**SUPPLEMENTARY MATERIAL**

**Obese-asthma syndrome: multiple inflammatory patterns, one key solution**

## 1) METHODS

The following criteria were used to select asthmatic patients: (1) a clinical history of asthma; (2) either bronchodilator responsiveness (>12 % and 200 ml improvement in forced expiratory volume in 1 s (FEV1) after 180 µg salbutamol metered-dose inhaler) or AHR (PC20 methacholine < 8 mg/ml). None of the subjects had received systemic corticosteroids for one month or longer prior to evaluation. In addition, no patient was receiving or had received biological asthma treatment. None of the subjects were current smokers [1,2].

Asthma severity (mild, moderate, severe) was established according to the pharmacological treatment used to control the disease [3,4] and asthma control was assessed using the asthma control test (ACT). Asthmatic patients with elevated specific IgE against one or more allergens were classified as atopic. Finally, patients were divided into two groups of more or less than 300 blood eosinophils/µl.

All participant's demographic and clinical characteristics were recorded (table 1). A CD4+ T cell phenotyping analysis was performed in a randomly selected subgroup of patients (Table 2) while 6 OA patients underwent BS (Table 3).

The study was performed with written informed consent from participating subjects and approved by the Ethics Committee of Hospital Clínic of Barcelona (2018/4015).

### 1.1 Measurement of serum cytokine levels

Interferon gamma (IFN-γ), tumor necrosis factor receptor 1 (TNFR1), Interleukin (IL)-4, IL-13, IL-17A, IL-1β, thymic stromal lymphopoietin (TSLP), and IL-8 levels were quantified by Luminex®

 
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multiplex immunoassay using Human ProcartaPlex Mix&Match kits (Thermo Fisher Scientific, Vienna, Austria) on a Luminex 200 analyzer. The procedure was carried out in accordance with the manufacturer's protocol.

### 1.2 CD4+ T cells phenotyping

*a) Cell stimulation*

To determine the different types of T cells in each sample, $2 \times 10^6$ cells/ml were initially mixed with GolgiStop TM (5 µL for every 10 ml of cell culture; BD Pharmingen, San Diego, CA), containing monensin (an intracellular protein transport inhibitor), to prevent the cytokine release from the cells and then stimulated with 50 ng/mL phorbol myristate acetate (BioLegend, San Diego, CA) and 1 µg/mL calcium ionomycin (BioLegend, San Diego, CA) for 4 h. Thereafter, the cells were harvested and fixed with the Cytofix TM buffer (BD Pharmingen, San Diego, CA) and permeabilized with the Perm/Wash TM buffer (BD Pharmingen, San Diego, CA).

*b) Cellular staining*

Cell staining was performed using the Human Th1/Th2/Th17 Phenotyping Kit (BD Pharmingen, San Diego, CA) containing anti-CD4, anti-IL-17A, anti-IFN-γ, and anti-IL-4 antibodies conjugated with PerCp, PE, FITC, and APC (BD Pharmingen, San Diego, CA), respectively. In addition, Zombie Violet TM Fixable Viability Kit (BioLegend, San Diego, CA) staining was used to measure cell viability. Unstained cells served as negative control.

*c) Flow cytometric analysis*

The stained cells were processed using a four-color FACSCanto II flow cytometer instrument (BD Biosciences, Erembodegem, Belgium). The frequency of Th1, Th2, and Th17 was determined among the gated CD4+ T cells expressing IFN-γ, IL-4, and IL-17, respectively. Typically, 30.000 $CD4^+$ lymphocytes were acquired. The data were analysed by FACSDiva TM software (BD Biosciences, Erembodegem, Belgium) (Figure 1).

 
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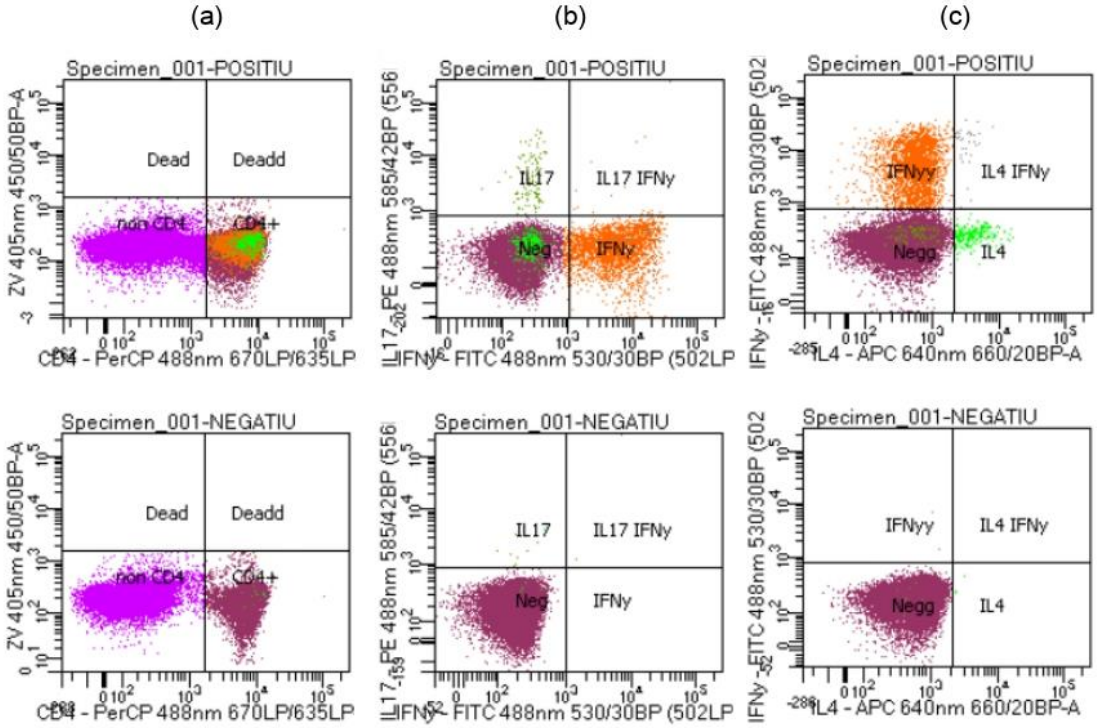
**Supplementary Figure 1.** Typical flow cytometry dot plot displaying the increased frequency of CD4+ alive cells **(a)** expressing IFNy, IL-17 **(b)**, and IL-1, IFN-γ **(c)** in stimulated cells *(upper)*, compared with the unstimulated controls *(lower)*.




### 1.3 Phenotyping CD4+ T cells

*a) Basal*

Since the intensity of T-cell response is more informative than the proportion of the cells responding, the investigation was developed by examining the intensity of IFN-γ (Th1 inflammation marker), IL-4 (Th2 inflammation marker), and IL-17 (Th17 inflammation marker), in CD4+ T-cell subsets. The strength of expression of these cytokines was assessed by calculating the median fluorescence intensity (MFI).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### 1.4 Statistical analysis

Clinical and experimental data were reported as the median and 25$^{th}$ to 75$^{th}$ percentile. Differences between groups were analysed using nonparametric tests: Mann–Whitney U test (unpaired data), Wilcoxon rank test (paired data), or Kruskal–Wallis H test followed by *post hoc* Dunn' multiple comparisons test. Correlation coefficients were calculated using the Spearman rank method. All analyses were performed using GraphPad Prism version 8.4 for Windows, (GraphPad Software, La Jolla, CA). Statistical significance was defined as p value < 0.05.

## 2) RESULTS

### Demographic and clinical characteristics of participants

We first analysed demographic and clinical data of HC, NOA and OA patients (Supplementary table 1). Various cytokines (IFN-γ, TNFR1, IL-4, IL-13, IL-17, IL-1b, TSLP and IL-8) were measured in all three groups (Supplementary figure 2).

**Supplementary Table 1.** Demographic and clinical characteristics of participants.

| | **HC (*n*, 33)** | **NOA (*n*, 14)** | **OA (*n*, 21)** |
|---|---|---|---|
| Age, years | 43.0 (37.5 - 50.5) | 52.0 (43.5 - 58.5) | 56.0 (50.0 - 61.0) |
| Female, n (%) | 24 (72.7) | 12 (85.7) | 16 (76.2) |
| BMI, kg/m2 | 22.5 (21.6 - 25.0) | 23.2 (22.3 - 24.9) | 38.4 (34.9 - 44.1) *# |
| Mild asthma, n (%) | N/A | 0 (0) | 5 (23.8) |
| Moderate asthma, n (%) | N/A | 6 (42.9) | 5 (23.8) |
| Severe asthma, n (%) | N/A | 8 (57.1) | 11 (52.4) |
| FVC, % predicted | 126.6 (122.2 - 136.3) | 132.1 (116.7 - 141.3) | 110.4 (94.1 - 122.5) *# |
| FEV1, % predicted | 101.0 (93.5 - 109.0) | 86.5 (75.2 - 103.8) | 85.0 (61.0 - 95.5) * |
| FEV1/FVC | 79.0 (76.0 - 83.0) | 71.0 (57.0 - 75.0) * | 77.0 (69.5 - 82.0) |

 
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|---|---|---|---|
| Use of ICS§, n (%) | N/A | 13 (92.8) | 16 (76.2) # |
| Atopia, n (%) | N/A | 8 (57.1) | 9 (42.8) |
| Serum total IgE, kU/L | 26.9 (16.5 - 80.2) | 95.5 (34.8 - 306.0) | 63.7 (14.4 - 273.0) |
| BEC, % | 2.9 (2.0 - 3.4) | 4.7 (3.6 - 6.3) * | 3.3 (2.3 - 5.0) |
| BEC, cells/µL | 200 (100 - 200) | 300 (200 - 425) * | 200 (150 - 350) |
| BEC ≥ 300 cells/µL, n (%) | 1 (3.0) | 10 (71.4) | 8 (38.1) |
| Metabolic syndrome, n (%) | N/A | N/A | 10 (47.6) |

*Data presented as medians ($25^{th}$-$75^{th}$ percentile). BEC, blood eosinophil count; BMI, body mass index; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in 1 s; HC, healthy controls; ICS, inhaled corticosteroids; IgE, immunoglobulin E; NOA, non-obese asthmatics; N/A, no applicable; OA, obese asthmatics. * p<0.05, compared with HC; # p<0.05 compared with NOA. § For NOA and OA patients who received ICS, the mean ± SD of the ICS dose in budesonide equivalents was 690.0 ± 573.9 and 1236.7 ± 857.5 µg/day, respectively*.

**Supplementary figure 2**. Serum cytokine levels comparison between asthmatic patients with and without obesity and healthy controls. Data presented as individual values and as medians (25th–75th percentile). HC, healthy controls; NOA, non-obese asthmatics; OA, obese asthmatics; IFN-γ, Interferon gamma; TNFR1, tumor necrosis factor receptor 1; IL, interleukin; TSLP, Thymic stromal lymphopoietin.

 
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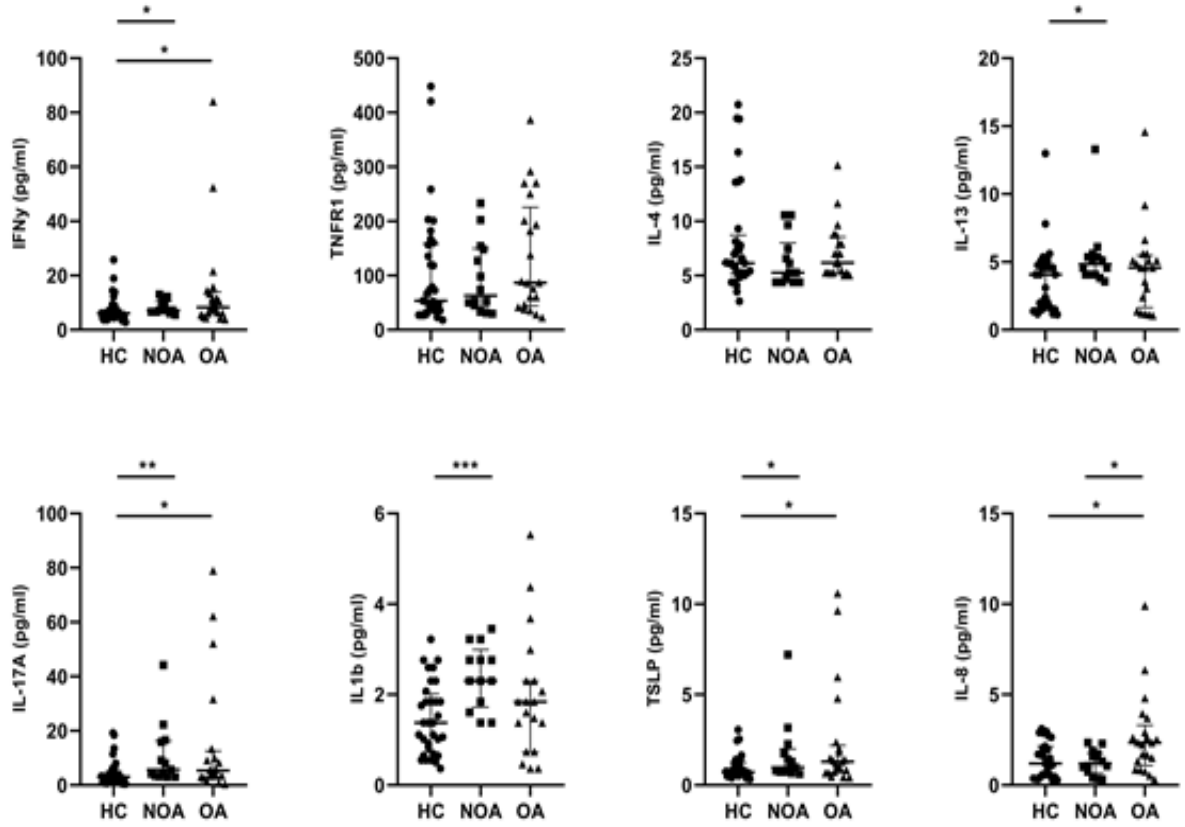


IL-17A and TSLP serum levels positively correlated with BMI (r=0.2895 and r=0.2976, respectively) and negatively with FEV1/FVC ratio (r=-0.2870 and r=-0.2663, respectively) (all $p$<0.005).

In addition, we found correlations between serum total IgE and TNFR1 (r = 0.3092, p = 0.0387), IFNγ (r = 0.3871, p = 0.0094), and TSLP (r = 0.3630, p = 0.0154). The MFI of IFNγ and IL-17 correlated positively with BMI (r = 0.5736, p = 0.0349 and r = 0.7363, p = 0.0037, respectively) in asthmatic participants with and without obesity, whereas the MFI of IL-4 correlated positively with TNFR1 (r = 0.4537, p = 0.0445), TSLP (r = 0.5062, p = 0.0228) and IL-8 (r = 0.5303, p = 0.0195) serum levels. Finally, the MFI of IL-17 from OA patients negatively correlated with FEV1/FVC ratio (r = -0.8193, p = 0.0171).

 
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When combined with clinical characteristics such as asthma severity or eosinophilia, no significant differences were found, although IL-4 serum levels were higher in atopic patients compared with non-atopic patients ($p$ = 0.0371).

**Characterization of patients according to CD4+ T cell study**

Secondly, in order to see the underlying inflammatory profile of our patients, IFN-γ, IL-4 and IL-17A levels were measured in a subset of the original HC, NOA and OA population. No statistically significant differences were found in these groups (expect for BMI and FVC%) (Supplementary table 2). Specifically, the characterization of OA patients according to these three cytokines identified three visual clusters (Manuscript Figure 1).

**Supplementary Table 2.** Demographic and clinical characteristics in patients with CD4+ T cell study

| | **HC (*n*, 9)** | **NOA (*n*, 11)** | **OA (*n*, 11)** |
|---|---|---|---|
| Age, years | 51.5 (42.5 - 64.5) | 58.0 (49.0 - 63.7) | 51.0 (42.7 - 56.7) |
| Female, n (%) | 7 (77.8) | 9 (81.8) | 10 (90.9) |
| BMI, kg/m2 | 22.7 (21.8 - 23.4) | 23.8 (22.3 - 25.4) | 42.1 (37.4 - 50.3) *# |
| Mild asthma, n (%) | N/A | 0 (0) | 3 (27.3) |
| Moderate asthma, n (%) | N/A | 3 (27.3) | 3 (27.3) |
| Severe asthma, n (%) | N/A | 8 (72.7) | 5 (45.4) |
| FVC, % predicted | 125.4 (123.3 - 141.8) | 136.5 (125.8 - 145.2) | 110.0 (99.9 - 122.3) # |
| FEV1, % predicted | 104.0 (98.2 - 111.0) | 95.0 (78.7 - 105.5) | 88.5 (76.2 - 97.5) |
| FEV1/FVC | 83.5 (73.0 - 84.2) | 71.5 (57.0 - 77.2) | 79.0 (71.7 - 82.0) |
| Use of ICS§, n (%) | N/A | 10 (90.9) | 8 (72.7) |
| Atopia, n (%) | N/A | 6 (54.5) | 4 (36.4) |
| Serum total IgE, kU/L | 44.5 (15.7 - 84.9) | 45.8 (33.9 - 131.4) | 117.5 (23.1 - 820.5) |
| BEC, % | 3.1 (2.2 - 3.5) | 5.4 (3.2 - 7.8) | 2.9 (1.5 - 4.8) |
| BEC, cells/µL | 200 (100 - 225) | 300 (200 - 425) | 200 (125 - 375) |

 
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|---|---|---|---|
| BEC ≥ 300 cells/µL, n (%) | 1 (9.1) | 7 (63.6) | 5 (45.4) |
| Metabolic syndrome, n (%) | N/A | N/A | 4 (36.4) |

*Data presented as medians ($25^{th}$-$75^{th}$ percentile). BEC, blood eosinophil count; BMI, body mass index; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in 1 s; HC, healthy controls; ICS, inhaled corticosteroids; IgE, immunoglobulin E; NOA, non-obese asthmatics; N/A, no applicable; OA, obese asthmatics. * p<0.05, compared with HC; # p<0.05 compared with NOA. § For NOA and OA patients who received ICS, the mean ± SD of the ICS dose in budesonide equivalents was 450.0 ± 224.8 and 793.3 ± 641.2 µg/day, respectively.*

**Bariatric Surgery**

Finally, 7 patients of the OA subgroup underwent bariatric surgery (Supplementary table 3). Their clinical and demographic characteristics are shown at baseline (V1) and at 6 months post-surgery (V2).

**Supplementary Table 3.** Demographic and clinical characteristics of patients who underwent Bariatric surgery.

| | **Obese asthmatics (*n*, 7)** | |
|---|---|---|
| | V1 | V2 (6 months post-surgery) |
| Female, n (%) | 7 (100) | 7 (100) |
| BMI, $kg/m^2$ | 45.1 (39.2 - 51.9) | 30.8 (26.6 - 38.0) * |
| Mild asthma, n (%) | 3 (42.8) | 6 (85.7) |
| Moderate asthma, n (%) | 2 (28.6) | 1 (14.3) |
| Severe asthma, n (%) | 2 (28.6) | 0 (0) |
| ACT | 18 (18 - 24) | 25 (25 - 25) * |
| FVC, % predicted | 110.4 (97.56 - 117.1) | 118.3 (100.0 - 139.8) |
| FEV1, % predicted | 86 (80 - 96) | 97 (91 - 110) |
| FEV1/FVC | 79 (77 - 82) | 81 (79 - 83) |

 
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| | | |
|---|---|---|
| Use of ICS§, n (%) | 4 (57.1) | 1 (14.3) |
| Atopia, n (%) | 2 (28.6) | 2 (28.6) |
| Serum total IgE, kU/L | 69.1 (18.1 - 994.3) | 24.6 (7.6 - 99.7) |
| BEC, % | 3.3 (1.2 - 4.1) | 2.7 (0.7 - 3.3) |
| BEC, cells/µL | 200 (100 - 300) | 200 (0 - 200) |
| BEC ≥ 300 cells/µL, n (%) | 2 (28.6) | 1 (14.3) |

*Data presented as medians ($25^{th}$-$75^{th}$ percentile). ACT, asthma control test; BEC, blood eosinophil count; BMI, body mass index; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; IgE, immunoglobulin E; OA, obese asthmatics; V1, visit 1; V2, visit 2. * p<0.05, compared with V1. § For NOA and OA patients who received ICS, the mean ± SD of the ICS dose in budesonide equivalents was 630.0 ± 651.9 and 100.0 ± 0 µg/day, respectively.*

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