LONG-TERM PROGNOSIS OF NEW ADULT-ONSET ASTHMA IN OBESE PATIENTS

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Supplementary material

Assessment of lung function, asthma control and inflammatory parameters

Lung function measurements were performed with a Vmax Encore 22 spirometer (Viasys Healthcare, Palm Springs, CA) that was calibrated daily. Finnish reference values were used [E1]. Post-bronchodilator measurements were taken 15 min after inhalation of salbutamol (400 µg). Fraction of exhaled nitric oxide (FeNO) was measured with a portable rapid-response chemiluminescent analyzer according to ATS standards [E2] (flow rate 50 mL/s; NIOX System, Aerocrine, Sweden). Venous blood was collected and white blood cell differential counts were determined. Total IgE levels were measured by using ImmunoCAP (Thermo Scientific, Uppsala, Sweden). Laboratory assays were performed in an accredited laboratory (SFS-EN ISO 15189:2013) of Seinäjoki Central Hospital.

Assessment of dispensed oral corticosteroids, ICS and adherence

Information on dispensed oral steroids and ICS was obtained from Finnish Social Insurance Institution [E3]. Only dispensed oral steroids having indication for asthma were taken into account. If indication was missing, medical records were examined and if no other possible indication was found, indication was assumed to be asthma. Methyl prednisolone was converted into mg of prednisolone to calculate total amount of dispensed oral steroids during the whole follow-up period as prednisolone mg. To calculate patient's average annual use in mg, total amount used during the whole follow-up period was divided by patient-specific years of follow-up. Adherence to ICS

medication was evaluated by comparing the patient´s dispensed doses to the prescribed doses for the whole 12-year period. Shortly, we converted all prescribed and dispensed ICS doses to budesonide equivalents and based on that information calculated annual and total 12-year adherence for each patient, as previously described [E3].

Bronchial challenge test

The bronchial challenge test was carried out with histamine by a dosimetric method with controlled tidal breathing by using a nebulizer [E4]. Buffered histamine diphosphate aerosol was inhaled by the subjects in 4-fold increasing doses (0.025 mg, 0.1 mg, 0.4 mg, 1.6 mg) at 5-min intervals. The end-point was either ≥15% fall in FEV_1 or the maximum histamine dose of 1.6 mg. After the provocation, 0.4 mg of salbutamol was given via spacer. Post-bronchodilatation FEV_1 was measured 15 min thereafter. $PD_{15}FEV_1$ value for histamine was calculated by interpolation [E5].

Table S1. Inclusion and exclusion criteria of SAAS-study.

Inclusion criteria	• a diagnosis of new-onset asthma made by a respiratory specialist
	• diagnosis confirmed by at least one of the following objective lung function measurements[1]:
	  ○ FEV_1 reversibility in spirometry of at least 15 % and 200 ml
	  ○ diurnal variability (≥ 20 %) or repeated reversibility (≥ 15 %/60 L/min) in PEF-follow-up
	  ○ a significant decrease in FEV_1 (15 %) or PEF (20 %) in response to exercise or allergen
	  ○ a significant reversibility in FEV_1 (at least 15 % and 200 ml) or mean PEF (20 %) in response to a trial with oral or inhaled glucocorticoids
	• symptoms of asthma
	• age ≥ 15years

Exclusion criteria	• physical or mental inability to provide signed informed consent
	• of note:
	  ○ patients with comorbidities, either other lung disease or any other significant disease were not excluded
	  ○ patients were not excluded because of smoking, alcohol use or any other lifestyle factor

[1]The objective lung function criteria reflect those of national and international guidelines valid in 1999-2002 and may not exactly follow those valid at the moment. FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow; SAAS, Seinäjoki Adult Asthma Study. The table has been previously published [E6].

Table S2. Characteristics of patients at baseline (asthma diagnosis) and 12 years of follow-up.

	Baseline (n=203)	**Follow-up (n=203)**	**p value**
Age (y)	46 (14)	58 (14)	
Male gender n (%)	85 (41.9)	85 (41.9)	
BMI (kg/m2)	27.1 (24.1-29.7)	28.1 (24.4-31.2)	<0.001
Smokers (ex/current) n (%)	103 (50.7)	107 (52.7)	0.125
Smoking history, pack-years	11 (5-20)	16 (7-30)	<0.001
Total IgE (kU/l)	84 (35-174)	61 (24-163)	0.046
Daily ICS use n (%)	16 (8.0)	155 (76.4)	<0.001
Blood eosinophils (10^9/l)	0.28 (0.15-0.42)†	0.17 (0.10-0.27)	<0.001
Pre-BD FEV_1 %	83 (71-92)	86 (76-96)	<0.001
Pre-BD FVC %	90 (80-100)	96 (87-106)	<0.001
Pre-BD FEV_1/FVC	0.75 (0.69-0.80)	0.73 (0.66-0.79)	<0.001
Post-BD FEV_1 %	88 (77-99)	90 (80-98)	0.013
Post-BD FVC %	94 (82-102)	99 (88-107)	<0.001
Post-BD FEV_1/FVC	0.79 (0.74-0.83)	0.75 (0.69-0.80)	<0.001
D_{LCO}, %	97 (19)	92 (19)	<0.001
D_{LCO}/VA, %	100 (17)	94 (17)	<0.001
AQ20 score	7 (4-10)	4 (2-7)	<0.001
Post-BD FEV_1/FVC <0.7 and pack-y ≥ 10 n (%)	16 (8.4)	29 (15.2)	0.001

Shown are mean (SD) or median (25-75 percentiles). †15.6 % with ongoing steroid –treatment. BMI = Body Mass Index, ICS = inhaled corticosteroid, BD = Bronchodilator, FVC = Forced vital capacity, D_{LCO}= Diffusing capacity, D_{LCO}/VA = Diffusing capacity adjusted by the alveolar volume, AQ20 = Airways Questionnaire**.** Comparisons were done by paired t-test (if normally distributed), related samples Wilcoxon signed rank test (if non-normally distributed) or McNemar test (categorical variables).

Table S3. Comparison of 12-year outcome of asthma in patients remaining obese to those who lost weight to become non-obese.

	Obese → obese	Obese → non-obese	P value
Subjects	42	7	
Females n (%)	24 (57.1)	4 (57.1)	>0.999
Age	59 (12)	65 (15)	0.304
BMI at diagnosis	33.2 (31.2-37.8)	30.9 (30.5-32.0)	**<0.001**
BMI at follow-up	34.3 (32.4-37.5)	28.1 (25.1-28.1)	**0.017**
Smoking history n (%)	25 (59.5)	3 (42.9)	0.443
Current smokers n (%)	6 (14.3)	0	0.574
Pack years of smokers	22 (16)	29 (12)	0.488
Atopic	12 (33.3)	2 (40.0)	>0.999
Daily ICS user n (%)	35 (83.3)	7 (100.0)	0.573
Self-reported daily ICS dose, µg as budesonide equivalent	1000 (800-1250)	800 (300-1400)	0.295
Average dispensed daily dose, µg budesonide equivalent	623 (284-841)	709 (451-776)	0.629
Average prescribed daily dose, µg budesonide equivalent	868 (797-1073)	800 (599-897)	0.282
Long-term adherence to ICS (%)	67 (40)	88 (21)	0.059
Asthma control[a]			0.320
Controlled n (%)	6 (14.3)	2 (28.6)	
Not controlled n (%)	36 (85.7)	5 (71.4)	
Reported using ≥1 oral steroid burst during follow-up, n (%)	21 (50.0)	2 (28.6)	0.424
Reported use of ≥1 oral steroid burst/2 previous yrs n (%)	19 (46.3)	2 (28.6)	0.445
Dispensed oral steroids/4 previous yrs (mg prednisolone)	150 (0-863)	0 (0-2000)	0.769
Dispensed oral steroids/total 12-year follow-up (mg prednisolone)	1275 (150-3003)	2600 (600-4040)	0.392
Any asthma-related unplanned hospitalization at follow-up n (%)	7 (16.7)	0	0.573
Pre-BD FEV_1 (% predicted)	80 (16)	86 (21)	0.406
Post-BD FEV_1 (% predicted)	83 (15)	90 (21)	0.292
Pre-BD FVC (% predicted)	88 (16)	102 (19)	**0.044**
Post-BD FVC (% predicted)	90 (15)	104 (20)	**0.033**
Pre-BD FEV_1/FVC	0.75 (0.70-0.79)	0.65 (0.62-0.73)	**0.049**
Post-BD FEV_1/FVC	0.74 (0.08)	0.69 (0.10)	0.161
ACT	20 (16-22)	21 (13-23)	0.769
AQ20	7 (3-9)	5 (4-8)	0.900
Blood eosinophils (*10^9/l)	0.19 (0.09-0.28)	0.15 (0.06-0.23)	0.457
Blood neutrophils (*10^9/l)	4.3 (1.3)	4.1 (1.1)	0.742
Total IgE (kU/l)	61 (29-127)	17 (5-198)	0.208
FeNO (ppb)	7 (5-15)	9 (4-16)	0.886
IL-6 (pg/ml)	3.5 (1.9-5.4)	2.1 (1.2-4.9)	0.242
hs-CRP (mg/l)	2.8 (1.2-5.5)	0.9 (0.4-3.3)	0.101
FEV_1 decline (ml/yr)	-50 (38)	-30 (26)	0.170
FEV_1 decline (% predicted/yr)	-0.55 (1.07)	+0.15 (0.97)	0.115
Post-BD FEV_1/FVC<0.7 and pack years≥10 n (%)	6 (14.3)	3 (42.9)	0.105

[a]based on GINA 2010 guideline. Abbreviations: ACT= asthma control test, AQ20=Airways Questionnaire, FeNO = fraction of nitric oxide in exhaled air, IL-6=interleukin 6, hs-CRP=high sensitivity C-reactive protein, IgE=immunoglobulin E, BD = Bronchodilator, FVC = Forced vital capacity, FEV_1=forced expiratory volume in 1 second. Comparisons were performed by chi-square-test (when categorical variables), independent samples t-test (if normally distributed continuous variable), independent samples Mann-Whitney U test (if non-normally distributed continuous variable).

Table S4. Predictors for ≥1 oral corticosteroid dispensing within 12 years from diagnosis by multivariate binary regression analysis.

	OR	95 % CI	P value
Female	2.70	1.27 to 5.74	0.010
BMI at dg <25			
BMI at dg 25-29.99	1.06	0.49 to 2.30	0.887
BMI at dg ≥30	3.29	1.18 to 9.21	0.023
Age of onset <45yrs			
Age of onset 45-60yrs	1.40	0.65 to 3.00	0.388
Age of onset >60yrs	1.70	0.55 to 5.25	0.359
Pack years at dg ≥10	1.81	0.74 to 4.43	0.192
B-eos at dg $<0.20*10^9$/l	2.61	1.19 to 5.74	0.017
pre-BD FEV_1 at dg >80 % predicted			
pre-BD FEV_1 at dg 60-80 % predicted	2.13	0.94 to 4.83	0.071
pre-BD FEV_1 at dg <60 % predicted	3.13	0.90 to 10.95	0.074
Asthma symptoms <16 yrs	1.60	0.70 to 3.64	0.265

BMI = Body Mass Index, BD = Bronchodilator, B-eos = blood eosinophils, FEV_1 = Forced expiratory volume in 1 second. Analyzed by multivariable binary logistic regression analysis. For BMI 25-29.99 and BMI≥30 at diagnosis, unadjusted ORs 1.17 (95% CI 0.61-2.24) and 3.36 (95% CI 1.40-8.06), respectively.

Table S5. Self-reported use of oral corticosteroids during 12-year follow-up in patients divided according to diagnostic BMI and blood eosinophil level.

	Low blood eosinophils at dg (<0.20)			**p-value**
	BMI<25 n=15	**BMI 25-29.99 n=32**	**BMI≥30 n=16**	
Oral steroids at follow-up n (%)	3 (20.0)	16 (50.0)	11 (68.8)	**0.023**
	High blood eosinophils at dg (≥0.20)			
	BMI<25 n=44	**BMI 25-29.99 n=53**	**BMI≥30 n=32**	
Oral steroids at follow-up n (%)	9 (20.5)	12 (22.6)	12 (37.5)	**0.198**

dg=diagnosis, BMI=body mass index. Comparisons performed by Chi-square test.

Table S6. Comparison of asthma outcome at 12-year follow-up visit in obese (BMI≥30) patients with high (≥0.20) and low (<0.20) blood eosinophils at diagnosis.

	Obese and high b-eos at dg	Obese and low b-eos at dg	P value
Subjects	32	16	
Age, yrs	45 (13)	52 (11)	0.069
Females n (%)	19 (59.4)	8 (50.0)	0.555
Smoking history n (%)	17 (53.1)	11 (68.8)	0.363
Current smoker n (%)	3 (9.4)	3 (18.8)	0.386
Pack years of smokers	25 (16)	19 (13)	0.388
BMI dg	32.2 (30.9-37.9)	33.3 (31.0-34.7)	0.759
BMI change (dg-follow-up)	+0.9 (3.6)	+0.1 (4.8)	0.518
Atopic (skin-prick test)	11 (42.3)	3 (21.4)	0.299
Asthma control[a]			0.956
Well controlled	5 (15.6)	2 (12.5)	
Partially controlled	10 (31.3)	5 (31.3)	
Uncontrolled	17 (53.1)	9 (56.3)	
Pre-BD FEV_1 (% pred)	84 (16)	75 (17)	0.094
Post-BD FEV_1 (% pred)	87 (16)	78 (16)	0.057
Pre-BD FEV_1/FVC	0.74 (0.07)	0.70 (0.10)	0.121
Post-BD FEV_1/FVC	0.75 (0.07)	0.71 (0.11)	0.142
FEV_1 decline during follow-up (ml/year)	-49 (30)	-46 (47)	
Asthma control test score	20 (15-22)	20 (17-22)	0.629
AQ20 score	7 (4-9)	6.5 (3-8)	0.403
Blood neutrophils (*10^9/l)	4.3 (1.3)	4.2 (1.3)	0.963
FeNO (ppb)	8 (5-16)	7 (5-12)	0.682
IL-6 (pg/ml)	3.3 (2.0-5.2)	3.7 (1.4-6.3)	0.827
hsCRP (mg/l)	3.1 (1.5-5.9)	1.2 (0.6-2.7)	0.049
Reported using oral steroids during follow-up, n (%)	12 (37.5)	11 (68.8)	0.066
Oral steroid dispensings during 12-year follow-up (mg prednisolone per year)	64 (12-241)	168 (86-353)	0.043
Daily ICS user at follow-up n (%)	28 (87.5)	14 (87.5)	>0.999
Self-reported ICS dose of daily users in budesonide eq µg	1000 (788-1500)	800 (500-1000)	0.383
ICS dispensings (average daily dose in budesonide eq µg)	676 (357-860)	694 (400-819)	0.872
Average adherence to ICS (dispensed/prescribed, %)	69 (38)	77 (36)	0.491
≥1 Hospitalizations, all respiratory-related, n (%)	13 (40.6)	6 (37.5)	>0.999
All visits to healthcare/12 yrs	17 (11-26)	25 (11-35)	0.399
Unplanned visits to healthcare/12 yrs	3 (1-8)	10 (0-15)	0.311
Pack years ≥10 and post-BD FEV_1/FVC<0.7 n (%)	5 (15.6)	4 (25.0)	0.457
Number of comorbidities	2 (1-4)	3 (1-4)	0.456

[a]based on GINA 2010 guideline. Data shown as mean (SD), median (25-75 percentiles) or n (%). BD=bronchodilator, FEV_1=forced expiratory volume in 1 second, FVC=forced vital capacity, IL=interleukin, hsCRP=high-sensitivity C-reactive protein, AQ20=Airways Questionnaire 20, FeNO= fraction of nitric oxide in exhaled air, ICS=inhaled corticosteroid, COPD=chronic obstructive pulmonary disease. Comparisons were performed by chi-square-test (when categorical variables), independent samples t-test (if normally distributed continuous variable), independent samples Mann-Whitney U test (if non-normally distributed continuous variable).

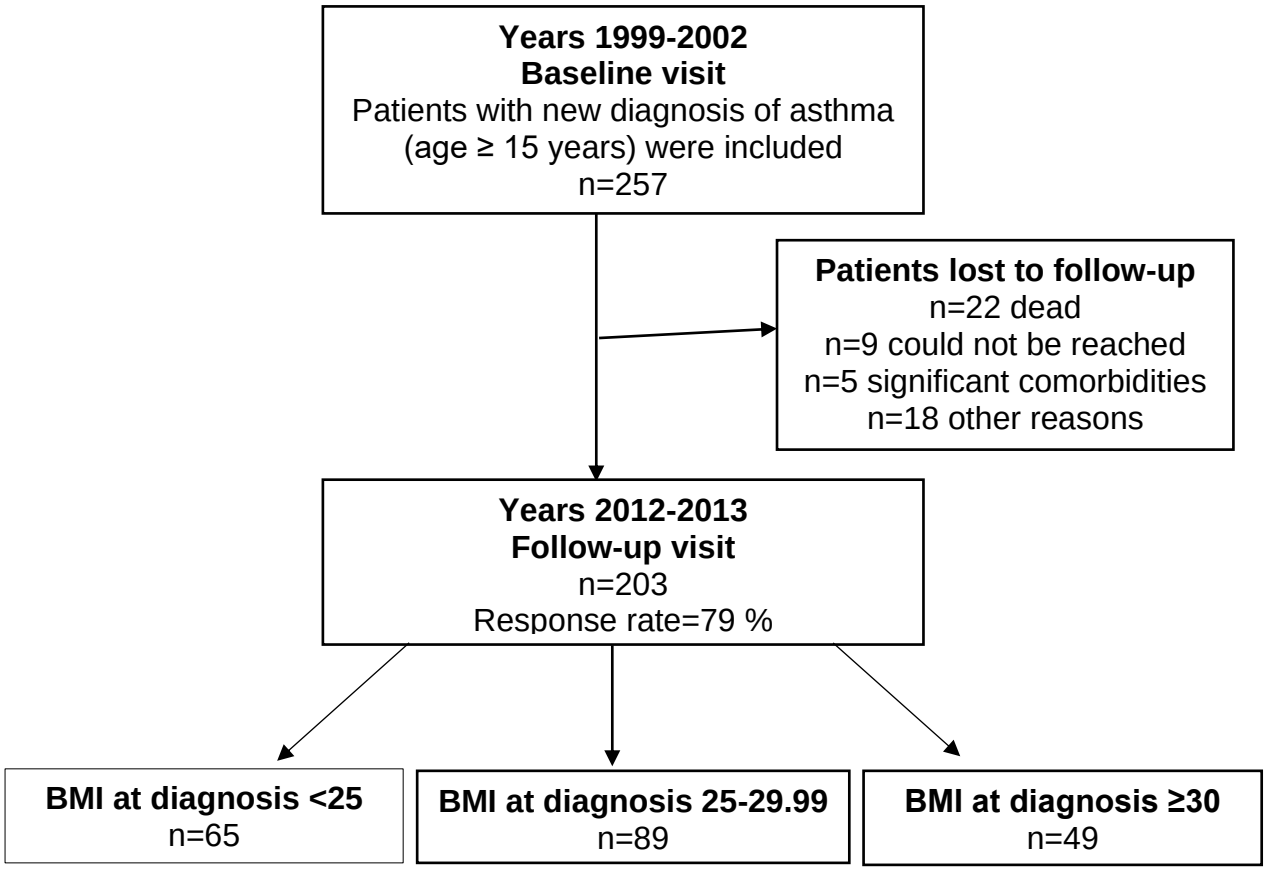

Figure S1. Flow chart of the study.

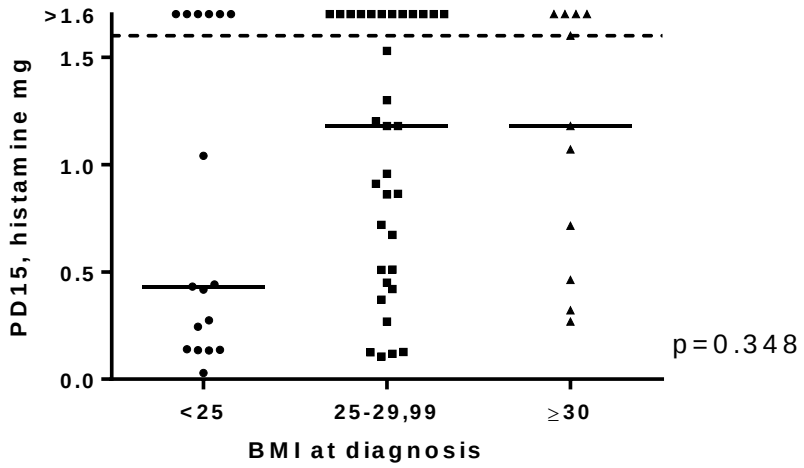

Figure S2. Airway hyperresponsiveness to histamine. PD15 >1.6mg was regarded as no hyperreactivity. Shown lines are medians. P-value was determined by Kruskal-Wallis test.

E-supplement references

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E4. Sovijärvi A, Malmberg LP, Reinikainen K, Rytilä P, Poppius H. A rapid dosimetric method with controlled tidal breathing for histamine challenge. Repeatability and distribution of bronchial reactivity in a clinical material. *Chest* 1993; 104:164-70.

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E6. Kankaanranta H, Ilmarinen P, Kankaanranta T, Tuomisto LE. Seinäjoki adult asthma study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialized care. *NPJ Prim Care Resp. Med*. 2015;25:15042.