

Combination of Systemic Inflammatory Biomarkers in Assessment of Chronic Obstructive Pulmonary Disease: Diagnostic Performance and Identification of Networks and Clusters

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Table 1. Levels of cytokines in all participants regarding their smoking status.

	healthy non-smokers N = 48	healthy smokers N = 47	COPD non-smokers N = 5	COPD former smokers N = 75	COPD smokers N = 29	P
IL-1 α (pg/ml)	0.30 (0.30 - 0.71)	0.30 (0.30 - 1.78)	0.30 (0.30 - 1.43)	0.40 (0.30 - 1.84)	1.57 (0.30 - 2.66) ^{1,2}	0.010
IL-1 β (pg/ml)	0.10 (0.10 - 0.16)	0.10 (0.10 - 2.76)	0.62 (0.28 - 2.24) ^{1,2}	8.24 (0.56 - 26.23) ^{1,2}	9.65 (1.52 - 17.54) ^{1,2}	<0.001
IL-6 (pg/ml)	4.41 (3.29 - 6.17)	5.37 (3.79 - 8.01)	10.40 (2.88 - 15.51)	36.05 (10.75 - 72.66) ^{1,2,3}	33.05 (11.09 - 52.03) ^{1,2,3}	<0.001
IL-8 (pg/ml)	6.22 (4.34 - 11.33)	6.36 (3.99 - 11.17)	3.25 (2.32 - 6.92)	8.20 (3.61 - 20.22)	9.65 (4.39 - 17.54)	0.172
TNF α (pg/ml)	0.35 (0.35 - 0.53)	0.70 (0.35 - 3.04) ¹	0.35 (0.35 - 0.71)	8.18 (0.46 - 23.32) ^{1,2,3}	11.08 (1.13 - 17.55) ^{1,2,3}	<0.001

Data were tested by Kruskal-Wallis one-way analysis of variance and presented as median with IQR. Results were statistically significant if P<0.05.

Afterwards, post-hoc analysis was performed.

IL-1 α – interleukin-1alpha; IL-1 β – interleukin-1beta; IL-6 – interleukin-6; IL-8 – interleukin-8; TNF α – tumour necrosis factor alpha.

¹statistically significant in comparison to healthy non-smokers;

²statistically significant in comparison to healthy smokers;

³statistically significant in comparison to COPD non-smokers.

Table 2. Values of 95th percentile of the parameters determined in healthy non-smokers that are used in network analyses.

network analysis of all cytokines	
parameter	95 th percentile value
IL-1 α (pg/ml)	6.18
IL-1 β (pg/ml)	0.10
IL-6 (pg/ml)	10.48
IL-8 (pg/ml)	26.14
TNF α (pg/ml)	2.65
network analysis of selected parameters	
parameter	95 th percentile value
IL-1 β (pg/ml)	0.10
IL-6 (pg/ml)	10.48
TNF α (pg/ml)	2.65
CRP (mg/l)	5.15
Fbg (g/l)	4.2
eATP (μ mol/l)	1.10
eHsp70 (ng/ml)	0.74

Table S3. Clinical characteristics and concentrations of cytokines (IL-1 β , IL-6, TNF α), common inflammatory parameters (CRP, Fbg) and DAMPs (eATP, eHsp70) in COPD patients according to the five clusters after unsupervised hierarchical clustering analysis.

parameter	CLUSTER 1 N=19	CLUSTER 2 N=11	CLUSTER 3 N=36	CLUSTER 4 N=28	CLUSTER 5 N=14	P	after adjusted P-value *
FEV ₁ (% pred.)	61.8 (40.4 – 61.8)	26.0 (20.8 – 30.0)	43.8 (29.4 – 56.9)	54.5 (34.4 – 64.0)	27.3 (23.1 – 30.6)	<0.001	1 vs. 2 1 vs. 3 1 vs. 5 2 vs. 3 2 vs. 4 3 vs. 5 4 vs. 5
DLCO	55.7 (45.1 -74.4)	44.6 (37.5 – 46.2)	50.1 (36.3 – 74.2)	56.7 (36.2 – 70.0)	40.7 (34.8 – 44.6)	0.319	NS
exacerbations (N)							
0	8	1	13	13	3	0.130	NS
1	5	4	14	9	4		
≥2	5	5	3	2	5		
≥1 + H	1	1	6	4	2		
mMRC	1 (1 – 2)	3 (2 – 3)	1 (1 – 2)	1 (1 – 2)	2 (2 – 3)	<0.001	1 vs. 2 1 vs. 5 2 vs. 3 2 vs. 4 3 vs. 5 4 vs. 5
CAT	15 (11 - 20)	20 (16 – 27)	17 (13 – 24)	17 (8 – 21)	22 (14 – 26)	0.016	NS
SGRQ-C	32.5 (24.1 – 49.8)	59.5 (45.5 – 79.4)	47.3 (31.3 – 62.5)	35.2 (20.5 – 47.1)	64.2 (54.7 – 69.0)	<0.001	1 vs. 2 1 vs. 5 2 vs. 4 3 vs. 4 4 vs. 5

CODEx	1 (1 - 3)	6 (5 - 6)	4 (2 - 5)	2 (1 - 4)	5 (4 - 6)	<0.001	1 vs. 2 1 vs. 5 2 vs. 3 2 vs. 4 3 vs. 5 4 vs. 5
IL-1 β (pg/ml)	0.38 (0.24 - 0.65)	0.21 (0.12 - 3.82)	13.22 (1.08 - 25.90)	8.81 (3.90 - 20.25)	47.55 (12.98 - 56.40)	<0.001	1 vs. 3 1 vs. 4 1 vs. 5 2 vs. 3 2 vs. 4 2 vs. 5 3 vs. 5 4 vs. 5
IL- 6 (pg/ml)	3.84 (1.87 - 9.26)	10.72 (1.56 - 14.73)	39.91 (29.98 - 72.15)	37.67 (19.08 - 63.71)	118.73 (40.21 - 189.82)	<0.001	1 vs. 3 1 vs. 4 1 vs. 5 2 vs. 3 2 vs. 4 2 vs. 5 3 vs. 5 4 vs. 5
TNF α (pg/ml)	0.35 (0.35 - 0.35)	0.35 (0.35 - 4.22)	12.31 (0.77 - 20.00)	9.22 (5.77 - 18.12)	27.56 (18.48 - 56.77)	<0.001	1 vs. 3 1 vs. 4 1 vs. 5 2 vs. 3 2 vs. 4 2 vs. 5 3 vs. 5 4 vs. 5
CRP (mg/l)	2.21 (1.09 - 4.53)	1.75 (0.98 - 2.96)	5.34 (3.82 - 8.35)	1.24 (0.89 - 1.77)	1.21 (0.77 - 3.21)	<0.001	1 vs. 3 2 vs. 3 3 vs. 4

							3 vs. 5
Fbg (g/l)	3.8 (3.6 – 4.5)	3.0 (2.5 – 3.3)	4.5 (4.2– 5.1)	3.5 (3.1 – 3.7)	3.7 (3.5 – 3.9)	<0.001	1 vs. 2 1 vs. 3 1 vs. 4 2 vs. 3 2 vs. 4 2 vs. 5 3 vs. 4 3 vs. 5
eATP (μ mol/l)	1.19 (0.93 – 1.52)	1.91 (1.84 – 2.23)	1.63 (1.24 – 2.04)	1.38 (1.12 – 1.52)	1.94 (1.92 – 2.33)	<0.001	1 vs. 2 1 vs. 3 1 vs. 5 2 vs. 4 3 vs. 4 3 vs. 5 4 vs. 5
eHsp70 (ng/ml)	0.99 (0.58 – 1.49)	3.41 (2.94 – 6.84)	2.06 (1.09 – 2.96)	0.82 (0.51 – 1.54)	3.45 (3.15 – 5.10)	<0.001	1 vs. 2 1 vs. 3 1 vs. 5 2 vs. 3 2 vs. 4 3 vs. 4 3 vs. 5 4 vs. 5

DAMP – damage-associated molecular pattern; FEV₁– forced expiratory volume in one second; DLCO – diffusing capacity for carbon monoxide; exacerbations – number of exacerbations reported in previous year; mMRC – modified Medical Research Council; CAT – COPD Assessment Test; SGRQ-C – St George’s Respiratory Questionnaire for COPD patients; CODEx – comorbidities, obstruction, dyspnoea, previous exacerbations; IL-1 β – interleukin-1beta; IL-6 – interleukin-6; TNF α – tumour necrosis factor alpha; CRP – C-reactive protein; Fbg – fibrinogen; eATP – extracellular adenosine-triphosphate; eHsp70 – extracellular heat shock protein 70; H – hospitalization; NS – not significant after the adjustment of P-value.

* significant differences between the groups after the adjustment of P-value by Benjamini-Hochberg method.