Supplementary information

Title: Smoking exposure is associated with airway eosinophilic activation and autoimmunity in severe asthma

Supplementary methods

Inclusion criteria in SATS and SEPIA:

SATS:

Inclusion criteria: A physician's diagnosis of asthma and high-dose inhaled corticosteroids (ICS) treatment (≥1600 µg budesonide or equivalent) with a second controller (long acting beta-agonist (LABA), theophylline or leukotriene-antagonist) for the previous year or OCS for ≥ 50 % of the previous year.

Exclusion criteria: age below 18 years, pregnancy or judged by the investigator to be unable to comply with the study protocol.

SEPIA:

Inclusion criteria: Informed consent, a valid asthma diagnosis either according to patient medical record (positive bronchial provocation test, positive reversibility test or PF-variability) or by positive asthma test at screening (mannitol or reversibility test). Eosinophilic phenotype defined by at least 3% sputum eosinophils, severe asthma according to ERS/ATS guidelines; i.e. treatment with high-dose inhaled corticosteroid (ICS) (≥ 1600 µg budesonide or equivalent) plus a second controller, either long-acting beta-2-agonist (LABA), leukotriene antagonist (LTRA), theophylline, or long-acting muscarinergic antagonist (LAMA).

Exclusion criteria: Current treatment with oral corticosteroids (any dose), current pregnancy, age under 18 years, co-morbidities such as malignant lung disease, severe bronchiectasis, severe emphysema, lung

fibrosis, significant cardiac disease, treatment with anti-asthma biologicals (anti-IgE, anti-IL5, anti-IL5-receptor, anti-IL4/13-receptor) or other immunosuppressants within the last 16 weeks, lower airway infection requiring antibiotics within the last 6 weeks, and change in maintenance ICS dose within the preceding 4 weeks.

Clinical assessments

Dynamic and static lung function measurements (spirometry and body plethysmography) as well as gas diffusion testing was performed on a *MasterScreen Pneumo* spirometer and a *MasterScreen BodyBox* (Jaeger, Würzburg, Germany). Spirometry was conducted in accordance with standard ERS protocol[1] [and] predicted values were based on NHANES reference data[2]. Fractioned exhaled nitrogen oxide (FeNO) was measured on the *Ecomedics CLD88sp* (Ecomedics AG, Duernten, Switzerland) in the SATS study, while the SIGNATURE study used the *Niox Vero* device (Circassia Limited, Uppsala, Sweden)[3].

Atopy was assessed by IgE to a standard panel of ten aeroallergens: pollen from birch (*betula verrucosa*), grass (*phleum pratense*), or mugwort (*artemisia vulgaris*); dander from horse (*equus caballus*), cat (*felius domesticus*) or dog (*canis familiaris*); house dust mites (*dermatophagoides pteronyssinus* or *dermatophagoides farinae*); or mold (*alternaria alternatia* and *cladosporium herbarum*[4].

Sputum induction

In the SIGNATURE study, patients with an FEV_1 over 70% were induced with mannitol; if this proved ineffective, incremental doses of saline was used instead. Patients with an FEV_1 under 70% were induced with isotonic saline (0.9%) following a reversibility test. In the SATS study, patients with an FEV_1 over 70% were induced with incrementally increasing doses of hypertonic saline,

while patients with an FEV_1 under 70% were induced with isotonic saline (0.9%)[5, 6]. In both studies, sputum was assessed within an hour of collection, using the “plug selection method”[7].

Supplementary tables

Table S1: Baseline characteristics, patients with severe eosinophilic asthma

Baseline characteristics	< 10 pack years	≥10 pack years	Healthy controls	P-value
Total No., n	31	27	22	-
Sex, females n (%)	14 (45.2%)	6 (22.2%)	13(59%)	0.029
BMI (kg/m^2)	27.1 ± 4	27.4 ± 5	24.4 ± 4	0.47
Mean age (yr), range	50(20-71)	58(40-80)	32(20-63)	<0.001
Adult onset asthma, n (%)	15(48.3)	17(63.0)	NA	0.30
Pack years, total	0(0-1)	17(10-69)	0(0-0)	<0.001
Daily ICS dose, µg	1600(1600-6400)	1600(1600-3200)	NA	0.09
Daily OCS dose, mg	0(0-5)	0(0-12.5)	NA	0.01
ACQ-5 score	1.8 ± 1.0	2.2 ± 0.9	NA	0.08
Exacerbation rate	1.29 ± 1.3	2.63 ± 2.5	NA	0.02
FEV_1 (%predicted)	72.1 ± 21.6	70.9 ± 19.7	106.7 ± 12.9	<0.001
FVC (%predicted)	93.6 ± 21.4	86.4 ± 17.1	111.7 ± 15.5	<0.01
FEV_1/FVC-ratio	0.63 ± 0.12	0.64 ± 0.11	0.82 ± 0.06	<0.001
Atopy$^{€}$, no. (%)	21(67.7)	14(51.9)	0(0)	<0.001
Total serum IgE (kU/L)	228 (4-13500)	196 (12-2480)	22 (3-137)	<0.001
Total leukocyte count	7.4 ± 2.4	7.8 ± 2.4	6.5 ± 2.3	0.19
Blood eosinophils	0.42 ± 0.43	0.54 ± 0.	0.12 ± 0.07	<0.001
Blood neutrophils	4.4 ± 1.9	4.4 ± 2.0	3.3 ± 1.4	0.18
Blood lymphocytes	1.8 ± 0.7	1.5 ± 0.6	2.3 ± 0.9	0.10
FeNO, ppb	29(9-84)	28(7-219)	12(9.5-16.5)	<0.01
% Sputum eosinophils (range)	7.3(3.0-76.0)	12.5(3.3-92.3)	0 (0-2.8)	<0.001
% Sputum neutrophils (range)	53.3(7.0-88.3)	44.8(2.3-93.3)	38.3(9.0-88.0)	0.45
% Sputum macrophages (range)	20.3(0.5-81.5)	18.3(0.8-65.3)	61.1(8.8-89.0)	<0.01
% Sputum lymphocytes (range)	0(0-4)	0(0-8)	0 (0-2.5)	0.90

Table S1: Baseline characteristics of the study population. Data are shown as mean±SD, median (IQR), numbers (n) or %. ICS: Inhaled cortocosteroids; OCS: oral corticosteroids, prednisolone. $^{€}$Atopy defined as a positive skin prick test or positive specific IgE.

Table S2: Baselines characteristics, SEPIA sub-study (2-weeks OCS treatment)

Baseline characteristics	Statistic	≥10 pack years (N=12)	< 10 pack years (N=11)	p-value
Sex (n females, %)	n / N (%)	1 / 11 (9.1%)	6 / 12 (50%)	0.069
Age at inclusion (yr)	Median (Range)	58 (41-68)	51 (28–71)	0.52
BMI (kg/m^2)	Mean, SD	26.7±4.1	25.7±4.8	0.70
Exacerbation rate (events/yr)	Mean, SD	2.36±1.03	1.42±0.67	0.024
Smoking history (pack years)	Median (Range)	15 (12–25)	0 (0–1)	<0.001
Smoking cessation, duration (yr)	Median (Range)	7.0 (2.0–20.0)	NA	-
ACQ5 score	Mean, SD	1.85±0.84	2.37±0.87	0.22
ICS dose (µg/day)	Median (Range)	1.600 (1.600– 3.200)	1.600 (1.600– 3.200)	0.31
Nasal polyposis (n, %)	n / N (%)	5 / 11 (45%)	5 / 12 (42%)	>0.9
Atopy$^{\epsilon}$ (n positive, %)	n / N (%)	9 / 11 (82%)	5 / 12 (42%)	0.089
FeNO(ppb)	Median (Range)	39.0 (12.0–67.0)	26.0 (9.0–59.0)	0.21
Total IgE	Median (Range)	160 (4-2480)	289 (50-1260)	0.33
FEV1 (%pred.)	Mean, SD	78.6±22	80.8±21	>0.9
FVC (%pred)	Mean, SD	98.5±19	96.0±25	0.62
FEV1/FVC ratio	Mean, SD	65.0±7.0	66.0±11.0	0.43
Sputum eosinophils (%)	Median (Range)	20 (5–86)	10 (4–90)	0.42
Sputum neutrophils (%)	Median (Range)	37 (4–79)	54 (4–91)	0.11
Sputum macrophages (%)	Median (Range)	42 (4–65)	19 (1–52)	0.052
Sputum lymphocytes (%)	Median (Range)	0 (0–10)	0(0–1.50)	0.20
Blood total leukocyte (x10^9cells/L)	Mean, SD	6.62±1.70	7.35±2.34	0.40
Blood neutrophils (x10^9cells/L)	Mean, SD	3.65±0.97	4.04±1.18	0.42
Blood eosinophil (x10^9cells/L)	Mean, SD	0.38±0.16	0.27±0.64	0.24
Blood lymphocyte (x10^9cells/L)	Mean, SD	1.85±0.41	1.64±0.91	0.83
CRP (mg/L)	Mean, SD	5.5±6.3	5.2±9.7	0.41
Anti-EPX status (n positive, %)	n / N (%)	4 / 11 (36%)	1 / 12 (8.3%)	0.20
FEG score	Median (Range)	1.00 (1.00–3.00)	0.00 (0.00–3.00)	0.028

Table S2: Baseline characteristics of the study population in the Sepia study. Data are shown as mean±SD, median (IQR), numbers (n) or %. ICS: Inhaled corticosteroids. $^{\epsilon}$Atopy defined as a positive skin prick test or positive specific IgE.

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