T2-high asthma phenotypes across life span

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Online Data Supplement

Study design and procedures

Recruiting centres of the ALLIANCE cohort are five pediatric specialist centers (Hannover, Lubeck, Munich, Marburg and Cologne) and two adult specialist centers (LungenClinic Grosshansdorf and Research Centre Borstel), all of which belong to the German Center for Lung Research (DZL). Recruitment started in 2013. Participants with preschool wheeze and asthma had annual study visits while healthy controls were only seen once. Study visits were postponed if patients had upper respiratory tract infections or asthma exacerbations (adults) or increased body temperature >38.5°C in the past two weeks (children). A questionnaire covering respiratory symptoms like wheeze and cough during the previous 12 months, previous medical history, including pre-and postnatal conditions, environmental exposures, childcare, and family history was answered by caregivers and adult subjects, respectively.

Definitions for eczema, hay fever, parental history of asthma, asthma exacerbations, active and passive smoking, asthma control, body mass index (BMI) and others are specified in detail in table E2 and have been published elsewhere (E1).

Spirometry and FeNO were measured in all participants ≥6 years and quality was controlled according to published guidelines (E2, E3). Positive bronchodilator response was assessed after two (children) or four puffs (adults) of albuterol. In children, FeNO was measured with a single breath manoeuver using the chemoluminescence analyzer CLD 88 (EcoMedics AG, Duernten, Switzerland) in all pediatric centers. In one center (Lubeck), FeNO was initially measured using an electrochemical sensor (NO VARIO Analyzer, Filt, Berlin, Germany). In adults the NIOX MINO (Circassia AB, Uppsala, Sweden) was used.

Differential blood count was performed in on-site routine hospital laboratories. Specific immunoglobulin E was measured centrally by Euroline™ (Euroimmun, Germany) against a panel of aeroallergens including house dust mite, grass pollen, mugwort, ribwort, plantain, common silver birch, ragweed, hazel, alder, cat, dog, horse, cladosporium, aspergillus fumigatus, alternaria alternata, penicillium notatum and food allergens including tomato, apple, kiwi, cod, bovine serum albumin, casein, beta-lactoglobulin, alpha-lactoglobulin, milk, egg yolk and egg white protein, soy, sesame, rye, wheat, almond, walnut, hazelnut and peanut.

Atopy was defined as at least one allergen-specific IgE $\geq$0.7 kU/L from a comprehensive allergen panel using an immunoblot based method (Euroline^{TM}). We based this cut-off on previous publication from the ALLIANCE cohort showing improved sensitivity and specificity for detecting clinical allergy against food and pollen when using a cut-off of $\geq$0.7 kU/L compared $\geq$0.35 kU/L (E4).

T2 cytokine analysis was performed centrally at the Institute of Laboratory Medicine and Pathobiochemistry, Philipps University Marburg. IL-5 and IL-13 were measured in TruCulture supernatants using a Bio-Plex Pro Human singleplex assays (Bio-Rad, USA) as per manufacturer instruction in children. For supernatants from adult samples, a Bio-Plex Pro Human multiplex assay (Bio-Rad, USA) was used to measure IL-4, IL-5 and IL-13. Sputum in adults was collected for cell differentiation per cytospin according to local clinical standards (E5). The following dataset versions were used for the analysis: 20200420_V4-0 (children) and 20180731_V2-1 (adults).

Supplement References

E1. Fuchs O, Bahmer T, Weckmann M, Dittrich AM, Schaub B, Rosler B, Happle C, Brinkmann F, Ricklefs I, Konig IR, Watz H, Rabe KF, Kopp MV, Hansen G, von Mutius E. The all age asthma

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E2. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, McCarthy K, McCormack MC, Oropez CE, Rosenfeld M, Stanojevic S, Swanney MP, Thompson BR. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med* 2019; 200: e70-e88.

E3. American Thoracic S, European Respiratory S. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912-30.

E4. Skevaki C, Tafo P, Eiringhaus K, Timmesfeld N, Weckmann M, Happle C, Nelson PP, Maison N, Schaub B, Ricklefs I, Fuchs O, von Mutius E, Kopp MV, Renz H, Hansen G, Dittrich AM, Group AS. Allergen extract- and component-based diagnostics in children of the ALLIANCE asthma cohort. *Clin Exp Allergy* 2021: 51(10): 1331-1345.

E5. Pedersen F, Zissler UM, Watz H, Rabe KF, Hohlfeld JM et al. (2019) Rating sputum cell quality in clinical trials for asthma and COPD treatment. International journal of chronic obstructive pulmonary disease 14: 195–198

Supplementary Figure Legends

Figure E1: Overview of participants included in the ALLIANCE study

Study design. Patients with data for blood eosinophils and atopy were included into the analysis. NA = not available, LPS = lipopolysaccharide, FeNO = fractional exhaled nitric oxide, y = year, t = time-point, t00 = baseline, t12 and t24 are the first and second follow-up, after 12 and 24 months, respectively.

Figure E2: Distribution of blood eosinophil counts by age-groups

Boxplots show the distribution of blood eosinophils for (A) children with preschool wheeze (<6 years) or asthma (≥6 years) and (B) adults compared to age-matched healthy participants. The green lines represent the loess smoothed regression fit on the data values.

Figure E3: Distribution of blood eosinophils levels stratified by age in healthy children

Blood eosinophil of healthy controls among children stratified according age (n = 70 with age < 6 years and n = 205 with age ≥ 6 years).

Figure E4: Overlap of T2-high asthma patients with patients having increased FeNO and sputum eosinophils

The Venn diagram shows the overlap between patients with FeNO ≥ 35 ppb and sputum eosinophils (s-Eos) ≥ 3% and T2-high asthma defined by blood eosinophils (b-Eos) ≥ 360 cells/µL and atopy (at least one allergen-specific IgE ≥0.7 kU/L). Only adult asthmatics with available for all four biomarkers were included (n= 83).

Supplementary tables

Table E1: Exclusion criteria for ALLIANCE study participants

Children
Premature birth (<37 weeks of gestation)
Pulmonary malformations
Postnatal oxygen requirement >24 hours
Post-natal mechanical ventilation
Cystic fibrosis
Primary ciliary dyskinesia
Interstitial lung disease
Any cardiac malformation with increased pulmonary blood flow
Other chronic non-allergic comorbidities
Adults
Clinical signs of chronic obstructive pulmonary disease, specified in (11)
Signs or history of chronic bronchitis
Signs or history of emphysema

Table E2: Definitions of variable

Variable	**Definition**	**Studygroup**
Eczema	Doctor's diagnosis, reported by parents	Children, adults
Hay fever	Doctor's diagnosis, reported by parents	Children, adults
Parental history of asthma	Doctor's diagnosis, reported by parents	Children, adults
Exacerbation rate/ person/ year	Number of exacerbations requiring any systemic steroid treatment (children: any length of systemic steroid treatment; adults: at least 3 days) or up-titration of regular OCS per person per past 12 months	Children, adults
Inhaled corticosteroid dose	Categorization into low, medium and high according to GINA guidelines, at study visit	Children, adults
Age of asthma onset	Childhood onset of asthma (diagnosis <18 years), adult onset (diagnosis ≥18 years)	Adults
Passive smoking	Household exposure to tobacco smoke (indoors and on the balcony)	Children, adults
Smoking status	Categorization into never, current or former smoker	Adults
Asthma control	Classified according to GINA guidelines (9)	Children, adults
Asthma severity	Classified according GINA treatment steps (GINA Treatment steps 1 and 2: mild asthma, step 3: moderate asthma, steps 4 and 5: severe asthma)	Adults
Body mass index	weight (kg) / [height (m)]^2	Children, adults
Outcome of pre-school wheeze		**Children (< 6 yrs)**

Remission	Absence of any asthma symptoms and no intake of any asthma medication in the past 12 months
Intermittent asthma	2-4 months with ICS treatment and/or 1-2 wheeze episodes (albuterol treatment for wheeze for more than 2/7 days) in the past 12 months
Asthma	At least 5 months with ICS treatment and/or treatment with a biological and/or at least one exacerbation (hospitalisation or treatment with systemic steroids) and/or at least 3 wheeze episodes (albuterol treatment for wheeze for more than 2/7 days) in the past 12 months and/or uncontrolled asthma (according to GINA guidelines) at time of the study visit
Unclear	Any intake of medication or symptoms not covered by the categories above

OCS = oral corticosteroids, ICS = inhaled corticosteroids, GINA = Global Initiative for Asthma, and BMI = body mass index, yrs= years.

Table E3: Characteristics of the ALLIANCE study participants

	Children				Adults	
	Healthy (<6 y)	**Wheeze (<6 y)**	**Healthy (≥6 y)**	**Asthma (≥6 y)**	**Healthy (≥18 y)**	**Asthma (≥18 y)**
Number of subjects	n=75	n=276	n=210	n=282	n=64	n=218
Sex, n (% females)	36 (48.0)	94 (34.1)	103 (49.0)	98 (34.8)**	29 (45.3)	122 (56.0)
Age (y), mean (SD)	3.28 (1.66)	3.07 (1.45)*	11.75 (3.35)	10.67 (3.09)**	50.03 (17.50)	51.97 (13.65)
Atopy, n (%)	27 (46.6)	80 (34.3)**	99 (50.0)	212 (81.9)****	21 (33.3)	135 (63.7)***
Exacerbations, n (%)	0 (0.0)	109 (50.0)	0 (0.0)	60 (23.7)	0 (0.0)	124 (57.1)
b-Eos (cells/µL), mean (SD)	231.72 (154.98)	405.11 (379.67)***	241.13 (208.69)	488.85 (371.15)****	176.88 (108.51)	368.86 (327.30)***
FeNO (ppb), mean (SD)	NA	NA	18.12 (28.53)	28.91 (40.86)**	17.88 (9.70)	38.26 (40.48)***
FEV_1 (z-score), mean (SD)	NA	NA	0.09 (0.94)	-0.42 (1.35)****	-0.15 (0.76)	-1.68 (1.45)****
FVC (z-score), mean (SD)	NA	NA	-0.02 (0.92)	0.08 (1.25)	0.21 (0.87)	-0.49 (1.13)****
FEV_1/FVC (z-score), mean (SD)	NA	NA	0.21 (1.14)	-0.80 (1.15)****	-0.60 (0.77)	-1.99 (1.34)****
FEF_{25-75} (z-score), mean (SD)	NA	NA	0.10 (1.15)	-0.91 (1.28)****	-0.49 (0.73)	-1.93 (1.29)****
ICS, n (%)	NA	105 (38.46)	NA	203 (73.29)	NA	194 (88.99)
LTRA, n (%)	NA	25 (9.16)	NA	34 (12.27)	NA	33 (15.14)
LABA, n (%)	NA	33 (12.09)	NA	122 (44.04)	NA	180 (82.57)
LAMA, n (%)	NA	NA	NA	NA	NA	57 (26.15)
OCS, n (%)	NA	0 (0.0)	NA	3 (1.08)	NA	49 (22.48)
Omalizumab, n (%)	NA	0 (0.0)	NA	4 (1.44)	NA	15 (6.88)

Comparison between two groups was performed using unpaired t-test and frequency distribution was compared using the Chi-square test. Statistical significance indicated by $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$. Patients with wheeze and asthma were compared to healthy participants of the same age group. Percentages might not add up to 100% due to rounding. ^{NA}Lung function not performed in children <6 y. Data about LAMA use was not obtained in children. SD = standard deviation, b-Eos = blood eosinophils, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, FEF_{25-75} = forced expiratory flow at 25%-75% of FVC, FeNO = fractional exhaled nitric oxide, ppb = parts per billion, ICS = inhaled corticosteroids, LTRA = leukotriene receptor antagonist, LABA = long-acting beta-agonist, LAMA = long-acting muscarinic-antagonists, OCS = oral corticosteroids, NA = not applicable, and y = year.

Table E4: Biomarker distribution of T2 inflammation across all phenotypes

	Atopy-only	Eos-only	T2-high	T2-low	p-value
b-Eos cut-off ≥470 (cells/µL)					
Children (≥6 y), n=165	**n=66**	**n=3**	**n=68**	**n=28**	
b-Eos (cells/µL), mean (SD)	268.78 (120.40)[b,d]	649.10 (200.02)[b,c]	825.90 (342.02)[d,d]	221.39 (115.64)[c,d]	<0.0001
FeNO (ppb), mean (SD)	22.47 (17.67)[a,c]	39.47 (52.46)	41.15 (57.60)[a,d]	12.93 (15.53)[c,d]	0.0001
FeNO, n (%): <20 (ppb)	36 (54.5)	2 (66.7)	30 (44.1)	26 (92.9)	
≥20-35 (ppb)	17 (25.8)	0 (0.0)	13 (19.1)	0 (0.0)	0.0004
≥35 (ppb)	13 (19.7)	1 (33.3)	25 (36.8)	2 (7.1)	
b-Eos cut-off ≥360 (cells/µL)					
Adults, n=211	**n=83**	**n=36**	**n=52**	**n=40**	
b-Eos (cells/µL), mean (SD)	182.65 (96.40)[d,d]	660.92 (418.73)[d,**d**]	607.39 (285.45)[d,*d*]	161.64 (94.23)[**d**, *d*]	<0.0001
FeNO (ppb), mean (SD)	30.43 (23.81)[a,b,c]	50.50 (35.67)[c,d]	48.83 (53.92)[b,d]	20.33 (12.38)[a,d,d]	<0.0001
FeNO, n (%): <25 (ppb)	42 (51.9)	8 (23.5)	17 (32.7)	26 (66.7)	
≥25-50 (ppb)	23 (34.5)	13 (38.2)	21 (40.4)	13 (33.3)	0.0003
≥50 (ppb)	16 (19.8)	13 (38.2)	14 (26.9)	0 (0.0)	
s-Eos (≥3%), n (%)	15 (21.4)	24 (77.4)	27 (60.0)	7 (23.3)	<0.0001

Calculations were based on different cutoff values for FeNO among children (≥6 years) and adults with asthma. Only patients with data available for FeNO were included. Kruskal-Wallis and Wilcoxon tests were used to compare biomarker between asthma phenotypes. Frequency distribution was compared using the Chi-square test. The (plain, underlined, bold, and/or italic) superscripts indicate for which phenotypes the continuous variables significantly differ. [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001 for contrasts (Wilcoxon test). FeNO = fractional exhaled nitric oxide, b-Eos = blood eosinophils, y = year, ppb = parts per billion, and s-Eos = sputum eosinophils.

Table E5: Phenotypes of T2 inflammation using FeNO and sputum eosinophils in adult asthma patients

Adult asthmatics	**FeNO-only (FeNO ↑, s-Eos ↓)**	**s-Eos-only (FeNO ↓, s-Eos ↑)**	**T2-high (FeNO ↑, s-Eos ↑)**	**T2-low (FeNO ↓, s-Eos ↓)**
Number of subjects, n = 180	**n (%)**	**n (%)**	**n (%)**	**n (%)**
FeNO (≥25 ppb), s-Eos, ≥2%	30 (16.7)	21 (11.7)	68 (37.8)	61 (33.9)
FeNO (≥25 ppb), s-Eos, ≥3%	37 (20.6)	16 (8.9)	61 (33.9)	66 (36.7)
FeNO (≥35 ppb), s-Eos, ≥2%	12 (6.7)	36 (20.0)	53 (29.4)	79 (43.9)
FeNO (≥35 ppb), s-Eos, ≥3%	17 (9.4)	29 (16.1)	48 (26.7)	86 (47.8)
FeNO (≥50 ppb), s-Eos, ≥2%	6 (3.3)	57 (31.7)	32 (17.8)	85 (47.2)
FeNO (≥50 ppb), s-Eos, ≥3%	8 (4.4)	47 (26.1)	30 (16.7)	95 (52.8)

Calculations were based on various cut-off values for fractional exhaled nitric oxide (FeNO) and sputum eosinophils among adults. Increased FeNO was defined as ≥25, ≥35, and ≥50 parts per billion (ppb). Increased sputum eosinophils were defined as ≥2 and ≥3% respectively. ↑ = increased and ↓ = decreased.

Table E6: Biomarker distribution of T2 inflammation in adults with asthma across all phenotypes using alternative blood eosinophil cut-offs

	Atopy-only	Eos-only	T2-high	T2-low	p-value
b-Eos cut-off ≥150 (cells/µL)					
Adults, n=211	n=29	n=55	n=106	n=21	
b-Eos (cells/µL), mean (SD)	79.02 (50.56)[d,d]	516.27 (394.28)[d,**d**]	419.36 (275.61)[d,*d*]	88.75 (40.99)[**d**,*d*]	<0.0001
FeNO (ppb), mean (SD)	27.25 (22.11)[a,a]	40.72 (32.49)[a,c]	40.39 (42.44)[a]	18.69 (12.75)[c]	0.0006
FeNO, n (%): <25 (ppb)	7 (24.1)	19 (36.5)	43 (41.0)	15 (71.4)	
≥25-50 (ppb)	5 (17.3)	20 (38.5)	37 (35.2)	6 (28.6)	0.0635
≥50 (ppb)	17 (58.7)	13 (25.0)	25 (23.8)	0 (0.0)	
s-Eos (≥3%), n (%)	4 (16.0)	29 (64.4)	38 (42.2)	2 (12.5)	<0.0001
b-Eos cut-off ≥300 (cells/µL)					
Adults, n=211	n=69	n=42	n=66	n=34	
b-Eos (cells/µL), mean (SD)	153.82 (78.42)[d,d]	611.93 (405.53)[d,**d**]	547.42 (278.47)[d,*d*]	134.05 (72.29)[**d**,*d*]	<0.0001
FeNO (ppb), mean (SD)	28.35 (21.82)[b,c]	47.10 (34.84)[c,d]	47.04 (49.82)[b,d]	19.79 (11.89)[d,d]	<0.0001
FeNO, n (%): <25 (ppb)	38 (56.7)	11 (28.2)	21 (31.8)	23 (67.6)	
≥25-50 (ppb)	18 (26.9)	15 (38.5)	26 (39.4)	11 (32.4)	0.0003
≥50 (ppb)	11 (16.4)	13 (33.3)	19 (28.8)	0 (0.0)	
s-Eos (≥3%), n (%)	11 (18.3)	27 (73.0)	31 (56.4)	4 (16.7)	<0.0001

T2 phenotypes are shown using alternative cut-off values for eosinophils (≥150 and ≥300 cells/µL, respectively) as sensitivity analysis. Kruskal-Wallis and Wilcoxon tests were used to compare between asthma phenotypes. Frequency distribution compared using the Chi-square test. The (plain, underlined, bold, and/or italic) superscripts indicate for which phenotypes the continuous variables significantly differ. [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001 for contrasts (Wilcoxon test). FeNO = fractional exhaled nitric oxide, ppb = parts per billion, b-Eos = blood eosinophils, s-Eos = sputum eosinophils, and y = year.

Table E7: Biomarker distribution of T2 inflammation in children with asthma across all phenotypes using alternative blood eosinophil cut-offs

	Atopy-only	Eos-only	T2-high	T2-low	p-value
b-Eos cut-off ≥150 (cells/µL)					
Asthma, n=165	n=12	n=25	n=122	n=6	
b-Eos (cells/µL), mean (SD)	92.21 (39.04)[d,d]	309.23 (166.70)[d,**d**]	596.58 (365.72)[d,**d**, *d*]	82.12 (37.01)[*d*]	<0.0001
FeNO (ppb), mean (SD)	15.06 (9.97)[a]	17.44 (23.20)[c]	34.27 (45.58)[a,b,c]	8.25 (4.48)[b]	<0.0001
FeNO, n (%): <20 (ppb)	10 (83.3)	22 (88.0)	56 (45.9)	6 (100.0)	
≥20-35 (ppb)	1 (8.3)	0 (0.0)	29 (23.8)	0 (0.0)	0.0004
≥35 (ppb)	1 (8.3)	3 (12.0)	37 (30.3)	0 (0.0)	
b-Eos cut-off ≥300 (cells/µL)					
Asthma, n=165	n=40	n=11	n=94	n=20	
b-Eos (cells/µL), mean (SD)	187.59 (73.73)[d,d]	448.09 (165.58)[b,d,**d**]	706.23 (347.35)[b,d,*d*]	164.73 (64.61)[**d**,*d*]	<0.0001
FeNO (ppb), mean (SD)	22.77 (19.13)[b]	22.54 (26.99)[a]	36.71 (50.46)[d]	11.88 (16.75)[a,b,d]	<0.0001
FeNO, n (%): <20 (ppb)	23 (57.5)	9 (81.8)	43 (45.7)	19 (95.0)	
≥20-35 (ppb)	7 (17.5)	0 (0.0)	23 (24.5)	0 (0.0)	0.0024
≥35 (ppb)	10 (25.0)	2 (18.2)	28 (29.8)	1 (5.0)	

T2 phenotypes are shown using alternative cut-off values for eosinophils (≥150 and ≥300 cells/µL, respectively) as sensitivity analysis. Kruskal-Wallis and Wilcoxon tests were used to compare between asthma phenotypes. Frequency distribution compared using the Chi-square test. The (plain, underlined, bold, and/or italic) superscripts indicate for which phenotypes the continuous variables significantly differ. [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001 for contrasts (Wilcoxon test). FeNO = fractional exhaled nitric oxide, ppb = parts per billion, b-Eos = blood eosinophils, s-Eos = sputum eosinophils, and y = year.

Table E8: Cytokines secreted after stimulation of whole blood with LPS or anti-CD3/CD28 in children

Children, Wheeze (<6 y)	**Atopy-only**	**Eos-only**	**T2-high**	**T2-low**	**p-value**
LPS (n=163)	n=29	n=24	n=26	n=84	
IL-5 (ng/mL)	4.54 (9.74)	4.11 (5.43)	4.33 (5.64)	4.26 (12.62)	0.8459
IL-13 (ng/mL)	6.19 (4.97)	6.50 (7.35)	5.81 (4.58)	5.05 (5.33)	0.7941
Anti-CD3/CD28 (n=157)	n=28	n=24	n=24	n=81	
IL-5 (ng/mL)	11.32 (32.57)	9.18 (32.36)	8.44 (28.17)	18.51 (18.51)	0.7218
IL-13 (ng/mL)	189.00 (300.32)	139.18 (358.35)	140.88 (338.63)	85.59 (219.79)	0.5489
Children, Asthma (≥6 y)	**Atopy-only**	**Eos-only**	**T2-high**	**T2-low**	**p-value**
LPS (n=182)	n=69	n=4	n=75	n=34	
IL-5 (ng/mL)	4.54 (9.74)	5.70 (11.80)	5.97 (11.65)	2.94 (9.40)	0.3042
IL-13 (ng/mL)	5.52 (4.44)	8.83 (6.69)	6.27 (5.54)	6.77 (6.66)	0.3200
Anti-CD3/CD28 (n=176)	n=68	n=5	n=69	n=34	
IL-5 (ng/mL)	8.95 (22.24)[b]	47.15 (26.89)[a]	19.89 (65.28)[b,b]	7.32 (16.74)[a,b]	0.0014
IL-13 (ng/mL)	150.26 (347.76)	472.66 (269.08)	216.92 (438.06)	187.01 (302.04)	0.1485

Median (interquartile range) levels of cytokines were compared between phenotypes using Kruskal-Wallis test (p-value). [a]$p<0.05$, [b,b]$p<0.01$ for contrasts between phenotypes (Wilcoxon test). Numbers of LPS and anti-CD3/CD28 samples within groups can vary due to sample availability. LPS = lipopolysaccharide, b-Eos = blood eosinophils, sIgE = specific Immunoglobulin E, and y = year.

Table E9: Cytokines secreted after stimulation of whole blood with LPS or anti-CD3/CD28 in adults

Adults with asthma (≥18 y)	Atopy-only	Eos-only	T2-high	T2-low	p-value
LPS (n=200)	n=81	n=34	n=46	n=39	
IL-4 (ng/mL)	0.51 (0.60)	0.45 (0.49)	0.42 (0.44)	0.37 (0.54)	0.3801
IL-5 (ng/mL)	0.42 (0.75)[a]	0.67 (0.84)[a,b]	0.45 (0.67)	0.29 (0.48)[b]	0.0149
IL-13 (ng/mL)	1.95 (2.14)[a]	2.00 (2.25)	1.67 (2.03)	1.36 (1.52)[a]	0.1212
Anti-CD3/CD28 (n=201)	n=82	n=34	n=46	n=39	
IL-4 (ng/mL)	2.19 (5.46)	2.73 (7.12)	3.27 (6.43)	1.83 (4.92)	0.4277
IL-5 (ng/mL)	40.36 (171.59)[a]	29.52 (176.25)	102.24 (223.91)[a,b]	19.31 (113.05)[b]	0.0851
IL-13 (ng/mL)	52.99 (200.33)	40.17 (242.02)	153.42 (354.91)[a]	33.89 (169.32)[a]	0.1121

Median (interquartile range) levels of cytokines were compared between phenotypes using Kruskal-Wallis test (p-value). [a]$p<0.05$, [b]$p<0.01$ for contrasts between phenotypes (Wilcoxon test). Numbers of LPS and anti-CD3/CD28 samples within groups can vary due to sample availability. LPS = lipopolysaccharide, b-Eos = blood eosinophils, sIgE = specific Immunoglobulin E, and y = year.

Table E10: Cytokines secreted after stimulation with LPS or anti-CD3/CD28 in adult asthma patients and adult healthy control subjects

	Atopy-only	Eos-only	T2-high	T2-low	p-value
Subjects <45 y, LPS					
Adult asthmatics (n=51)	n=27	n=4	n=14	n=6	
HC (n=18): IL-4 (ng/mL): 0.39 (0.40)					
IL-4 (ng/mL)	0.45 (0.67)	0.43 (0.55)	0.37 (0.46)	0.28 (0.21)	0.4531
HC (n=18): IL-5 (ng/mL): 0.64 (0.58)					
IL-5 (ng/mL)	0.42 (0.63)	1.10 (0.49)[a]	0.35 (0.79)	0.27 (0.09)[a]	0.1103
HC (n=18): IL-13 (ng/mL): 2.14 (1.23)					
IL-13 (ng/mL)	1.76 (1.20)[a]	2.09 (0.92)[a]	2.26 (2.26)	0.94 (1.00)[a,a]	0.1069
Subjects <45 y, Anti-CD3/CD28					
Adult asthmatics (n=51)	n=27	n=4	n=14	n=6	
HC (n=18): IL-4 (ng/mL): 1.12 (3.17)					
IL-4 (ng/mL)	2.21 (3.66)	2.42 (4.64)	2.33 (2.26)	3.19 (4.50)	0.9723
HC (n=18): IL-5 (ng/mL): 13.12 (91.74)					
IL-5 (ng/mL)	47.74 (142.00)	32.05 (78.51)	49.59 (117.09)	29.19 (51.91)	0.8918
HC (n=18): IL-13 (ng/mL): 26.01 (163.18)					
IL-13 (ng/mL)	69.03 (166.54)	25.81 (61.83)	88.82 (214.54)	81.15 (157.93)	0.7959
Subjects ≥45 y, LPS					
Adult asthmatics (n=149)	n=54	n=30	n=32	n=33	
HC (n=29): IL-4 (ng/mL): 0.49 (0.75)					
IL-4 (ng/mL)	0.52 (0.53)	0.45 (0.55)	0.43 (0.42)	0.37 (0.54)	0.7264
HC (n=29): IL-5 (ng/mL): 0.47 (0.59)					
IL-5 (ng/mL)	0.40 (0.76)	0.59 (0.85)[a]	0.51 (0.64)	0.31 (0.67)[a]	0.1009
HC (n=29): IL-13 (ng/mL): 1.99 (2.60)					
IL-13 (ng/mL)	2.10 (3.17)	1.99 (2.67)	1.63 (1.86)	1.36 (2.03)	0.2815
Subjects ≥45 y, Anti-CD3/CD28					
Adult asthmatics (n=150)	n=55	n=30	n=32	n=33	

HC (n=29): IL-4 (ng/mL): 2.96 (8.83)					
IL-4 (ng/mL)	2.17 (6.49)	2.73 (7.12)	5.33 (8.82)[a]	1.83 (5.25)[a]	0.1783
HC (n=29): IL-5 (ng/mL): 47.59 (144.0)					
IL-5 (ng/mL)	32.16 (187.90)[a]	29.52 (179.05)[a]	133.57 (267.58)[a,a,b]	19.31 (112.76)[b]	0.0434
HC (n=29): IL-13 (ng/mL): 97.13 (422.75)					
IL-13 (ng/mL)	52.46 (210.79)[a]	50.02 (328.25)	183.26 (427.79)[a,a]	33.89 (172.56)[a]	0.0546

Comparison of cytokine levels in adult asthmatics are based on age stratification <45 y and ≥45 y. Median (interquartile range) levels of cytokines were compared between phenotypes using Kruskal-Wallis test (p-value). [a,a]$p<0.05$ for contrasts between phenotypes (Wilcoxon test). Numbers of LPS and anti-CD3/CD28 samples within groups can vary due to sample availability. LPS = lipopolysaccharide, and y = year, HC = Healthy controls (values for HC are shown in italics).

Figures

Figure E1: Overview of participants included in the ALLIANCE study

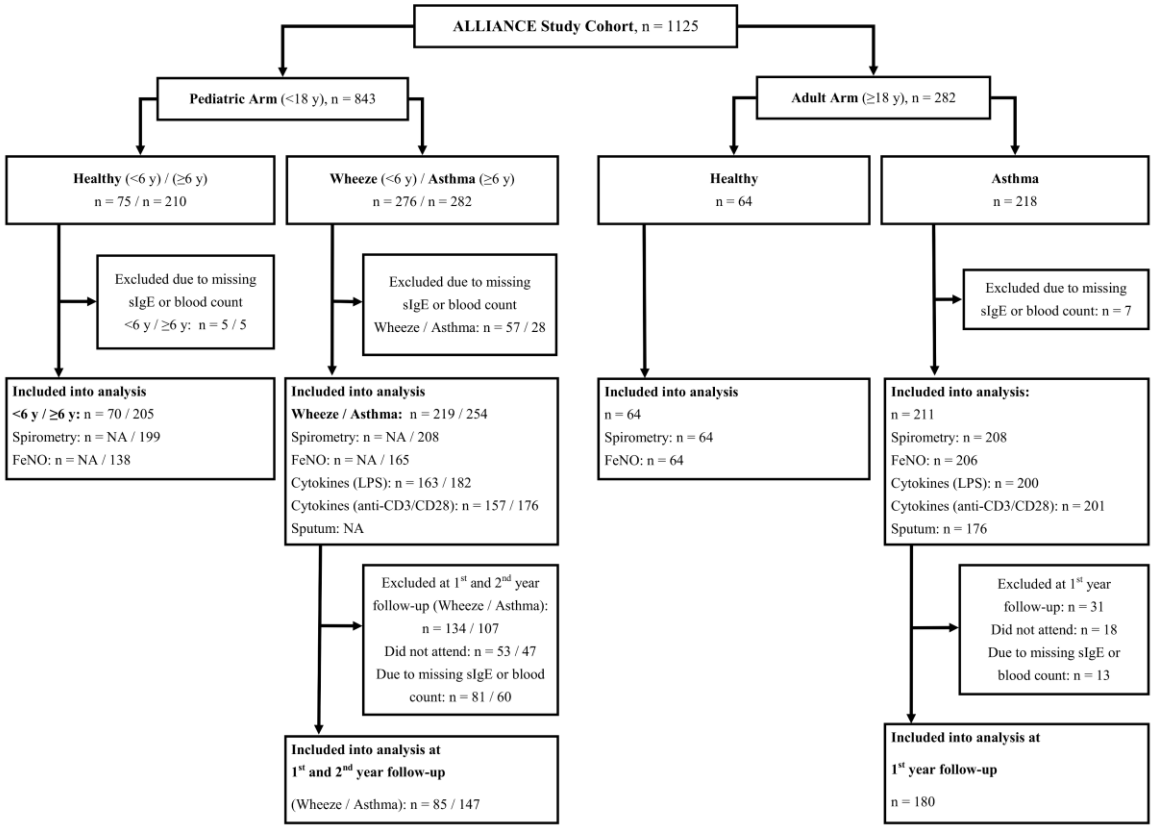

Figure E2: Distribution of blood eosinophil count by age-groups

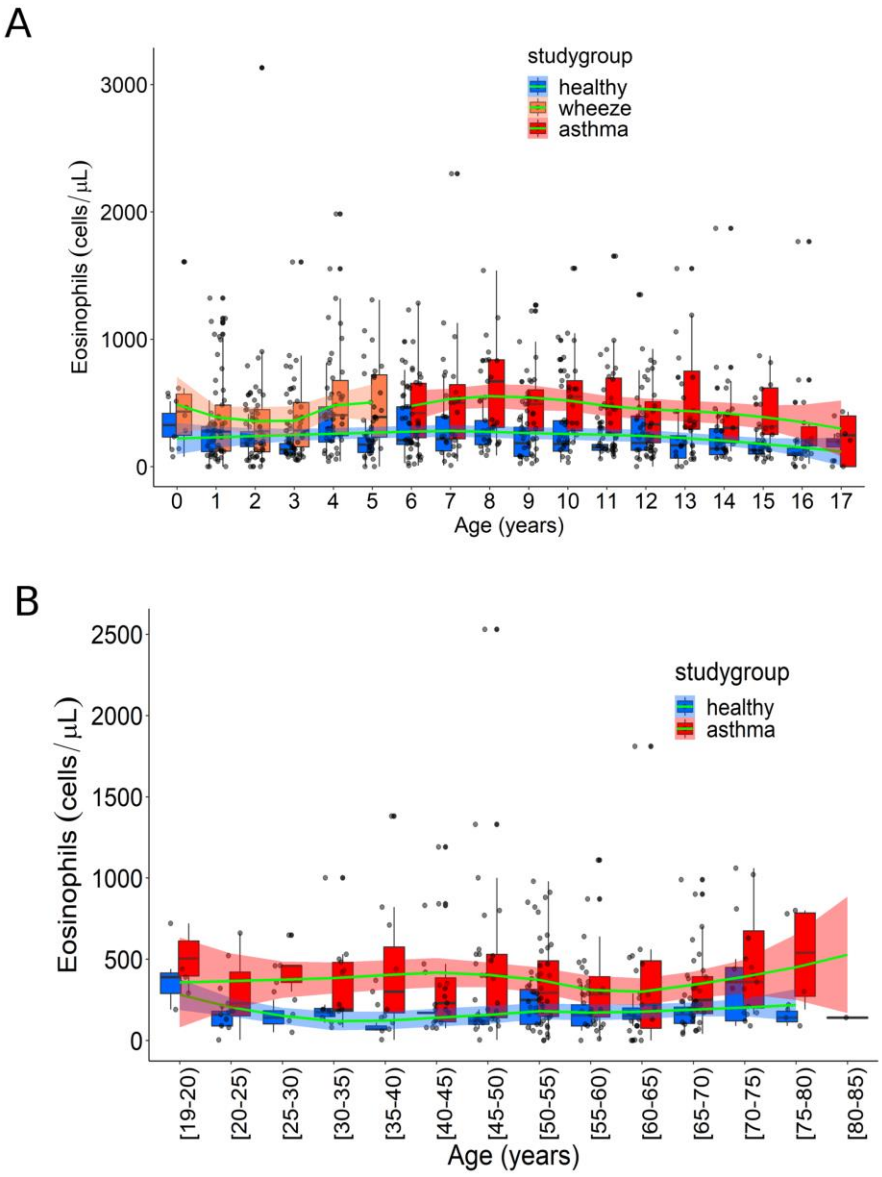

Figure E3: Distribution of blood eosinophils levels stratified by age in healthy children

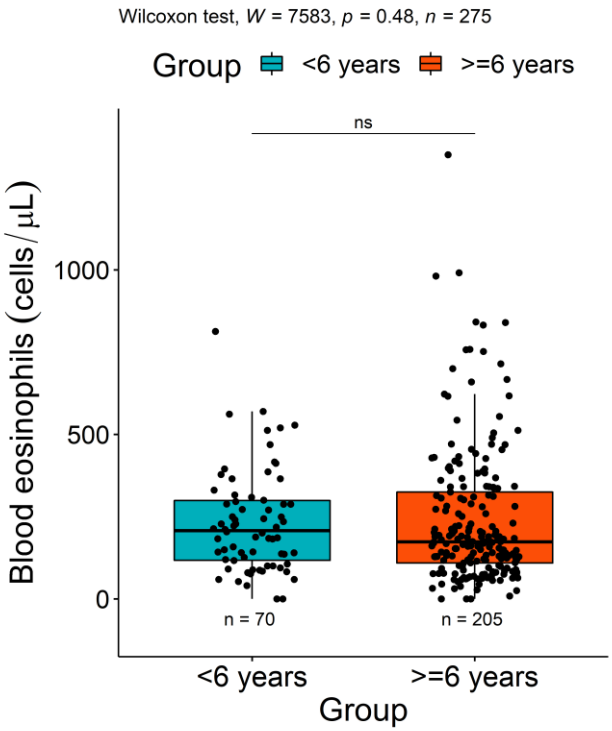

Figure E4: Overlap of T2-high asthma patients with patients having increased FeNO and sputum eosinophils