardiovascular disease	16 (72.7)	33 (61.1)	NS
Statin use	1 (4.5)	7 (13.0)	NS
ICS use	1 (4.5)	5 (9.3)	NS
Physiology, mean ± SD			
BMI, kg/m ²	26.04±4.69	27.93±5.50	NS
FEV1, liters	2.63±0.72	2.79±0.94	NS
FEV1 _% , predicted	80.28±20.92	88.36±23.03	NS
FVC, liters	3.92±0.83	3.91±1.11	NS
FEV1/FVC, %	66.51±10.98	70.63±11.18	NS
Biomarker profile, n (%)			
Smokers with no elevated cytokine levels ("non-inflamed" smokers)	9 (40.9)	27 (50.0)	NS
Smokers with ≥2 elevated cytokine levels ("inflamed" smokers)	8 (36.4)	11 (20.4)	NS
Smokers with ≥3 elevated cytokine levels ("inflamed" smokers)	5 (22.7)	6 (11.1)	NS
Single biomarkers and pneumoproteins			

Table S3 (continued)

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Characteristics	Smoking status		P
	Heavy smokers (>40 pack/years) (n=22)	Other smokers (<40 pack/years) (n=54)	
White blood cells			
Leukocytes ($\times 10^9/L$)	7.4 (6.7; 8.6)	6.7 (5.3; 8.5)	NS
Lymphocytes ($\times 10^9/L$)	2.3 (1.8; 2.9)	2.1 (1.7; 2.6)	NS
Granulocytes ($\times 10^9/L$)	4.6 (3.6; 5.3)	3.7 (3.1; 5.0)	NS
Cytokines			
IL-4, pg/mL	1.4 (0.7; 2.1)	1.1 (0.8; 1.8)	NS
IL-6, pg/mL	2.5 (1.3; 5.1)	1.3 (0.6; 2.5)	0.007 ^{###}
IL-8, pg/mL	2.5 (1.4; 8.8)	1.5 (0.6; 5.2)	NS
IL-10, pg/mL	3.2 (1.8; 5.4)	2.4 (1.1; 4.0)	NS
hs-CRP, mg/L	3.9 (2.9; 7.7)	2.6 (1.6; 5.3)	0.017 ^{###}
TNF- α , pg/mL	1.2 (0.7; 2.0)	0.9 (0.6; 1.7)	NS
Enzyme			
MMP9, ng/mL	9.8 (7.4; 14.4)	9.1 (5.7; 13.2)	NS
Pneumoproteins			
SP-D, ng/mL	947.3 (466.1; 1,195.0)	627.1 (407.9; 1,018.6)	0.039 ^{###}
CC16, ng/mL	5.4 (4.2; 8.4)	5.3 (4.4; 7.5)	NS
Other biomarkers			
TIMP, ng/mL	1,447.5 (1,058.3; 1,916.4)	1,272.5 (901.4; 1,531.4)	NS
$\alpha 1$ -antitripsin, mg/dL	254.3 (236.5; 285.8)	218.4 (186.8; 242.3)	0.006 ^{###}
NproBNP, pg/mL	39.0 (17.1; 63.2)	32.7 (18.2; 64.5)	NS
Vitamin D	14.7 (8.7; 20.0)	16.4 (8.4; 22.1)	NS

*, statistically significant factors; #, independent samples *t*-test, ##, Pearson Chi-square test; ###, non-parametric: Mann-Whitney U test for distribution. SD, standard deviation; IQR, interquartile range; COPD, chronic obstructive pulmonary disease; ICS, inhaled corticosteroid; BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; hs-CRP, high-sensitivity C-reactive protein; TNF- α , tumor necrosis factor- α ; MMP9, matrix metalloproteinase 9; CC16, Clara cell secretory protein 16; SP-D, surfactant protein D; TIMP, tissue inhibitor of metalloproteinases; NTproBNP, NTpro-brain natriuretic peptide; NS, non-significant.