**SUPPLEMENTARY**

**S****mall airway dysfunction in asthma is associated with perceived respiratory symptoms, non-type 2 airway inflammation and poor responses to therapy**

**Design overview and subjects**

Study I was a cross-sectional study to explore effects of small airway dysfunction (SAD) on perceptual respiratory symptoms in stable asthmatics during bronchoconstriction who were recruited from the Asthma Clinic of West China Hospital at Sichuan University. Asthma was diagnosed according to American Thoracic Society (ATS) guidelines and Global Initiative for Asthma (GINA). Individuals were ineligible if they had a medical contraindication to methacholine (Mch) challenge testing (MCT), an acute exacerbation of asthma in previous 4 week, FEV1 < 80% predicted, other respiratory diseases, or were pregnant or breast feeding. The goal of study II also as a cross-sectional study, was to assess the relationship between SAD and local airway inflammation in adults with stable asthma who underwent sputum induction from the Australasian Severe Asthma Network (ASAN). This study had the inclusion and exclusion criteria similar to study I. Adult patients (≥18 years of age) with stable asthma, and confirmed by variable airflow obstruction, were recruited. The subjects were excluded if they were pregnant or breast feeding, had cognitive impairment, current solid organ malignancy, an acute exacerbation of asthma in previous 4 week or FEV1 < 80% predicted. Study III with a prospective cohort design, aimed to explore the effect of small airway dysfunction on anti-asthma response in the subjects who were followed up from Study II.”

Table S1. Response of respiratory symptoms and lung function to methacholine in asthma patients grouped by small airway dysfunction in study I.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | Non-SAD | SAD | *F*/X2 | *P* |
| n | 62 | 123 |  |  |
| Symptom changes |  |  |  |  |
| Dyspnea |  |  |  |  |
|  ∆VAS | 19.24±25.23 | 27.98±25.17 | 4.96 | 0.027 |
|  Slope of ∆VAS/∆FEF25-75 | 38.36±49.57 | 101.37±297.72 | 11.08 | 0.0009 |
|  ∆Borg score | 1.43±1.52 | 1.69±1.54 | 1.23 | 0.192 |
|  Slope of ∆Borg /∆FEF25-75 | 2.99±3.19 | 6.29±19.45 | 4.74 | 0.0294 |
| Chest tightness |  |  |  |  |
|  ∆VAS | 16.15±18.29 | 23.45±23.14 | 4.69 | 0.032 |
|  Slope of ∆VAS/∆FEF25-75 | 32.22±35.05 | 95.98±291.77 | 7.82 | 0.0052 |
| Cough |  |  |  |  |
|  ∆VAS | 16.81±24.03 | 20.83±26.68 | 1.00 | 0.319 |
|  Slope of ∆VAS/∆FEF25-75 | 36.66±52.58 | 86.70±294.58 | 1.40 | 0.2372 |
| Wheezing |  |  |  |  |
|  ∆VAS | 20.03±21.95 | 24.48±24.72 | 1.44 | 0.232 |
|  Slope of ∆VAS/∆FEF25-75 | 40.51±44.05 | 98.43±293.75 | 3.72 | 0.0539 |
| Total symptoms  |  |  |  |  |
|  ∆VAS | 72.23±63.64 | 96.73±75.35 | 4.82 | 0.0294 |
|  Slope of ∆VAS/∆FEF25-75 | 147.76±128.87 | 381.64±1138.97 | 11.84 | 0.0006 |
| Lung Function |  |  |  |  |
| ∆FEV1, % | -29.17±7.86 | -27.67±7.34 | 1.527 | 0.218 |
| ∆FEV1, L | -0.96±0.36 | -0.70±0.23 | 14.58 | <0.001 |
| ∆FVC, % | -16.30±10.96 | -20.41±11.76 | 5.18 | 0.024 |
| ∆FVC, L | -0.60±0.46 | -0.64±0.31 | 1.42 | 0.202 |
| ∆FEV1/FVC, % | -13.87±8.44 | -8.98±7.83 | 15.16 | <0.001 |
| ∆FEF25-75, % | -50.75±11.06 | -38.21±15.34 | 32.63 | <0.001 |

SAD=small airway dysfunction, VAS=visual analogue scale.

Table S2. The relationship between inflammatory profiles and small airway dysfunction in study II.

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Statistics | Unadjusted | Adjusted# |
| FEF25-75, L/s | FEF25-75, % predicted | FEF25-75, L/s | FEF25-75, % predicted |
| IFN-γ, pg/mL | Correlation Coefficient | -.243\* | -.222\* | -.228\* | -.213\* |
|  | Sig. (2-tailed) | 0.018  | 0.032  | 0.031 | 0.044 |
| IL-13, pg/mL | Correlation Coefficient | -0.163  | -0.161  | -0.167 | -0.158 |
|  | Sig. (2-tailed) | 0.118  | 0.120  | 0.118 | 0.138 |
| IL-17A, pg/mL | Correlation Coefficient | -0.200  | -0.169  | -0.176 | -0.147 |
|  | Sig. (2-tailed) | 0.053  | 0.103  | 0.099 | 0.169 |
| IL-1β, pg/mL | Correlation Coefficient | -0.157  | -0.153  | -0.133 | -0.114 |
|  | Sig. (2-tailed) | 0.130  | 0.141  | 0.213 | 0.286 |
| IL-4, pg/mL | Correlation Coefficient | -0.096  | -0.109  | -0.084 | -0.093 |
|  | Sig. (2-tailed) | 0.355  | 0.296  | 0.431 | 0.386 |
| IL-5, pg/mL | Correlation Coefficient | -0.026  | -0.039  | -0.038 | -0.054 |
|  | Sig. (2-tailed) | 0.806  | 0.708  | 0.724 | 0.617 |
| IL-6, pg/mL | Correlation Coefficient | -0.063  | -0.076  | -0.021 | -0.040 |
|  | Sig. (2-tailed) | 0.549  | 0.469  | 0.842 | 0.711 |
| IL-8, pg/mL | Correlation Coefficient | -.254\* | -.219\* | -.251\* | -.217\* |
|  | Sig. (2-tailed) | 0.013  | 0.034  | 0.017 | 0.040 |
| TNF-α, pg/mL | Correlation Coefficient | -0.128  | -0.160  | -0.129 | -0.153 |
|  | Sig. (2-tailed) | 0.219  | 0.123  | 0.229 | 0.152 |
| Eosinophil, %  | Correlation Coefficient | -0.061  | -0.049  | -0.091 | -0.083 |
|  | Sig. (2-tailed) | 0.575  | 0.654  | 0.416 | 0.458 |
| Neutrophil, % | Correlation Coefficient | -.230\* | -0.178  | -.224\* | -0.167 |
|  | Sig. (2-tailed) | 0.033  | 0.101  | 0.043  | 0.134 |
| Lymphocyte, % | Correlation Coefficient | -0.068  | -0.097  | -0.065 | -0.108 |
|  | Sig. (2-tailed) | 0.534  | 0.373  | 0.560 | 0.335 |
| Macrophage, % | Correlation Coefficient | .257\* | .215\* | .242\* | .203 |
|  | Sig. (2-tailed) | 0.017  | 0.047  | 0.028  | 0.067 |

IFN = Interferon; IL=Interleukin; SD=standard deviation; TNF = tumor necrosis factor.

\* *P* < 0.05;

# Spearman’s partial correlation adjusted for age, gender, BMI, smoking, FEV1%, ICS.

Table S3. Agreement analysis between LLN and FEF25-75% in assessing small airway dysfunction.

|  |  |
| --- | --- |
|  | Definition of SAD by LLN  |
| LLN defined-SAD | LLN defined-non-SAD |
| Definition of SAD by 65% predicted FEF25-75  | SAD | 111 | 12 |
| Non-SAD | 3 | 59 |

LLN = lower limits of normal; SAD = small airways dysfunction;

Sensitivity：97.37%；Specificity：83.10%；

Table S4. Response of respiratory symptoms to methacholine challenge testing in asthma patients grouped by small airway dysfunction defined by LLN in study I.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | LLN-defined SAD | LLN-defined non-SAD | *F*/X2 | *P* |
| n | 114 | 71 |  |  |
| Symptom changes |  |  |  |  |
| Dyspnea |  |  |  |  |
|  ∆VAS | 26.96±24.77 | 21.11±25.48 | 2.226 | 0.027 |
|  Slope of ∆VAS/∆FEF25-75 | 102.28±308.85 | 44.92±55.82 | 2.677 | 0.007 |
|  ∆Borg score | 1.59±1.51 | 1.57±1.55 | 0.429 | 0.668 |
|  Slope of ∆Borg /∆FEF25-75 | 6.33±20.27 | 3.44±3.42 | 1.246 | 0.213 |
| Chest tightness |  |  |  |  |
|  ∆VAS | 23.32±23.44 | 17.00±18.81 | 2.166 | 0.032 |
|  Slope of ∆VAS/∆FEF25-75 | 99.16±303.65 | 36.65±43.55 | 2.492 | 0.013 |
| Cough |  |  |  |  |
|  ∆VAS | 19.52±25.34 | 18.70±25.66 | -0.092 | 0.927 |
|  Slope of ∆VAS/∆FEF25-75 | 86.41±306.07 | 44.67±64.64 | 0.607 | 0.544 |
| Wheezing |  |  |  |  |
|  ∆VAS | 24.30±24.50 | 21.37±23.02 | 0.625 | 0.532 |
|  Slope of ∆VAS/∆FEF25-75 | 100.79±305.88 | 45.51±49.68 | 1.522 | 0.128 |
| Total symptoms  |  |  |  |  |
|  ∆VAS | 94.11±75.81 | 78.18±65.14 | 2.196 | 0.029 |
|  Slope of ∆VAS/∆FEF25-75 | 388.64±1187.43 | 171.75±150.33 | 2.576 | 0.010 |

LLN = lower limits of normal; SAD = small airways dysfunction;

Table S5. Inflammatory profiles and phenotypes in patients with asthma grouped by small airways dysfunction defined by LLN in study II.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | LLN-defined SAD | LLN-defined non-SAD | t/χ2/Z | P |
| n | 60 | 87 |  |  |
| Sputum |  |  |  |  |
|  Eosinophil, %  | 0.00 (0.00, 2.75) | 0.00 (0.00, 0.25) | -0.952 | 0.341 |
|  Neutrophil, % | 37.25 (11.25, 53.38) | 24.25 (10.25, 57.25) | -0.222 | 0.825 |
|  Lymphocyte, % | 1.00 (0.50, 2.00) | 0.50 (0.25, 1.47) | -1.310 | 0.190 |
|  Macrophage, % | 58.75 (33.88, 84.75) | 69.75 (42.50, 86.25) | 0.900 | 0.368 |
| Cytokines in sputum |  |  |  |  |
| IFN-γ, pg/mL | 1.96 (1.68, 2.68) | 1.74 (1.41, 2.01) | -2.565 | 0.010 |
| IL-13, pg/mL | 3.39 (2.18, 5.79) | 2.72 (1.96, 3.92) | -0.165 | 0.869 |
| IL-17, pg/mL | 3.39 (2.01, 5.08) | 2.10 (1.59, 3.62) | -2.211 | 0.027 |
| IL-1β, pg/mL | 18.14 (7.07, 73.97) | 16.83 (8.52, 38.58) | 0.932 | 0.351 |
| IL-4, pg/mL | 29.26 (2.25, 70.57) | 17.69 (2.25, 48.38) | -1.802 | 0.071 |
| IL-5, pg/mL | 1.47 (1.01, 2.71) | 1.13 (0.84, 1.79) | -1.623 | 0.105 |
| IL-6, pg/mL | 19.57 (5.64, 68.85) | 21.64 (5.34, 40.57) | -0.672 | 0.502 |
| IL-8, pg/mL | 1636.00 (576.05, 3925.00) | 1406.00 (769.16, 2615.00) | -0.910 | 0.363 |
| TNF-α, pg/mL | 14.05 (4.09, 55.14) | 11.56 (3.76, 17.92) | -1.412 | 0.158 |

LLN = lower limits of normal; SAD = small airways dysfunction; FENO = fractional exhaled nitric oxide; IFN = Interferon; IL=Interleukin; SD=standard deviation; TNF = tumor necrosis factor

Table S6. Treatment responsiveness across small airway dysfunction (SAD) and Non-SAD subjects defined by LLN in study III.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | LLN-defined SAD | LLN-defined non-SAD | t/χ2/Z | P |
| n | 30 | 35 |  |  |
| Spirometry |  |  |  |  |
|  ΔFEF25-75, L/s | 0.024±0.56 | -0.02±0.67 | 0.291 | 0.772 |
|  ΔFEF25-75, % | 2.78±35.64 | -0.77±26.31 | 0.460 | 0.647 |
| ΔFEV1, L | -0.05 (-0.16, 0.18) | 0.02 (-0.16, 0.18) | 0.507 | 0.612 |
| ΔFEV1, % | -1.94 (-7.85, 8.28) | 0.74 (-4.77, 5.50) | 0.224 | 0.823 |
| ΔFEV1 ≥ 12%, n (%) | 5 (16.7) | 2 (5.7) | 2.016 | 0.234 |
| Response in asthma control |  |  |  |  |
| ΔACQ | -0.13±0.60 | -0.44±0.87 | 1.645 | 0.103 |
| ΔACQ ≥ 0.5，n (%) | 6(20.0) | 18(51.4) | 6.851 | 0.009 |
| ΔACT | 1.20±3.61 | 3.20±4.41 | -2.009 | 0.049 |
| ΔACT ≥ 3.0，n (%) | 9(30.0) | 20(57.1) | 4.816 | 0.028 |

LLN = lower limits of normal; SAD = small airways dysfunction;

Δ= change from baseline; ACQ = Asthma Control Questionnaire; ACT = Asthma Control Test; AQLQ = Asthma Quality of Life Questionnaire.