EUROPEAN RESPIRATORY journal
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original research article

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Please cite this article as: Maison N, Omony J, Illi S, *et al*. T-high asthma phenotypes across life span. *Eur Respir J* 2022; in press (https://doi.org/10.1183/13993003.02288-2021).

T2-high asthma phenotypes across life span

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NM, JO, EVM, RG contributed to conceptualization, methodology, data analysis and presentation of the published work and wrote the manuscript. SI and TB contributed to conceptualization, methodology, provided scientific advice, specifically critical review, commentary or revision of the manuscript. JO and SI performed data curation and statistical analysis. DT conducted management activities to annotate, scrub and maintain research data. CS, AMD, MW, CH and BS planned and supervised cytokine measurement and data curation, provided scientific advice, specifically critical review, commentary or revision of the manuscript. MM and SF contributed to patient recruitment. ER, HR, MVK, KFR and GH provided scientific advice, specifically critical review, commentary or revision of the manuscript. KFR, MVK, GH, EVM are Principal Investigators of the DZL ALL Age Asthma Cohort and secured funding and conceived ideas, formulation and evolution of overarching research goals and aims.

Funding

This research was supported by the German Center for Lung Research (Federal Ministry of Education and Research) and the Wilsing Stiftung, Cologne.

Conflict of Interest:

N. Maison, J. Omony, S. Illi, D. Thiele, A.M. Dittrich, C. Happle, M. Meier, S. Foth and R. Grychtol have nothing to disclose. C. Skevaki reports grants and personal fees from Hycor Biomedical, Bencard Allergie, grants and personal fees from Thermo Fisher Scientific as well as grants from Mead Johnson Nutrition (MJN), Universities Giessen and Marburg Lung Centre, the German Centre for Lung Research (DZL), University Hospital Giessen and Marburg, Deutsche Forschungsgemeinschaft (DFG). T. Bahmer reports grants from the Federal Ministry for Education and Research (BMBF) for the German Center for Lung Research (DZL) and personal fees from AstraZeneca, GlaxoSmithKline, Novartis, Roche, Chiesi. M. Weckmann reports grants from Federal Ministry for Education and Research (BMBF), University of Luebeck and German Academic Exchange Service. B. Schaub reports grants from DFG, BMBF, the EU as well from GlaxoSmithKline, Sanofi and Novartis. H. Renz reports grants from German Center for Lung Disease (DZL) and Universities Giessen Marburg Lung Center. M.V. Kopp reports grants and personal fees from Allergopharma GmbH and Vertex GmbH. Additional, personal fees from Sanofi GmbH, Infectopharm GmbH and Leti GmbH. E. Rietschel reports personal lecture payments for Nutricia Milupa GmbH and Novartis Pharma and honoraria for participation in advisory boads for MICE-Mylan, Novartis Pharma GmbH, Boehringer-Ingelheim GmbH. K.F. Rabe recieved personal payments or honoraria from AstaZeneca, Boehringer Ingelheim, Chiesi Pharmaceuticals, Novartis, Sanofi & Regeneron, GlaxoSmithKline, Berlin Chemie and Roche. K.F. Rabe also discloses participation on Data Safety Monitoring Boards/ Advisory Boards for AstraZeneca and Sanofi Regeneron and leadership or fiduciary role in the German Center for Lung Research (DZL), German Chest

Society (DGP) and American Thoracic Society (ATS). G. Hansen reports grants from German Federal Ministry of Education and Research (BMBF) and German Research Foundation (DFG) as well as personal fees from Sanofi GmbH, MedUpdate, and Abbvie. E. von Mutius reports grants from the German Center for Lung Research (DZL) as well as royalties/ licenses held by Elsevier GmbH, Gerog Thieme Verlag, Springer Verlag GmbH, Elsevier Ltd; furthermore, consultation fees were received from the Chinese University of Hongkong, European Commission, HiPP GmbH, AstraZeneca. E.v.M also received payments and/or support for meetings/ travel from the Massachusetts Medical Society, Springer-Verlag GmbH, Elsevier Ltd, Böhringer Ingelheim Internation GmbH, European Respiratory Society (ERS), University Utrecht, Salzburg, Colorado and Imperial College London, Springer Medizin Verlag GmbH, Japanese Society of Pediatric Allergy and Clinical Immunology, Klinkum Rechts der Isar, Paul-Martini-Stiftung; further support for meetings/ travel was granted by Verein zur Förderung der Pneumologie am Krankenhaus Groshansdorf, Pneumologie Development Mondial Congress & Events GmbH, American Academy of Allergy, Asthma & Immunology, Margaux Orange, Volkswagen Stiftung, Österreichische Gesellschaft für Allergologie & Immunologie, OM Pharma SA, Hanson Wade Ltd, iKOMM GmbH, DSI Dansk Bornestma Center, American Thoracic Society, HiPP GmbH, E. von Mutius has patent EP2361632, EP1411977, EP1637147 and EP 1964570 (licensed to Protectimmun) furthermore patent LU101064 is pending. E. von Mutius participates in the following Data monitoring or Advisory Boards: EXPANSE, BEAMS External Scientific Advisory Board, Journal of Allergy and Clinical Immunology: in Practice, Children's Respiratory and Environmental Workgroup (CREW), International Scientific & Societal Advisory Board of Utrecht Life Sciences, External Review Panel of the Faculty of Veterinary Science (University of Utrecht), Gottfried Wilhelm Leibniz Programme, Asthma UK for Applied Research, Advisory Board of The Lancet Respiratory Medicine, CHILD (Canadian Healthy Infant Longitudinal Development Study).

Word Count: 3689

Abbreviations

ALLIANCE: ALL Age Asthma Cohort.

BMI: Body mass index.

CI: Confidence interval.

DZL: German Center for Lung Research.

FeNO: Fractional exhaled nitric oxide.

FEV_1: Forced Expiratory Volume in 1 second.

FVC: Forced vital capacity.

FEF_{25-75}: Forced expiratory flow at 25% - 75% of FVC.

GINA: Global Initiative for Asthma.

IgE: Immunoglobulin E.

IL: Interleukin.

IQR: interquartile range.

LPS: Lipopolysaccharide.

PBMC: Peripheral blood mononuclear cell.

SD: standard deviation.

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Abstract

Rationale: In adults, personalized asthma treatment targets patients with T2-high and eosinophilic asthma phenotypes. It is unclear whether such classification is achievable in children.

Objectives: To define T2-high asthma with easily accessible biomarkers and compare resulting phenotypes across all ages.

Methods: In the multicenter clinical ALL Age Asthma Cohort (ALLIANCE), 1125 participants (n=776 asthmatics, n=349 controls) were recruited and followed for 2 years (1 year in adults). Extensive clinical characterization (questionnaires, blood differential count, allergy testing, lung function and sputum induction (in adults) was performed at baseline and follow-ups. Interleukin (IL)-4, IL-5 and IL-13 were measured after stimulation of whole blood with LPS or anti-CD3/CD28.

Measurements and Main Results: Based on blood eosinophil counts and allergen-specific serum IgE antibodies (sIgE), patients were categorized into four mutually exclusive phenotypes: 'Atopy-only', 'Eosinophils-only', 'T2-high' (eosinophilia + atopy) and 'T2-low' (neither eosinophilia nor atopy). The T2-high phenotype was found across all ages, even in very young children in whom it persisted to a large degree even after two years of follow-up. T2-high asthma in adults was associated with childhood onset suggesting early origins of this asthma phenotype. In both children and adults, the T2-high phenotype was characterized by excessive production of specific IgE to allergens ($p<0.0001$) and, from school age onwards, by increased production of IL-5 after anti-CD3/CD28 stimulation of whole blood.

Conclusions: Using easily accessible biomarkers, patients with T2-high asthma can be identified across all ages delineating a distinct phenotype. These patients may benefit from therapy with biologicals even at younger age.

Word count: 250

Keywords: atopy, eosinophil, phenotype, biomarkers, pediatric, adult.

Introduction

Asthma is a heterogeneous disease comprising several endotypes and clinical phenotypes. Adult studies have identified type 2 (T2) inflammation as key immune response in asthma pathobiology, resulting in the broad classification of T2-high and T2-low asthma [1]. T2 inflammation is characterized by increased secretion of IL-4, IL-5 and IL-13 by T cells or innate cells, associated with clinical features such as allergic sensitization and bronchoconstriction, eosinophilic airway inflammation and airway mucus production. While T2-high asthma was initially described by molecular signatures of T2 cytokines in affected airway samples [2], simpler and clinically available biomarkers like fractional exhaled nitric oxide (FeNO), total or specific IgE, blood or sputum eosinophils are now being used [3]. These biomarkers in combination with T2 targeting monoclonal antibodies have opened up new personalized treatment strategies, especially for severe asthma [4, 5].

Asthma affects patients from all age groups ranging from preschool children to senior adults but comparative studies regarding T2 inflammation across all age groups are scarce [5-7]. This could ultimately lead to adoption of adult definitions of T2-high asthma to children. However, children often need age-adjusted cut-offs for biomarkers which has been recognized in regards to FeNO, but might also hold true for blood eosinophils [8]. Many biomarkers used in adults are difficult to assess in children of younger age groups, i.e. sputum or even FeNO, which limits the possibilities of age-spanning comparisons. Furthermore, the lack of knowledge about age-appropriate definitions of T2 inflammation impedes research on long-term asthma trajectories from child- to adulthood and comparative investigations into clinical phenotypes and therapeutic success. Although T2 targeting drugs are currently primarily used in severe asthma, future applications in

moderate asthma to influence long-term outcome are potentially conceivable but age-specific and cross-age research into biomarkers for T2-high asthma will be prerequisite first.

The aim of this study was to investigate in the multicenter ALLIANCE cohort whether such classification is feasible in children of all age groups and adults using routine biomarkers like blood eosinophil counts and allergen-specific IgE antibodies.

We assessed whether the classification results in comparable phenotypes in children and in adults and assessed stability over time. Furthermore, we characterized patient categories by clinical features and immune response profiling across a broad age range from infancy to old age. These analyses reveal age-specific and age-spanning characteristics of T2-high asthma and facilitate future research into personalized therapeutic and preventative strategies.

Methods

Study design

The ALLIANCE cohort of the German Center for Lung Research (DZL) is a prospective multi-center asthma cohort recruiting in five pediatric specialist centers (Hannover, Lubeck, Munich, Marburg and Cologne) and two adult specialist centers (LungenClinic Grosshansdorf and Research Centre Borstel). All local ethics committees approved the study protocol. Parents of study participants <18 years and study participants ≥8 years gave written informed consent. The study was registered at ClinicalTrials.gov (pediatric arm: NCT02496468, adult arm: NCT02419274).

Inclusion criteria were age-adapted: Children aged 6 months to 5 years were eligible for inclusion if they had at least two episodes of wheeze during the past 12 months based on parental report (‘preschool wheezer’). Children ≥6 years and adults were included based on a history of doctor-

diagnosed asthma according to the Global Initiative for Asthma (GINA) guidelines [9] and German guidelines [10]. Current or former smoking was not an exclusion criterion. Age- and sex-matched healthy controls were recruited into both arms if they were never diagnosed with asthma or preschool wheeze but irrespective of other allergic diseases. Spirometry and FeNO were measured in all participants ≥6 years. Laboratory tests included differential blood count (all participants), sputum cytology (only adults) and specific immunoglobulin E against 36 allergens in all patients measured by Euroline™ (Euroimmun, Germany). For cytokine measurements, one milliliter of whole blood was stimulated with lipopolysaccharide (LPS) or anti-CD3/CD28 (TruCulture®, Myriad Rbm, Austin, TX, USA) for 48 hours at 37°C. Supernatant was collected and stored at -80°C. T2 Cytokines were measured centrally using Bio-Plex assays (Bio-Rad, USA). Details regarding methods, study design and definition of clinical variables are specified in the online supplement and published elsewhere [11].

Statistical analysis

Blood eosinophil counts from healthy subjects in the ALLIANCE cohort were used to define increased blood eosinophils as counts above the 90th percentile (figure 1 and E2). This resulted in a cut-off of ≥470 cells/µL in children of all age groups and ≥360 cells/µL in adults. Eosinophil counts did not differ significantly between healthy children <6 years and ≥6 years (figure E3). Atopy was defined as specific IgE ≥0.7 kU/L against at least one of 36 aero – or food allergens or by summing up all allergen-specific IgEs to all allergens to reflect the degree of sensitization [12, 13]. Details on the allergen panels can be found in the supplementary methods section. Asthma phenotypes in children were defined as: Atopy-only (b-Eos <470 cells/µL and any sIgE ≥0.7 kU/L), Eos-only (b-Eos ≥470 cells/µL, all sIgE <0.7 kU/L), T2-high (b-Eos ≥470 cells/µL, any sIgE ≥0.7

kU/L), and T2-low (b-Eos <470 cells/μL, all sIgE <0.7 kU/L). The same phenotype definitions were applied in adults but with a b-Eos cut-off of ≥360 cells/μL.

Demographics and clinical categorical variables were compared across phenotypes (Chi-square test). Means between groups were compared using the unpaired t-test. Kruskal-Wallis and Wilcoxon testing was used to compare continuous variables across phenotypes. To investigate the independent contribution of atopy (discrete variable) and blood eosinophils (continuous variable) to asthma, we used multivariable logistic regression models - adjusting for age, gender, atopic comorbidities, parental history of asthma, active and passive smoking, siblings and day care including two-way interaction between covariates using 95% CI and Wald test p-value. The best models were selected using the Akaike Information Criterion (backward variable selection, using p <0.3 in univariable model). Model fit and predictive accuracy were assessed using the Receiver Operating Area Under the Curve [14]. Statistical significance was set at p <0.05 and descriptive statistics were summarized as mean (standard deviation, SD), range (interquartile range, IQR) and number (%). Data was analysed using R version 4.0.2 [15]. In children and adults, standard curve-derived cytokine values were analyzed. Spirometry values were analyzed as z-scores [16].

Results

Subject characteristics

Demographic and clinical information at baseline was collected for 1125 subjects from three age groups: 282 children aged 6-18 years with asthma (‘children and adolescents’), 218 adults with asthma (‘adults’) and 276 children <6 years with ‘preschool wheeze’ additionally to healthy controls in all age groups (see figure E1 and table E3 in the online data supplement). As asthma cannot be confidently diagnosed in children <6 years, the term ‘preschool wheeze’ was chosen for

this age group, although some children with recurrent wheeze at preschool age will develop early onset asthma.

Blood eosinophils and FeNO were higher and atopy was more prevalent in pediatric and adult asthma patients compared to healthy control subjects. FEV_1, FEV_1/FVC and FEF_{25-75} were significantly lower in adult and pediatric asthma patients than in healthy controls (see table E3 in the online supplement). In preschool wheezers, atopy was less prevalent compared to healthy controls while blood eosinophils were increased.

Classification of phenotypes

Using the 90th percentile as a cut-off for blood eosinophilia and a clinically relevant cut-off for atopy (≥0.7 kU/L) [13], subjects were categorized into four mutually exclusive groups: Atopy-only, Eos-only, T2-high with both eosinophilia and atopy, and T2-low with neither atopy nor eosinophilia (table 1). Comparing the distribution of phenotypes within each age group, the T2-high phenotype was most prevalent in children and adolescents (40.2%) followed by adults (24.6%) and preschool children (16.9%). FeNO was significantly increased in the T2-high group compared to T2-low and Atopy-only group in all age groups while no difference was seen between T2-high groups and the Eos-only group (table E4).

Next, we compared our classification to other proposed biomarkers for T2 inflammation as FeNO and sputum eosinophils [17, 18]. A combination of FeNO ≥35 ppb and sputum eosinophils ≥3% resulted in a similar prevalence (26.7%) (table E5) of the T2-high phenotype compared to our definition based on increased b-Eos and atopy (24.6%) (table 1). However, overlap of patients with a T2-high asthma definition based on FeNO and sputum eosinophils and a T2-high asthma definition based on atopy and blood eosinophils was only 20% in adult patients (figure E4). Among

the cases classified as T2-high by FeNO and sputum eosinophils (n=31, 37%), nineteen subjects were classified as b-Eos-only, seven as Atopy-only and only two subjects were classified as T2-low (three cases had missing information on atopy). By increasing the cut-off to FeNO≥50 ppb and s-Eos≥3%, the proportion of patients classified as T2-high phenotype reduced as one would expect, given that patients with values below this cut-off would belong to the T2-low category (table E5). For phenotype definition in adults and children, we also ran a sensitivity analysis using b-Eos cut-off values of ≥150 and ≥300 cells/μL, often used for prescribing biologicals [4]. Results regarding markers of airway inflammation as FeNO and sputum eosinophils remained similar in adults, (table E4 and E6) but changed considerably in children, especially using the lowest eosinophil cut-off (table E7), again pointing to the need of higher cut-off values in children.

Clinical characteristics and associated features of the T2-high phenotype

The most defining feature of the T2-high phenotype across all age groups was a high degree of atopy as assessed by the sum of all allergen-specific IgEs (table 3). In contrast, allergic comorbidities (eczema, hay fever) were similarly present in the T2-high and Atopy-only group in subjects >6 years and adults. In preschool children, eczema was specifically elevated within the T2-high group. Furthermore, FeNO was increased in both children and adults in the T2-high phenotype compared to Atopy-only and T2-low, but similar compared to Eos-only (table 2).

Some characteristics of the T2-high phenotype were only seen in certain age groups (table 2). In adults, patients with T2-high asthma were younger and had more often childhood-onset asthma. Also, asthma severity and asthma control differed between all phenotypes; T2-high asthma patients showed higher severity and higher exacerbation rate / person / year than Atopy-only, but were overall less severely affected than patients from T2-low and Eos-only.

Phenotypes were less contrasting in children. In children and adolescents, no differences occurred between the four phenotypes regarding lung function, exacerbations, asthma control or ICS use. Similar results were seen for preschool wheezer, with the exemption of more ICS use in both Atopy-only and T2-high groups. We saw no association of active and passive smoking, neutrophils and number of siblings with any of the phenotypes in any age group. Daycare attendance as proxy for increased exposure to pediatric infections was less likely in wheezers with Eos-only and T2-low phenotype, however, children with these phenotypes were also younger (table 3).

In n=71 children included with pre-school wheeze we assessed the outcome at the first follow-up visit ≥ 6 years of age based on questionnaire data (supplementary table E2). Persistence of symptoms consistent with an asthma diagnosis was highest in the T2-high group (n=15, 73.3%), followed by Atopy-only (n=13, 38.5%). The atopy-only group showed a high proportion of patients with "intermittent asthma symptoms" (n=5, 38.5%). Remission of symptoms was highest among T2-low subjects (n=36, 50.0%) (figure 2).

Cytokine levels across phenotypes

High levels of IL-5 production after anti-CD3/CD28 stimulation of whole blood were observed in school-age children with the eosinophilic phenotypes T2-high and Eos-only (table E8). A trend for higher IL-5 secretion was also seen in adults, in both atopic phenotypes, T2-high and Atopy-only especially in the patients ≥45 years (table E9, table E10). Furthermore, adults showed increased IL-5 levels after LPS stimulation but only in the Eos-only phenotype and with lower levels than after anti-CD3/CD28 stimulation. No significant differences were seen for IL-5 in preschool children. IL-4 did not differ between the four phenotypes in adults. Equally, no phenotype-specific changes were seen for IL-13 in all age groups.

Prevalence of phenotypes across age groups

T2-high groups were not uniformly distributed across ages but showed lower proportions in younger children (<6 years) and older patients (≥45 years) (figure 3). The phenotype Eos-only was almost exclusively seen in very young children <3 years and adults above 45 years with increasing prevalence particularly in adults >60 years.

Persistence of phenotypes

Longitudinal stability of phenotypes was assessed after one and two years in children, and after one year in adults. Overall, stability of the T2-high phenotype was slightly higher in children than in adults. After one year of follow-up, 73.3% (preschool children) and 77.0% (children and adolescent) retained the T2-high phenotype in contrast to 60.0% (18-45 years) and 51.2% (≥45 years) in adults. A high stability of the T2-high phenotype was furthermore found for preschool children, with 66.7% retaining their phenotype for two years, while the proportion of children and adolescents with persistent T2-high asthma reduced from 77.0% to 50.8% after two years, respectively (figure 4A-D).

Individual contributions of atopy and blood eosinophils to disease risk across age groups

Our T2 phenotype definition was based on clinical experience. We were therefore interested in understanding the individual contribution of eosinophils and specific IgE levels to 'asthma risk' after adjusting for potential confounders like atopic comorbidities, parental history, active and passive smoking, siblings, day care and sex (figure 5). All models had a good performance fit (AUC = 0.760 to 0.969). The findings support the predominating role of atopy to preschool wheeze and asthma risk until mid-adulthood. In addition to atopy, eosinophils clearly influenced asthma

risk in children and adolescents (figure 5). Conversely, blood eosinophil levels but not atopy was a significant contributor to asthma risk in adults ≥45 years, coinciding with increasing frequencies of asthma patients with the Eos-only phenotype and late asthma onset (table 2).

Discussion

In the ALLIANCE cohort, patients of all age groups displayed a T2-high phenotype defined by the presence of eosinophilia and atopy, with highest prevalence of T2-high asthma in school-aged children and young adults. Moreover, adults with T2-high asthma were significantly more likely to have childhood onset asthma and children with preschool wheeze and T2-high phenotype were more likely to develop asthma at the age of 6 years. Across all ages, T2-high was consistently and strongly ($p<0.0001$) associated with a high degree of atopy as assessed by the sum of all allergen-specific IgEs. Specifically, in children with T2-high asthma, we also found an augmented IL-5 response after T cell stimulation and both, atopy and eosinophils contributed to disease risk particularly in this age group. T2-high asthma defined by atopy and blood eosinophilia thus outlines a phenotype linked to onset in childhood, which tracks to or re-occurs in adulthood. There is increasing evidence that asthma is not one disease but rather a syndrome consisting of many phenotypes and possibly distinct underlying endotypes [19]. In adult patients, molecular phenotyping of lower airway samples has revealed a T2 signature in a significant proportion of subjects [2, 20]. The term was first used to describe a subgroup of mild-moderate adult asthmatics with an IL-13 inducible gene signature of the airway epithelium, which coincided with increased airway and blood eosinophils, higher sensitization levels and good response to ICS [2]. Afterwards, several cohorts confirmed similar T2 cytokine-driven molecular signatures in either mucosal biopsies or sputum cells, mainly in adults with severe asthma [21, 22] but also children [21]. Increased FeNO, blood or sputum eosinophils and sensitization against allergens were associated

in most studies with a T2-high airway gene signature with sensitization being specifically important in younger adults and children [2, 23].

There is still no consensus about a definition of a T2-high phenotype across studies and age groups. In clinical routine, invasive procedures assessing sputum or airway samples are difficult to perform, particularly in young children, advocating for more easily accessible proxies of a T2-high signature like blood eosinophils and measures of atopy. For blood eosinophilia, we used the 90^{th} percentile cut-off for b-Eos of our healthy control population, which amounted to ≥470 cells/µL in children and ≥360 cells/µL in adults. Atopy was defined as at least one allergen-specific IgE ≥0.7 kU/L based on previously established clinical relevant cut-offs [13]. This phenotype definition was also reflective of T2 airway inflammation: FeNO levels (children and adults) and sputum eosinophils (adults) were increased in both the T2-high and eosinophil phenotype. Children and adolescents with T2-high asthma also showed increased IL-5 production after T cell stimulation with anti-CD3/CD28 in comparison with the Atopy-only group. This observation might indicate an augmented propensity of T cells to respond to activation, which is not solely dependent on atopy, suggesting that our definition is clinically useful for identifying patients with an underlying T2 endotype.

The most distinct clinical feature in the T2–high phenotype across all age groups of the ALLIANCE cohort was a high degree of sensitization against allergens compared to all other phenotypes, particularly Atopy-only. While atopy was defined as binary variable for the phenotype definitions using sIgE ≥0.7 kU/L as a cut-off, this does not reflect the degree of sensitization for which the sum of all allergen-specific IgEs is a better measure. Accordingly, the sum of all allergen-specific IgEs was significantly higher in the T2-high than in the Atopy-only group, even though both groups were defined as “atopic” (preschool wheeze ($p=0.013$), asthmatic children ($p=0.032$),

and asthmatic adults (p=0.012)). Atopy is a complex trait that does not necessarily result in allergic disease. Recently, subgroups of atopy with varying clinical relevance were identified by latent class analyses showing that early and multiple sensitizations are not only the strongest predictor of asthma, hospital admissions and lung function deficits, but also of increased production of IgE towards aeroallergens [24, 25]. These findings suggest that early and augmented production of specific IgEs resulting in an increased sum of sIgE reflects the early life origins of T2-high across all-age groups. This notion is supported by the high percentage of adults with the T2-high phenotype reporting childhood onset of asthma and the association between T2-high phenotype and asthma outcome at school age in the children with preschool wheeze. Additionally, the T2-high phenotype showed a high percentage of children retaining the T2-high phenotype at the second follow-up particularly in the preschool group. Due to the broad inclusion criteria for children below the age of six years, our cohort comprises all phenotypes of preschool wheeze, ranging from children with transient symptoms to children with a high risk of developing childhood asthma according to the GINA definition. Our definition of T2-high, however, seems useful to identify preschool children at increased risk of asthma persistence. Additive effects of blood eosinophils and atopy for prediction of asthma at school age has been shown by others as well [26, 27]. Confirmation by longer follow-up of the ALLIANCE cohort is needed.

Intriguingly an increasing proportion of the study population had an Eos-only phenotype at both ends of the age spectrum. However, further analysis showed marked age-dependent differences. In adults ≥45 years, eosinophils but not atopy was associated with asthma diagnosis. Clinically, the Eos-only phenotype was characterized by increased severity, OCS use and high exacerbation rates in parallel with less GINA control. Interestingly, the Eos-only group in adults also showed an increase in IL-5 after stimulation with LPS, pointing towards additional and distinct pathways of

IL-5 production apart from specific T cell receptor stimulation. In preschool children, the Eos-only group did not markedly differ from other phenotypes, apart from less ICS usage. No association with IL-5 production was seen after either stimulation. Eosinophils may thus adopt different roles in disease pathology in both age groups. They may confer tissue damage and bronchoconstriction in asthmatic patients but might also promote anti-viral innate host defense in viral-induced wheeze [28, 29]. This is of particular relevance should T2 targeting biologicals be licensed for use in that age group in the future.

Several studies have shown that patients with severe asthma, T2-high or eosinophilic asthma benefit from biologic therapies targeting eosinophils or related inflammatory pathways, which have become standard treatment of severe asthmatics. Our blood eosinophil cut-off of ≥360 cells/µL in adults is approximately in line with recommendations for prescribing T2 targeting antibodies [4, 10]. However, we and others [8] have identified higher eosinophil levels in healthy children, raising the need for more research into clinically relevant cut-offs for prescribing T2 targeting biologicals in children.

The early origins of the T2-high phenotype seen in our study population in addition to the specific association of increased IL-5 secretion upon T cell stimulation raises the question of future therapeutic use of T2 targeting biologicals also for children and adolescents with non-severe asthma for example to mitigate asthma exacerbations. It is furthermore conceivable that such therapies might also be used for secondary prevention as currently investigated for omalizumab [30].

We acknowledge some limitations of our study. Our definition of the four phenotypes was based on biomarkers available in all age groups but other definitions of T2-high asthma exist, especially

in adult clinical practice [4]. Overlap between distinct definitions and biomarkers of T2 inflammation is often only moderate as we have seen in the adult arm of our cohort and which has been reported by other studies as well [31-33]. FeNO and sputum are difficult to obtain in young children in a standard clinical setting, and we were therefore not able to include these biomarkers in our age-spanning analysis. Since the collection of sputum and FeNO data in preschool children is not easily available, new approaches of assessing airway inflammation should be part of future research.

While most categories had balanced numbers of study participants, the Eos-only group in school-aged children was too small for reasonable comparisons. The same applies to pediatric patients with severe asthma or frequent exacerbations. Furthermore, the majority of ALLIANCE asthma patients were under long-term therapy with inhaled corticosteroids and 22.3% of the adult asthmatics were on regular oral corticosteroids, which both influence blood eosinophil numbers [34] and may, therefore, have biased the phenotype categorization. Our population is Caucasian and results may not be generalizable to other ethnic groups. Lastly, we only considered T2 cytokines IL-13, IL-4 and IL-5 in our analysis and not ratios between T2 cytokines and counter-balancing cytokines as IL-10 as previous authors have done [35]. Our work shows the importance of including patients from childhood to adulthood in studies investigating asthma phenotypes. While many authors still restrict research into asthma phenotypes to either children or adults, a more inclusive approach as utilized by the ALLIANCE cohort reveals similarities and differences between age groups with better precision and will improve uncovering of age-spanning trajectories. To our knowledge, this is the first study on T2-high asthma phenotypes across all ages. We found a high age-dependent occurrence of this phenotype in the ALLIANCE patient population and identified it already in early childhood using easily available biomarkers. Future studies need to

confirm the trajectories described in this cross-sectional analysis. Confirmation of the early T2-high phenotype in childhood might ultimately facilitate personalized preventative or therapeutic strategies in the future.

Acknowledgments

We are thankful to our patients and healthy participants for their invaluable contribution to this work. We are indebted to our study participants and their families for participating in the study and the staff of the participating hospitals and primary care practices and our cooperation partners within the DZL for the support and recruitment. We also thank the study nurses Nicola Korherr, Arzu Yilmazsubasi, Nicole Rahmanian, Petra Hundack-Winter, Susann Prange, Corinna Derworth, Johanna Döhling, Romina Pritzkow, Eva Wittmer, Nadine Weissheimer, and Elvira Ehlers-Jeske, Nasanin Schröder, the data managers Edith Riess, Julia Kontsendorn, the lung function technicians Elvira Kolling, Andrea Suender, Annegret Telsemeyer, Dunja Tennhardt, Christiane Staabs, Cornelia Stolpe, and Ines Krömer as well as the lab technicians Isolde Schleich, Tatjana Netz, Jana Bergmann, Anika Dreier and Christin Albrecht for their invaluable assistance and support. The BioMaterial Bank Nord is supported by the German Center for Lung Research. The BioMaterialBank Nord is member of popgen 2.0 network (P2N) which is supported by a grant from the German Ministry for Education and Research (01EY1103).

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Figure Legends

Figure 1: Distribution of blood eosinophils counts of healthy children and adults

Distribution of blood eosinophil levels among healthy children (n = 275) and healthy adults (n = 64) in the ALLIANCE cohort. Lines indicate median (red), 90th percentile (blue) and literature-based cut-offs (black for 150 cells/µL and green for 300 cells/µL).

Figure 2: Asthma outcome of children included as preschool wheezers

Clinical outcome of children included with preschool wheeze was assessed at the first visit ≥ 6 years and classified as remission (dark green), asthma (red), intermittent asthma (orange), unclear status (light green). Mean age was 6.7 years (SD = 0.65). Children were grouped according to T2 phenotypes at baseline.

Figure 3: Prevalence of T2 phenotypes across all age groups

(A) T2 phenotypes in children with preschool wheeze (<6 years) or asthma (≥6 years), and (B) in adults with asthma. Phenotypes were defined as: Atopy-only (children: b-Eos <470 cells/µL or adults: b-Eos <360 cells/µL and any sIgE ≥0.7 kU/L), Eos-only (children: b-Eos ≥470 cells/µL or adults: b-Eos ≥360 cells/µL, all sIgE <0.7 kU/L), T2-high (children: b-Eos ≥470 cells/µL or adults: b-Eos ≥360 cells/µL, any sIgE ≥0.7 kU/L), and T2-low (children: b-Eos <470 cells/µL or adults: b-Eos <360 cells/µL, all sIgE <0.7 kU/L). b-Eos = blood eosinophils, and sIgE = specific Immunoglobulin E.

Figure 4: Longitudinal stability of T2 phenotypes

Asthma phenotypes are shown at baseline and at two follow-ups after 12 (t12) and 24 (t24) months in children. Adults had one follow-up after 12 months (t12). Clustering was done according to baseline phenotype. (A) Children with preschool wheeze (<6 y) n=85. (B) Children with asthma (≥6 y) n=147, (C) adults with asthma (18–45 y) n=46, (D) adults with asthma (≥45 y) n=134. b-Eos = blood eosinophils, sIgE = specific Immunoglobulin E, and y = year.

Figure 5: Multivariable logistic regression models – association between atopy and blood eosinophils with preschool wheeze and asthma

Contribution of atopy and blood eosinophils is shown for risk of preschool wheeze and asthma after adjustment for confounders. (A) Children with wheeze versus healthy children (<6 years). (B) Children with asthma versus healthy children (≥6 years). (C) Adult asthma patients versus healthy adults (≥18 to <45 years), and (D) adult asthma patients versus healthy adults (≥45 years). Atopy was defined as at least one allergen with sIgE ≥0.7 kU/L. aOR = adjusted odds ratio, and CI = confidence interval.

Tables

Table 1: Distribution of T2 phenotypes across age groups

	Atopy-only	**Eos-only**	**T2-high**	**T2-low**
	n (%)	n (%)	n (%)	n (%)
Children: Wheeze (<6 y), n=219	40 (18.26)	31 (14.16)	37 (16.89)	111 (50.68)
Asthma (≥6 y), n=254	105 (41.34)	6 (2.36)	102 (40.16)	41 (16.14)
Adults: Asthma, n=211	83 (39.34)	36 (17.06)	52 (24.64)	40 (18.96)

Frequencies of the four T2 phenotypes are shown for children with preschool wheeze or asthma and adults with asthma.

Table 2: Association between T2 phenotypes and clinical features

	Atopy-only	Eos-only	T2-high	T2-low	p-value
Children, Wheeze (<6 y), n=219	**n=40**	**n=31**	**n=37**	**n=111**	
Age (SD), years	3.99 (1.42)[d,**d**]	2.39 (1.08)[d,d]	4.03 (1.43)[d,*d*]	2.66 (1.21)[**d**,*d*]	<0.0001
ICS use, n (%)	22 (55.0)	7 (22.6)	21 (56.8)	33 (30.0)	0.0009
ICS dose, n (%)					
Low	13 (68.4)	5 (71.4)	16 (80.0)	19 (67.9)	0.1835
Medium	6 (31.6)	1 (14.3)	2 (10.0)	9 (32.1)	
High	0 (0.0)	1 (14.3)	2 (10.0)	0 (0.0)	
GINA control, n (%)					
Uncontrolled	9 (22.5)	14 (45.2)	17 (45.9)	29 (26.6)	0.1605
Partly controlled	14 (35.0)	9 (29.0)	9 (24.3)	39 (35.8)	
Controlled	17 (42.5)	8 (25.8)	11 (29.7)	41 (37.6)	
Exacerbations/person/yr, mean (SD)	1.0 (2.23)	1.27 (2.63)	0.89 (1.20)	0.58 (1.04)	0.3754
Children, Asthma (≥6 y), n=254	**n=105**	**n=6**	**n=102**	**n=41**	
Age (SD), years	11.72 (3.16)[a,c]	10.74 (2.18)	10.06 (2.81)[c]	10.27 (3.28)[a]	0.0009
FEV_1 (z-score), mean (SD)	-0.41 (1.09)	0.11 (1.45)	-0.34 (1.67)	-0.52 (0.96)	0.4803
FVC (z-score), mean (SD)	0.00 (1.00)	-0.02 (0.90)	0.19 (1.61)	0.01 (0.90)	0.9740
FEV_1/FVC (z-score), mean (SD)	-0.68 (1.22)	0.07 (1.00)[a]	-0.81 (1.09)[a]	-0.85 (1.07)	0.2163
FEF_{25-75} (z-score), mean (SD)	-0.79 (1.25)	-0.26 (1.29)	-0.96 (1.30)	-0.95 (1.06)	0.4121
ΔFEV_1 (%), mean (SD)	8.18 (7.45)	14.75 (2.81)	12.19 (13.20)	7.15 (7.15)	0.2365
FeNO (ppb), mean (SD)	22.47 (17.67)[a,c]	39.47 (52.46)	42.33 (57.69)[a,d]	13.11 (14.96)[c,d]	<0.0001
ICS use, n (%)	79 (76.7)	4 (66.7)	74 (73.3)	25 (61.0)	0.2868
ICS dose, n (%)					
Low	44 (58.7)	1 (25.0)	37 (56.1)	13 (54.2)	0.2805
Medium	27 (36.0)	3 (75.0)	19 (28.8)	9 (37.5)	
High	4 (5.3)	0 (0.0)	10 (15.2)	2 (8.3)	
GINA control, n (%)					
Uncontrolled	16 (15.4)	1 (16.7)	12 (11.9)	6 (14.6)	0.5980
Partly controlled	50 (48.1)	2 (33.3)	40 (39.6)	14 (34.1)	

Controlled	38 (36.5)	3 (50.0)	49 (48.5)	21 (51.2)	
Exacerbations/person/yr, mean (SD)	0.52 (1.47)	0.17 (0.41)	0.48 (1.07)	0.24 (0.94)	0.2867

Adults, Asthma, n=211	**n=83**	**n=36**	**n=52**	**n=40**	
Age (SD), years	50.00 (12.73)[a,b]	57.17 (11.12)[b,**b**]	48.52 (14.13)[b,**b**]	56.20 (14.51)[a,b]	0.0036
FEV_1 (z-score), mean (SD)	-1.58 (1.55)	-1.81 (1.36)	-1.88 (1.33)	-1.89 (1.46)	0.5609
FVC (z-score), mean (SD)	-0.54 (1.25)	-0.39 (1.05)	-0.50 (1.12)	-0.55 (0.95)	0.6960
FEV_1/FVC (z-score), mean (SD)	-1.81 (1.32)	-2.33 (1.27)	-2.19 (1.38)	-1.82 (1.42)	0.1136
FEF_{25-75} (z-score), mean (SD)	-1.68 (1.29)[a,a]	-2.18 (1.21)[a]	-2.26 (1.26)[a]	-1.78 (1.38)	0.0692
ΔFEV_1 (%), mean (SD)	8.73 (9.96)	7.17 (8.87)[a]	10.13 (8.31)[a,a]	6.52 (7.99)[a]	0.0572
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
ICS use, n (%)	69 (83.1)	36 (100.0)	46 (88.5)	37 (92.5)	0.0471
ICS dose, n (%):					
Low	26 (37.7)	1 (2.8)	9 (19.6)	11 (29.7)	0.0058
Medium	28 (40.6)	20 (55.6)	20 (43.5)	13 (35.1)	
High	15 (21.7)	15 (41.7)	17 (37.0)	13 (32.1)	
OCS use, n (%)	10 (12.0)	17 (47.2)	8 (15.4)	12 (30.0)	0.0001
Pediatric asthma onset, n (%)	33 (39.8)	4 (11.1)	29 (56.9)	6 (15.0)	<0.0001
Severity grade, n (%)					
Mild	32 (38.6)	0 (0.0)	12 (23.1)	5 (12.5)	<0.0001
Moderate	28 (33.7)	9 (25.0)	17 (32.7)	14 (35.0)	
Severe	23 (27.7)	27 (75.0)	23 (44.2)	21 (52.5)	
GINA control, n (%)					
Uncontrolled	26 (31.3)	25 (69.4)	21 (40.4)	14 (35.0)	<0.0001
Partly controlled	25 (30.1)	7 (19.4)	21 (40.4)	21 (52.5)	
Controlled	32 (38.6)	4 (11.1)	10 (19.2)	5 (12.5)	
Exacerbations/person/yr, mean (SD)	1.21 (2.12)[b]	2.83 (2.91)[b]	2.27 (3.16)	2.75 (3.62)	0.0025

The p-values are based on Chi-square and Kruskal-Wallis 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). ICS use and ICS dose referred to medication taken at the time of the study visit in children and adults. ICS doses were categorized into mild, moderate and high according to GINA guidelines. Exacerbation rate was defined exacerbations per person per year with exacerbations requiring any systemic steroid treatment (children) or systemic steroids for at least 3 days

(adults) or up-titration of regular OCS per person in the past 12 months. Asthma severity was assessed according to GINA treatment steps and asthma control according to GINA control status. Data regarding bronchodilator response was only available for 94 children. GINA = Global initiative for asthma, b-Eos = blood eosinophils, sIgE = specific Immunoglobulin E, yr = year, SD = standard deviation, ICS = inhaled corticosteroid, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, FEF_{25-75} = forced expiratory flow at 25%-75% of FVC, ΔFEV_1 = percent increase of FEV_1 after albuterol administration (bronchodilator response), FeNO = fractional exhaled nitric oxide, ppb = parts per billion, and OCS = oral corticosteroids.

Table 3: Association between T2 phenotypes and clinical features

	Atopy-only	Eos-only	T2-high	T2-low	p-value
Children, Wheeze (<6 y), n=219	**n=40**	**n=31**	**n=37**	**n=111**	
Sex, n (% females)	12 (30.0)	11 (35.5)	10 (27.0)	38 (34.2)	0.8260
BMI, mean (SD)	16.32 (1.75)	16.68 (1.65)	16.16 (1.77)	16.44 (1.53)	0.2987
Passive smoker, n (%)	1 (2.6)	1 (3.2)	2 (5.4)	4 (3.6)	0.9267
Maternal history of asthma, n (%)	3 (7.9)	3 (10.3)	11 (29.7)	27 (24.8)	0.0345
Paternal history of asthma, n (%)	7 (18.4)	6 (20.7)	9 (24.3)	18 (16.5)	0.7565
Eczema, n (%)	13 (32.5)	2 (6.5)	21 (56.8)	16 (14.4)	<0.0001
Hay fever, n (%)	8 (20.0)	0 (0.0)	11 (29.7)	1 (0.9)	<0.0001
Sum of sIgE, mean (SD)	102.93 (157.99)[c,d,**d**]	6.57 (0.18)[d,d]	199.57 (166.49)[c,d,*d*]	6.63 (0.39)[**d**, *d*]	<0.0001
Siblings, n (%)	29 (72.5)	21 (67.7)	28 (75.7)	65 (58.6)	0.1732
Daycare, n (%)	31 (81.6)	19 (61.3)	33 (89.2)	80 (72.1)	0.0369
Eosinophil counts (cells/µL), mean (SD)	262.4 (138.8)[a,d,d]	821.9 (498.4)[d,**d**]	893.4 (355.0)[d,*d*]	208.6 (124.3)[a,**d**,*d*]	<0.0001
Neutrophil counts (cells/µL), mean (SD)	3164.5 (1193.4)	3622.9 (1187.7)	3750.7 (1640.4)	3408.7 (1488.8)	0.3147
Children, Asthma (≥6 y), n=254	**n =105**	**n=6**	**n=102**	**n=41**	
Sex, n (% females)	42 (40.0)	2 (33.3)	29 (28.4)	17 (41.5)	0.2832
BMI, mean (SD)	21.24 (6.22)[b]	18.67 (3.00)	18.90 (4.16)[b]	20.20 (5.37)	0.0116
Passive smoker, n (%)	20 (19.6)	0 (0.0)	12 (11.8)	4 (10.0)	0.2162
Maternal history of asthma, n (%)	25 (24.8)	0 (0.0)	28 (27.7)	12 (29.3)	0.4636
Paternal history of asthma, n (%)	22 (21.8)	0 (0.0)	25 (24.8)	6 (14.6)	0.3280
Eczema, n (%)	45 (42.9)	1 (16.7)	49 (48.0)	8 (19.5)	0.0079
Hay fever, n (%)	58 (55.2)	1 (16.7)	59 (57.8)	3 (7.3)	<0.0001
Sum of sIgE, mean (SD)	148.11 (126.58)[a,c,d]	6.84 (0.43)[c,c]	194.67 (156.64)[a,d,c]	6.59 (0.27)[d,d]	<0.0001
Siblings, n (%)	79 (75.2)	6 (100.0)	80 (78.4)	30 (73.2)	0.4919
Eosinophil counts (cells/µL), mean (SD)	256.6 (124.4)[d]	591.0 (145.5)[a,d]	804.5 (330.4)[a,d,**d**]	225.4 (112.6)[**d**,d]	<0.0001
Neutrophil counts (cells/µL), mean (SD)	3795.0 (2335.9)	3644.8 (1520.8)	3636.0 (1799.3)	3341.5 (953.5)	0.9943
Adults, Asthma, n=211	**n=83**	**n=36**	**n=52**	**n=40**	
Sex, n (% females)	51 (61.4)	22 (61.1)	20 (38.5)	26 (65.0)	0.0268
BMI, mean (SD)	28.11 (5.62)	27.95 (7.38)	27.66 (4.90)	27.39 (5.38)	0.9355

Active smoker, n (%)	6 (7.2)	0 (0.0)	3 (6.0)	4 (10.0)	0.3369
Ex-smoker	37 (44.6)	18 (50.0)	18 (34.6)	20 (50.0)	
Never smoker	40 (48.2)	28 (50.0)	31 (59.6)	16 (40.0)	
Maternal history of asthma, n (%)	12 (14.5)	2 (5.6)	3 (5.8)	7 (17.5)	0.1645
Paternal history of asthma, n (%)	10 (12.0)	4 (11.1)	5 (9.6)	2 (5.0)	0.6680
Eczema, n (%)	3 (3.6)	0 (0.0)	6 (11.5)	0 (0.0)	0.0169
Hay fever, n (%)	62 (74.7)	10 (27.8)	46 (88.5)	8 (20.0)	<0.0001
Sum of sIgE, mean (SD)	88.50 (95.65)$^{a,d,\underline{d}}$	12.98 (0.06)$^{d,\mathbf{d}}$	170.31 (215.69)$^{a,\mathbf{d},d}$	13.01 (0.14)$^{\underline{d},d}$	<0.0001
Eosinophil counts (cells/µL), mean (SD)	182.6 (96.4)$^{d,\underline{d}}$	660.9 (418.7)$^{d,\mathbf{d}}$	607.4 (285.4)$^{\underline{d},d}$	161.6 (94.2)$^{\mathbf{d},d}$	<0.0001
Neutrophil counts (cells/µL), mean (SD)	4650.4 (2294.8)	5272.1 (2609.5)	4636.1 (1705.3)	5259.6 (2874.9)	0.2791

The p-values are based on Chi-square and Kruskal-Wallis 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). SD = standard deviation, b-Eos = blood eosinophils, sIgE = specific Immunoglobulin E, y = year, and BMI = body mass index.

Figures

Figure 1

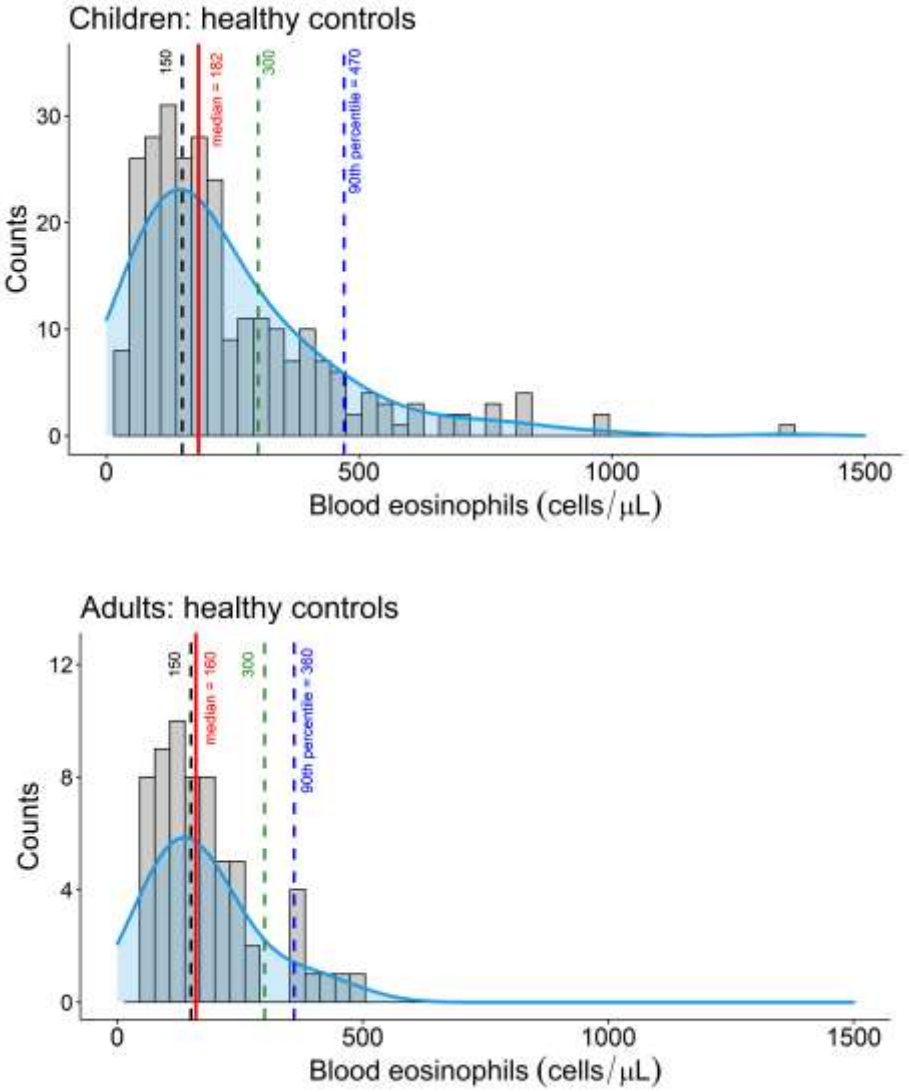


Figure 2

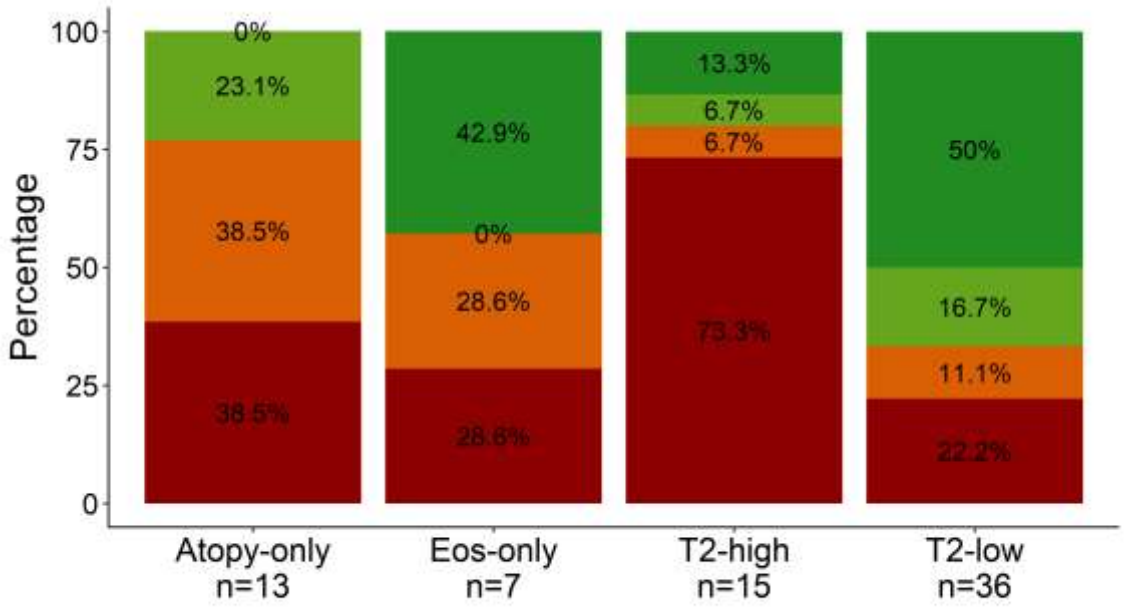

Percentage
100
75
50
25
0
0%
23.1%
38.5%
38.5%
42.9%
0%
28.6%
28.6%
13.3%
6.7%
6.7%
73.3%
50%
16.7%
11.1%
22.2%
Atopy-only
n=13
Eos-only
n=7
T2-high
n=15
T2-low
n=36

Figure 3

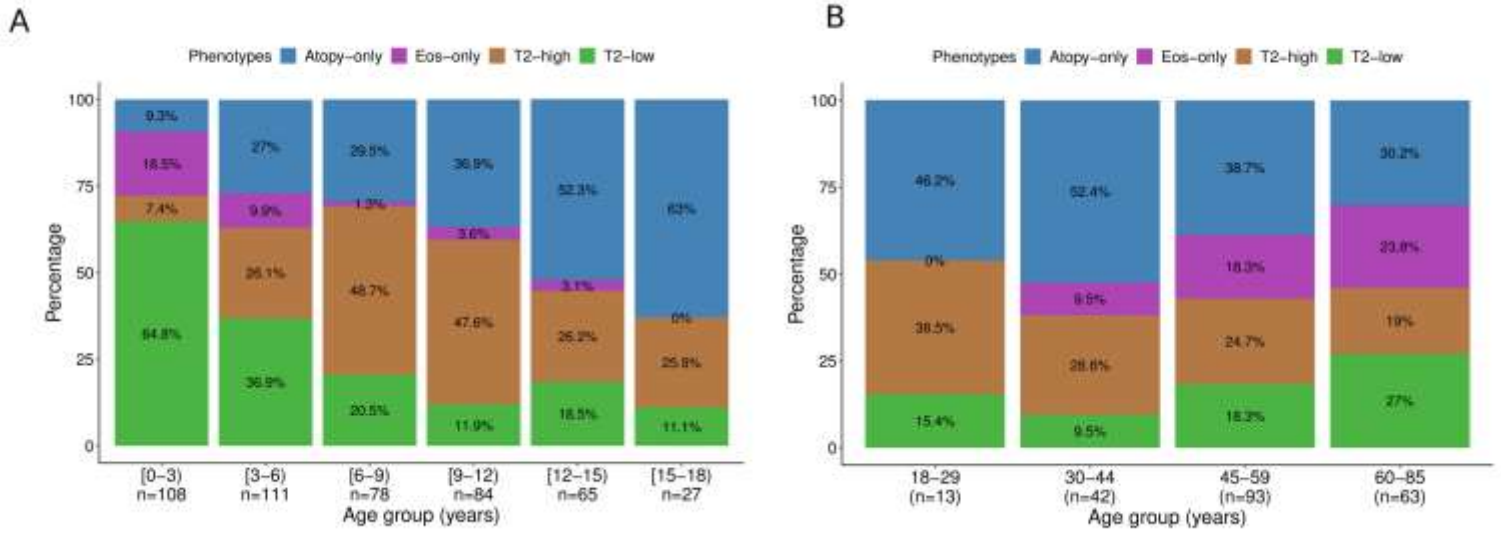

A
Phenotypes Atopy−only Eos−only T2−high T2−low
Percentage
100
75
50
25
0
9.3%
18.5%
7.4%
64.8%
27%
9.9%
26.1%
36.9%
29.5%
1.3%
48.7%
20.5%
36.9%
3.6%
47.6%
11.9%
52.3%
3.1%
26.2%
18.5%
63%
0%
25.8%
11.1%
[0−3) n=108
[3−6) n=111
[6−9) n=78
[9−12) n=84
[12−15) n=65
[15−18) n=27
Age group (years)
B
Phenotypes Atopy−only Eos−only T2−high T2−low
Percentage
100
75
50
25
0
46.2%
0%
38.5%
15.4%
52.4%
9.5%
28.6%
9.5%
38.7%
18.3%
24.7%
18.3%
30.2%
33.8%
19%
27%
18−29 (n=13)
30−44 (n=42)
45−59 (n=93)
60−85 (n=63)
Age group (years)

Figure 4

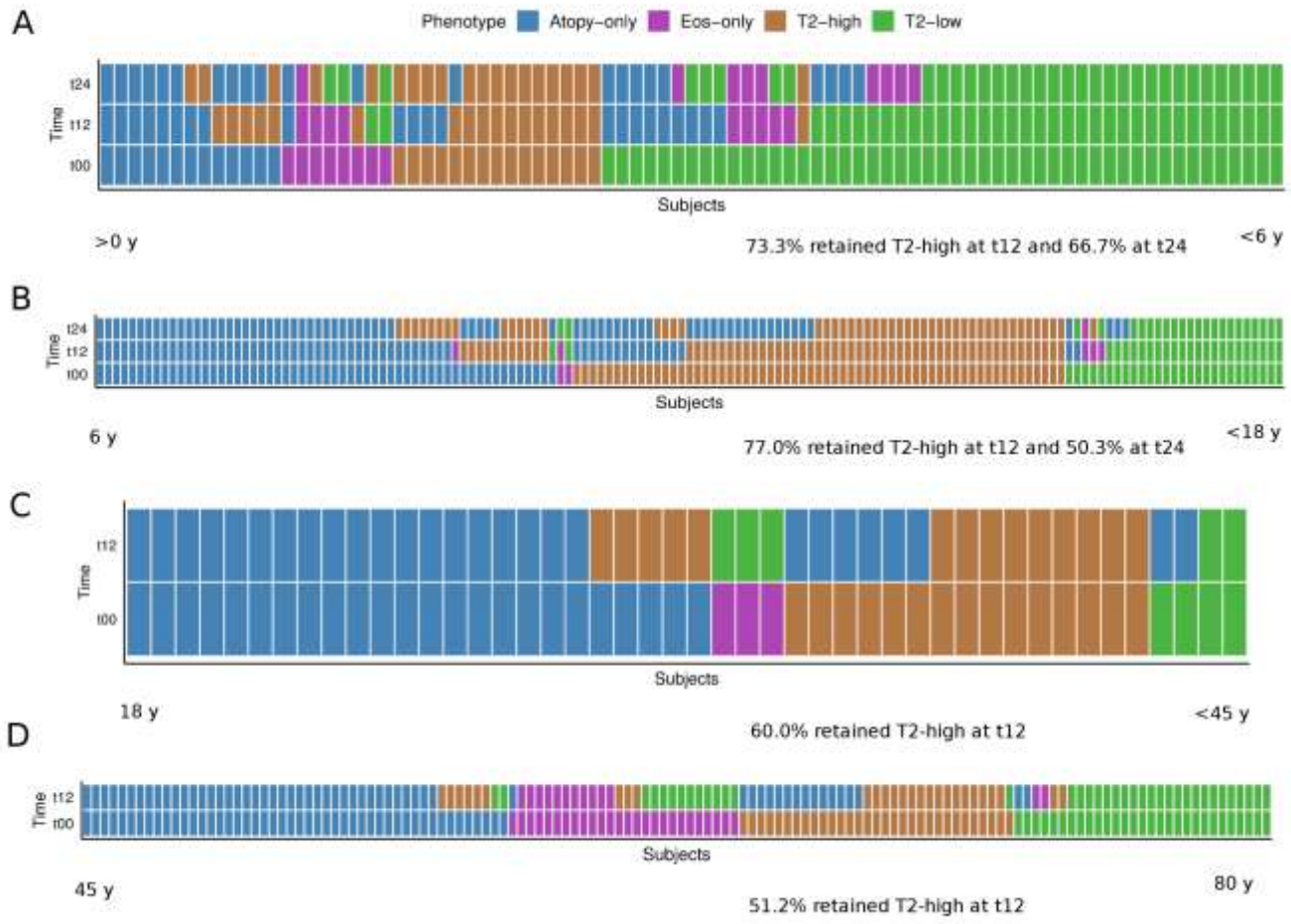
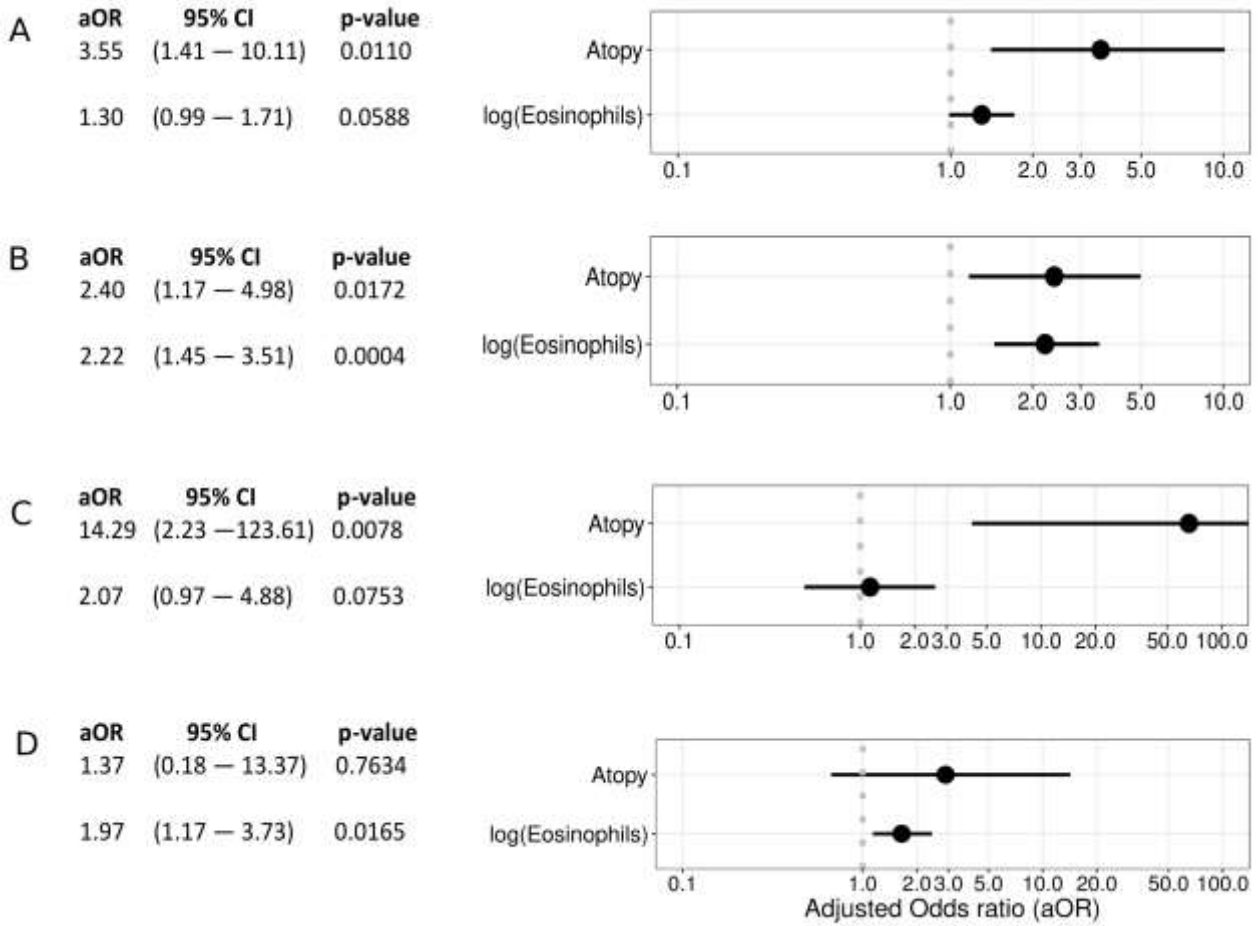

A
Phenotype
Atopy−only
Eos−only
T2−high
T2−low
Time
t24
t12
t00
Subjects
>0 y
73.3% retained T2-high at t12 and 66.7% at t24
<6 y
B
Time
t24
t12
t00
Subjects
6 y
77.0% retained T2-high at t12 and 50.3% at t24
<18 y
C
Time
t12
t00
Subjects
18 y
60.0% retained T2-high at t12
<45 y
D
Time
t12
t00
Subjects
45 y
51.2% retained T2-high at t12
80 y

Figure 5


A
aOR 95% CI p-value
3.55 (1.41 — 10.11) 0.0110
1.30 (0.99 — 1.71) 0.0588
Atopy
log(Eosinophils)
0.1 1.0 2.0 3.0 5.0 10.0
B
aOR 95% CI p-value
2.40 (1.17 — 4.98) 0.0172
2.22 (1.45 — 3.51) 0.0004
Atopy
log(Eosinophils)
0.1 1.0 2.0 3.0 5.0 10.0
C
aOR 95% CI p-value
14.29 (2.23 —123.61) 0.0078
2.07 (0.97 — 4.88) 0.0753
Atopy
log(Eosinophils)
0.1 1.0 2.03.0 5.0 10.0 20.0 50.0 100.0
D
aOR 95% CI p-value
1.37 (0.18 — 13.37) 0.7634
1.97 (1.17 — 3.73) 0.0165
Atopy
log(Eosinophils)
0.1 1.0 2.03.0 5.0 10.0 20.0 50.0 100.0
Adjusted Odds ratio (aOR)

T2-high asthma phenotypes across life span

Nicole Maison, Jimmy Omony, Sabina Illi, Dominik Thiele, Chrysanthi Skevaki, Anna-Maria Dittrich, Thomas Bahmer, Klaus Friedrich Rabe, Markus Weckmann, Christine Happle, Bianca Schaub, Meike Meier, Svenja Foth, Ernst Rietschel, Harald Renz, Gesine Hansen, Matthias Volkmar Kopp, Erika von Mutius*, Ruth Grychtol, and the ALLIANCE Study Group

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

cohort (ALLIANCE) - from early beginnings to chronic disease: a longitudinal cohort study. *BMC Pulm Med* 2018; 18: 140

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

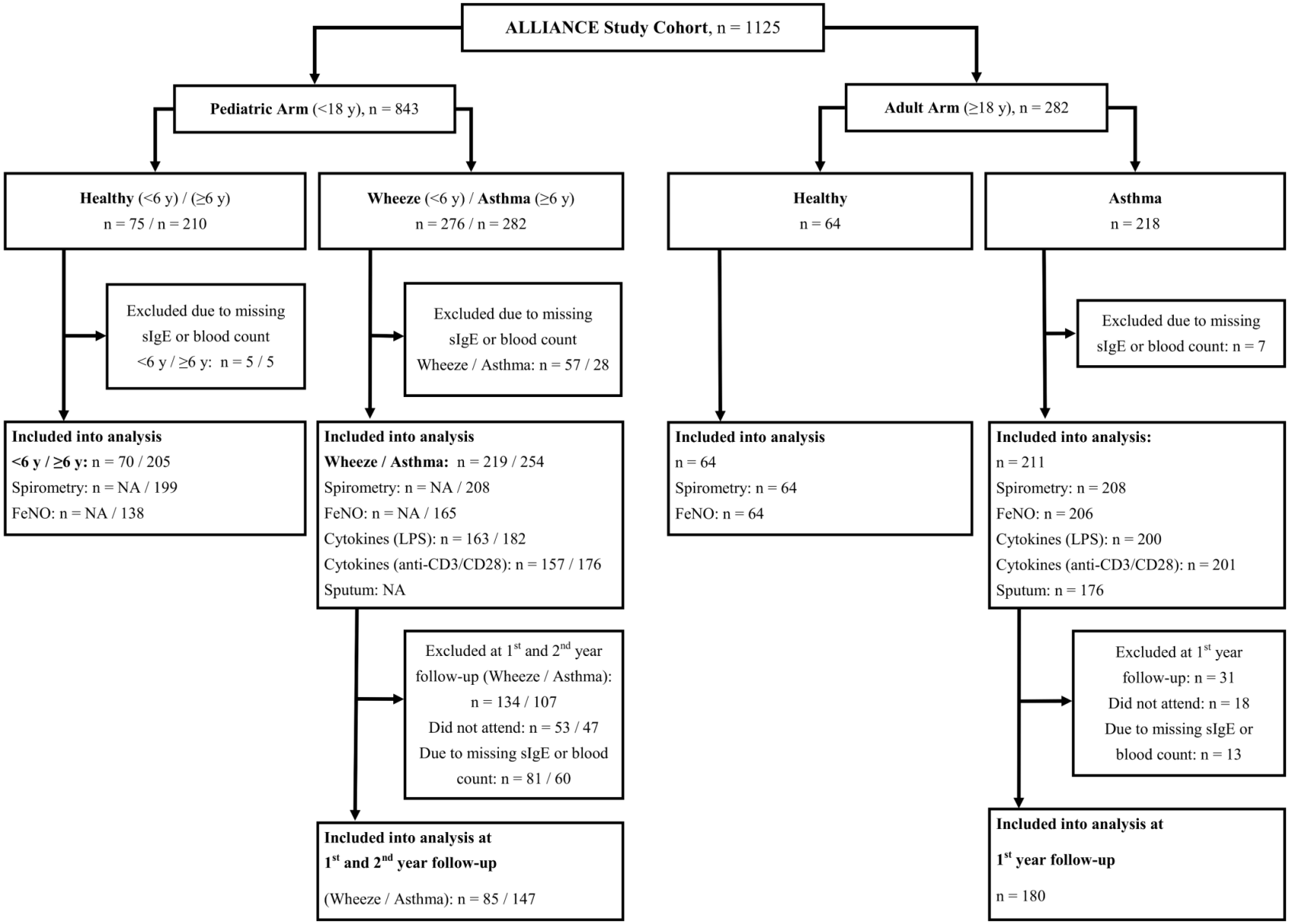


Figure E2: Distribution of blood eosinophil count by age-groups

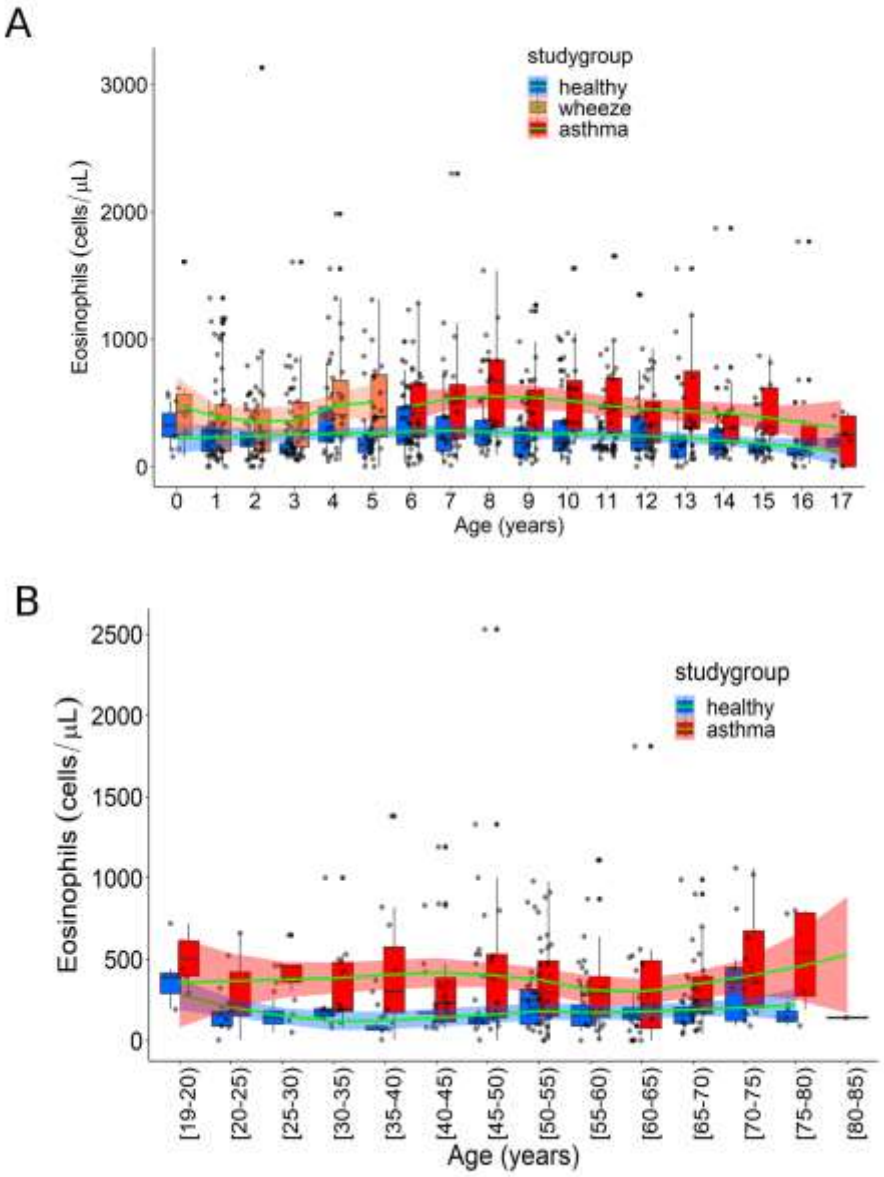


Figure E3: Distribution of blood eosinophils levels stratified by age in healthy children

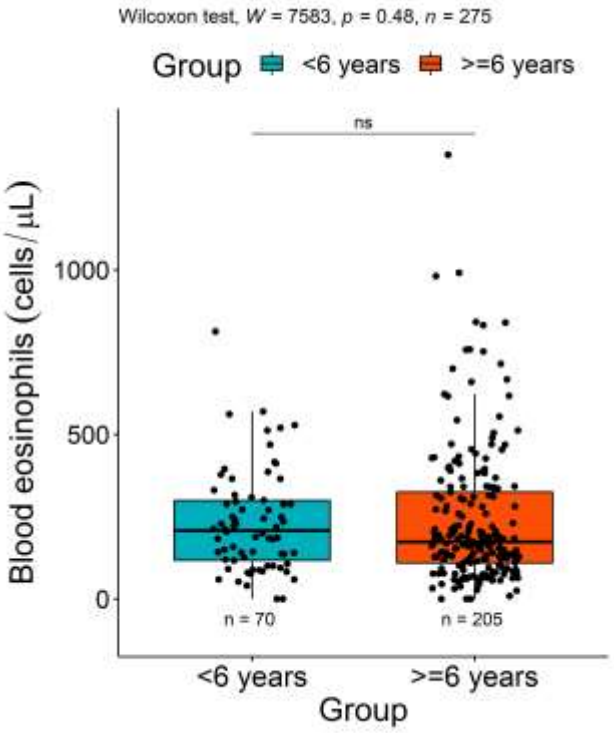


Figure E4: Overlap of T2-high asthma patients with patients having increased FeNO and sputum eosinophils