**Supplementary appendix**

### Supplement to: Nanoparticles in induced sputum – a window to airway inflammation among active smokers.

Table S1, Correlations between baseline characteristics and nanoparticles variables.

Table S2, Correlations between serum and sputum cytokines and nanoparticles variables

Table S3, Correlations between serum and sputum cytokine levels

Table S4, Correlations between sputum cytokine levels and baseline variables

Table S5, Comparison of cytokines in serum and sputum between COPD and non-COPD smokers.

Figure S1, Correlations between particle characteristics and variables among the COPD patients.

Table S1, Correlations between baseline characteristics and nanoparticles variables.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable (pg/ml) | NP mean size | | NP concentration | | Size variance | |
| r | p | r | p | r | p |
| Age, years | -0.20 | 0.31 | 0.31 | 0.08 | 0.05 | 0.80 |
| Pack years | -0.13 | 0.5 | 0.40 | 0.04 | 0.39 | 0.05 |
| CAT score | -0.42 | 0.02 | 0.11 | 0.53 | 0.46 | 0.01 |
| Pulmonary function tests: | |  |  |  |  |  |
| FVC, % predicted | 0.15 | 0.43 | 0.06 | 0.8 | -0.18 | 0.35 |
| FEV1, % predicted | 0.21 | 0.27 | 0.03 | 0.86 | -0.16 | 0.40 |
| FEV1/FVC | 0.23 | 0.23 | -0.10 | 0.59 | -0.16 | 0.40 |
| DLCO/VA, % predicted | 0.27 | 0.17 | -0.20 | 0.92 | -0.09 | 0.67 |
| 6-minute walk, % predicted | 0.03 | 0.88 | 0.29 | 0.12 | -0.24 | 0.21 |
| Impulse oscillometry: | |  |  |  |  |  |
| Resistance 5 Hz, kPa/L/s | -0.13 | 0.50 | 0.19 | 0.33 | -0.13 | 0.49 |
| Resistance 20 Hz, kPa/L/s | 0.15 | 0.43 | 0.03 | 0.87 | -0.01 | 0.95 |
| Area of reactance, kPa/L/s | -0.43 | 0.03 | 0.27 | 0.17 | -0.16 | 0.43 |
| Resonant frequency, Hz | -0.39 | 0.04 | 0.15 | 0.44 | -0.10 | 0.61 |
| Exhaled CO, PPM | -0.09 | 0.63 | -0.09 | 0.63 | -0.15 | 0.45 |

Abbreviations: NP, nanoparticles; CAT, Chronic Obstructive Pulmonary Disease assessment tool; FVC, forced vital capacity; FEV1, forced expiratory volume in first second; DLCO, diffusing capacity for carbon monoxide; VA, alveolar volume. Analyses were made using the spearman correlation coefficient.

Table S2, Correlations between serum and sputum cytokines and nanoparticles variables.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable (pg/ml)** | **NP mean size** | | **NP concentration** | | **Size variance** | |
| r | p | r | p | r | p |
| Serum IL-1β | -0.15 | 0.45 | -0.19 | 0.35 | -0.02 | 0.90 |
| Serum IL-4 | -0.08 | 0.68 | 0.01 | 0.95 | 0.06 | 0.77 |
| Serum IL-5 | -0.22 | 0.28 | -0.12 | 0.55 | 0.21 | 0.31 |
| Serum IL-6 | 0.01 | 0.95 | -0.07 | 0.74 | -0.16 | 0.41 |
| Serum IL-8 | -0.16 | 0.41 | 0.26 | 0.74 | -0.34 | 0.07 |
| Serum IL-10 | <0.01 | 0.99 | -0.21 | 0.28 | <0.01 | 0.99 |
| Serum IL-13 | -0.16 | 0.46 | 0.12 | 0.58 | 0.03 | 0.91 |
| Serum TNFα | 0.10 | 0.59 | 0.02 | 0.91 | 0.10 | 0.59 |
| Sputum IL-1β | -0.39 | 0.03 | 0.49 | <0.01 | -0.29 | 0.12 |
| Sputum IL-6 | -0.42 | 0.02 | 0.49 | <0.01 | -0.40 | 0.03 |
| Sputum IL-8 | -0.21 | 0.27 | 0.35 | 0.05 | -0.06 | 0.78 |
| Sputum IL-10 | -0.12 | 0.61 | 0.11 | 0.62 | -0.22 | 0.33 |
| Sputum IL-13 | -0.24 | 0.49 | 0.18 | 0.61 | -0.33 | 0.16 |
| Sputum TNFα | -0.50 | 0.01 | 0.59 | p<0.01 | -0.30 | 0.15 |

Abbreviations: NP, nanoparticles. Analyses were made using the spearman correlation coefficient.

Table S3, Correlations between serum and sputum cytokine levels

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Serum levels** | **Sputum levels (coefficient r)** | | | | | |
| IL-1β | IL-6 | IL-8 | IL-10 | IL-13 | TNFα |
| IL-1β | -0.275 | -0.304 | -.401\* | -0.133 | -0.109 | -0.032 |
| IL-4 | 0.041 | -0.272 | -0.179 | -0.226 | 0.006 | -0.061 |
| IL-5 | 0.040 | -0.253 | -0.235 | -0.383 | -0.385 | 0.140 |
| IL-6 | 0.145 | -0.159 | -0.161 | -0.312 | -0.089 | 0.054 |
| IL-8 | -0.037 | 0.291 | -0.074 | 0.279 | -0.024 | 0.111 |
| IL-10 | -0.072 | -0.294 | -0.354 | -0.315 | -0.092 | -0.051 |
| IL-13 | 0.340 | -0.530\* | -0.179 | -0.358 | -0.046 | -0.110 |
| TNFα | 0.051 | -0.051 | 0.117 | 0.088 | 0.299 | -0.214 |

\* *P* value <0.05 for the correlation. Correlations made using the Pearson correlation coefficient.

Table S4, Correlations between sputum cytokine levels and baseline variables.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | **Sputum levels (coefficient r)** | | | | | |
|  | IL-1β | IL-6 | IL-8 | IL-10 | IL-13 | TNFα |
| Age | -0.114 | -0.031 | 0.281 | 0.031 | -0.120 | 0.112 |
| Pack years | -0.121 | -0.139 | 0.178 | -0.027 | -0.325 | -0.287 |
| CAT score | 0.064 | 0.263 | 0.160 | -0.026 | 0.416 | -0.012 |
| FEV1, % predicted | -0.240 | -0.330 | -0.418\*\* | -0.160 | -0.415 | -0.283 |
| DLCOc/VA | -0.242 | -0.262 | -0.374 | -0.163 | -0.335 | -0.396 |
| IOS Area of reactance, kPa/L/s | 0.308 | 0.468\* | 0.655\*\* | 0.425\* | 0.395 | 0.267 |
| IOS Resonant frequency, Hz | 0.199 | 0.491\* | 0.612\*\* | 0.486\* | 0.306 | 0.380 |

Abbreviations: CAT, Chronic Obstructive Pulmonary Disease assessment tool; FEV1, forced expiratory volume in first second; DLCO, diffusing capacity for carbon monoxide; VA, alveolar volume; IOS, Impulse-oscillometry.

\* *P* value <0.05. \* *P* value <0.01. Correlations made using the Pearson correlation coefficient.

Table S5, Comparison of cytokines in serum and sputum between COPD and non-COPD smokers.

|  |  |  |  |
| --- | --- | --- | --- |
| Variable (pg/ml) | **COPD median (IQR)** | **Non-COPD median (IQR)** | ***p*** |
| Serum IL-1β | 1.7 (1-3) | 2.7 (1-3) | 0.76 |
| Serum IL-4 | 90 (32-118) | 97 (31-160) | 0.85 |
| Serum IL-5 | 3 (1-4) | 3 (2-4) | 0.86 |
| Serum IL-6 | 4 (2-5) | 5 (3-7) | 0.07 |
| Serum IL-8 | 15 (11-22) | 13 (9-19) | 0.75 |
| Serum IL-10 | 17 (6-36) | 25 (6-29) | 0.7 |
| Serum IL-13 | 5 (2-6) | 4 (3-6) | 0.98 |
| Serum TNFα | 16 (14-18) | 11 (8-16) | 0.09 |
| Sputum IL-1β | 26 (8-80) | 25 (4-29) | 0.53 |
| Sputum IL-6 | 114 (24-313) | 31 (9-192) | 0.25 |
| Sputum IL-8 | 4150 (1755-4884) | 1363 (916-4560) | 0.29 |
| Sputum IL-10 | 4 (2-6) | 3 (2-4) | 0.41 |
| Sputum IL-13 | 0.7 (0.3-0.7) | 0.3 (0.2-0.4) | 0.12 |
| Sputum TNFα | 3.4 (1.9-12) | 1.3 (0.7-11) | 0.23 |

Comparisons were made using the Mann-Whitney u test.

Figure S1, Correlations between particle characteristics and variables among the COPD patients.

**a**. Correlation between CAT score and particle concentration (E8 particles/ml). **b**. Correlation between area of reactance and nanoparticle concentration (E8 particles/ml). **c**. Correlation between serum IL-8 and particle concentration (E8 particles/ml). **d**. Correlation between serum IL-10 and particle concentration (E8 particles/ml).