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Levels of total IgE versus specific IgE during childhood for defining and predicting T2-high asthma

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ABSTRACT

Background: T2-high asthma is characterized by elevated blood eosinophils (b-eos), and/or fractional exhaled nitric oxide (FeNO), and/or being "allergy-driven", which is not well-defined.

Objective: To investigate the role of total and specific immunoglobulin E (tIgE/sIgE) for defining and predicting T2-high asthma in childhood as biomarkers of "allergy-driven".

Methods: We utilized data from the COPSAC2000 (n = 411) and COPSAC2010 (n = 700) mother-child cohorts with repeated measurements of tIgE, sIgE, b-eos and FeNO through childhood. We defined T2-high asthma by elevated b-eos ($\geq 0.3 \times 10^9$/L) and/or FeNO ($\geq$20 ppb) and analyzed association with elevated tIgE (age-specific cut-offs) and sIgE ($\geq$0.35 kU/L) using logistic regression at ages 7/10/13/18 years. Further, we analyzed the association between elevated tIgE and sIgE at age 0–4 years and later risk of T2-high asthma using logistic regression and ROC models.

Results: Elevated tIgE was associated with risk of T2-high asthma at all time points, whereas elevated sIgE showed similar results at ages 10/13/18 years. There was no overall model fit preference for a combination of tIgE and sIgE instead of tIgE or sIgE alone using Vuong's Likelihood-Ratio-Test, Akaike or Bayesian Information Criterion. Further, elevated tIgE at age 0–4 years was associated with later risk of T2-high asthma at all time points (AUC = 0.63–0.70, sensitivity = 0.62–0.81, specificity = 0.57–0.78), whereas elevated sIgE at 0–4 years was only associated with T2-high asthma at 18 years (AUC = 0.66, sensitivity = 0.45, specificity = 0.88). There were no significant differences in AUC values between tIgE and sIgE (DeLong's test).

Conclusion: Elevated tIgE and sIgE are equally useful stand-alone biomarkers for defining and predicting risk of T2-high asthma in childhood.

Keywords: Asthma, Pulmonary eosinophilia, Paediatric asthma

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INTRODUCTION

Childhood asthma affects 10–15% of all European school-aged children with an increasing prevalence globally over the past decades.[1-3] A common asthma inflammatory phenotype is T2-high asthma, which is defined by either 1) being "allergy-driven"; 2) elevated sputum eosinophil count ≥2% and/or elevated blood eosinophil count (b-eos) ≥0.15 10^9/L; and/or 3) elevated fractional exhaled nitric oxide (FeNO) ≥20 parts per billion (ppb).[4] The latter 2 have clearly defined international cut-off values used by The Global Initiative for Asthma (GINA), whereas "allergy-driven" is not well-defined and could be characterized by either age-specific elevated total immunoglobulin E (tIgE) and/or elevated specific immunoglobulin E (sIgE) ≥0.35 kU/L towards aeroallergens and/or relevant symptoms on exposure.[4]

Most difficult-to-treat and severe asthma in childhood and adolescence can be classified as T2-high, which usually debuts in early childhood.[5,6] T2-high asthma in children is often well-controlled with inhaled corticosteroids in low to moderate daily doses, possibly with the addition of a second controller. In refractory severe cases biological treatment targeting key inflammatory mediators are needed.[7-9] Therefore, early identification, prediction and correct diagnosis of T2-high asthma in childhood is important in order to develop personalized care and precision medicine.[10]

The objective of this study was to assess and compare the value of tIgE and sIgE from infancy until adulthood for defining and predicting the risk of T2-high asthma in childhood using existing data from the extensively characterized Copenhagen Prospective Studies on Asthma in Childhood 2000 (COPSAC2000) and 2010 (COPSAC2010) mother-child cohorts.[11,12] Additionally, we investigated whether aeroallergen skin prick test (SPT) positivity or parent-reported rhinitis symptoms could serve the same purpose for defining and predicting risk of T2-high asthma in childhood.

METHODS

The COPSAC2000 cohort

COPSAC2000 is a prospective study conducted at a single center in Denmark, comprising 411 children born between 1998 and 2001 who were at-risk by virtue of maternal physician-diagnosed asthma. Enrollment of the children occurred at 1 month of age with scheduled clinical visits every 6 months until 7 years of age and thereafter at 13 and 18 years along with acute visits as needed. Longitudinal data was collected, including detailed clinical and environmental measures, alongside multi-omics profiling, to understand early-life asthma and allergy development.[11]

The COPSAC2010 cohort

COPSAC2010 is a prospective study of 736 pregnant women and their 700 children born between 2008 and 2011 with similar endpoints as the COPSAC2000 cohort but using an unselected population. Scheduled clinical visits were done at 1 week, 1, 3, 6, 12, 18, 24, 30, and 36 months, and regularly afterwards until 6 years of age and thereafter at ages 8 and 10 years along with acute visit as needed. In addition to similar endpoints, COPSAC2010 incorporates assessments of body composition, metabolism, neurodevelopment and multi-omics profiling to provide a broader understanding of how early-life exposures and comorbidity influence asthma and allergy, and offering validation of findings in a larger, more diverse population.[12,13]

Type-2 biomarkers

Blood eosinophil count was measured at ages 6 and 18 months and 4, 7, 13, and 18 years for COPSAC2000, and at ages 18 months and 6 and 10 years for COPSAC2010.

FeNO was measured at scheduled visits at ages 5, 6, 7, 13 and 18 years and at acute visits for COPSAC2000 and at ages 6 and 10 years for COPSAC2010 using a NIOX Vero (Aerocrine AB, Solna, Sweden) and a Denox 88 (ECO Medics AG, Switzerland) in accordance with international guidelines.[14]

Total IgE was determined at the same ages as b-eos. We used age-specific cut-off values of 7.3, 13, 40, 63, 85, and 85 kU/L for ages 6 and 18 months and 4, 7, 13 and 18 years respectively as recommended by the manufacturer.[15,16]

Specific IgE levels for aeroallergens were determined at the same ages as b-eos for

COPSAC2000 and at 10 years of age for COPSAC2010. sIgE was measured at additional ages for COPSAC2010 without simultaneous tIgE measures. A screening cut-off value of ≥0.35 kU/L was used (ImmunoCAP, Phadiatop Infant™ and Phadiatop™, Thermo Fisher Scientific, Uppsala, Sweden) followed by analysis of individual allergen sIgE levels by ImmunoCAP in screening positive samples for dog, cat, horse, birch, house dust mites, timothy grass, mugwort, and molds.

Skin prick test for aeroallergens were determined at the same ages as b-eos for COPSAC2000 and at 10 years of age for COPSAC2010 using standard allergen extracts for the same 8 aeroallergens as sIgE. A positive SPT reaction was defined as an average wheal diameter that exceeded the negative control by ≥ 2 mm up to 18 months of age and ≥3 mm for ages beyond 18 months.

Diagnosis of asthma and rhinitis

Asthma: In both cohorts, troublesome lung symptoms defined as clinically significant cough, wheeze, or dyspnea explained to the parents as wheeze or whistling sounds, breathlessness, or recurrent troublesome cough severely affecting the well-being of the child were recorded in a daily diary from birth as a dichotomous score (yes/no). Asthma before age 7 years was prospectively determined by physicians employed at the COPSAC research unit using strict predefined standard operating procedure as described in **Supplementary methods**.[17,18] Asthma after age 7 years was objectively validated in accordance with the European Respiratory Society guidelines (Supplementary Table 1).[19]

T2-high asthma was defined by elevated b-eos ≥0.3 10^9/L and/or FeNO≥20 ppb.[4] This cut-off value for b-eos is the recommended upper limit for initiation of biologic treatment targeting type-2 inflammation in refractory asthma cases ensuring higher specificity for this study.[4]

Rhinitis was defined as parent-reported symptoms of at least 2 symptoms of runny nose, sneezing, itchy nose, or blocked nose in the absence of infection. This assessment was conducted during acute visits for both cohorts, and additionally at ages 7, 13 and 18 years for COPSAC2000, and at ages 6 and 10 years for COPSAC2010.

Statistical analysis

Descriptive statistics are presented as frequencies and proportions. Counts used for Venn diagram visualization were estimated in R and digitally drawn with ellipses using the software EulerAPE version 3 to achieve area-proportional dimensions.[20] Spearman's rank correlation coefficient was calculated for all type-2 biomarkers. Cross-sectional association analyses were done using logistic regression models calculating odds ratio (OR) with 95% confidence interval (CI) and associated p-values (P) using an α value of 0.05. Comparison of models for tIgE and sIgE was done using Vuong's Likelihood-Ratio-Test (LRT) for model selection and the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).[21] Predictive analysis with logistic regression models were performed with corresponding receiver operating characteristic (ROC) analysis estimating area under the curve (AUC) values with 95% CI, sensitivity, specificity, positive and negative predictive values to compare the predictive performance of elevated tIgE, sIgE, and SPT ever at age 0–4 years in relation to development of T2-high asthma compared to T2-low asthma as well as cross-sectionally. This analysis was repeated for rhinitis symptoms ever at age 0–6 years. Comparison of AUC values were done using DeLong's test. Statistical analyses were performed using R version 4.2.1 (R Core Team, 2022).

Ethics

The studies were approved by the Copenhagen Ethics Committee (COPSAC2000: KF 01–289/96, COPSAC2010: H–B-2008-093) and the Danish Data Protection Agency (COPSAC2000 & COPSAC2010: 2015-41-3696). Parental and participant consent was obtained.

RESULTS

In COPSAC2000, a total of 155 (55.8%) children had elevated tIgE, 48 (17.0%) had elevated sIgE, and 26 (10.6%) had a positive SPT during their first 4 years of life. Levels of sIgE and radioallergosorbent test (RAST) class distributions at various time points are detailed in Supplementary Table 2. Information on mono- and polysensitization, along with sIgE sensitization to specific aeroallergens, is provided in Supplementary Table 3. In COPSAC2000, 31

(14.9%) children experienced rhinitis symptoms during their first 6 years of life, which was 44 (6.7%) children in COPSAC2010. In COPSAC2000, the percentages of asthma cases at ages 7, 13, and 18 years were 14.0%, 17.1%, and 25.4%, respectively, while in COPSAC2010, it was 6.6% at age 10 years. In COPSAC2000, the percentages of T2-high asthma cases at ages 7, 13 and 18 years were 10.2%, 9.3%, and 15.2%, respectively, while in COPSAC2010, it was 3.6% at age 10 years. The distribution of children with asthma and elevated type-2 biomarkers in COPSAC2000 and COPSAC2010 is outlined in Table 1. No children included from either cohort had diagnoses of primary immune deficiency, including hyper IgE syndrome, recurrent severe infections, or parasitic infections at any point during the study.

The correlation between elevated tIgE, elevated sIgE, positive SPT and reported rhinitis symptoms is visualized in Fig. 1. In COPSAC2000, moderate Spearman's rank correlation coefficients were identified between tIgE and sIgE at ages 7, 13, and 18 years, for both healthy children (0.41, 0.41, 0.52) and children with asthma (0.44, 0.30, 0.49). In COPSAC2010, a moderate correlation between tIgE and sIgE was observed at age 10 years for both healthy children (0.41) and children with asthma (0.56). All p-values were less than 0.001 for the correlations between tIgE and sIgE. In COPSAC2000, symptoms of allergic rhinitis were more strongly correlated with sIgE than tIgE at all time points in both healthy children (0.39 vs 0.12) and children with asthma ($\geq$0.30 vs $\geq$ 0.12). Similarly, in COPSAC2010 at age 10 years, symptoms of allergic rhinitis were more strongly correlated with sIgE than tIgE in both healthy children (0.34 vs 0.19) and children with asthma (0.74 vs 0.61).

Visualizations of the overlap between elevated b-eos, elevated FeNO and either elevated tIgE or sIgE among children with a confirmed asthma diagnosis are shown for both cohorts in Fig. 2 at ages 7–18 years. We observed a similar overlap of asthma with elevated b-eos and/or FeNO and either elevated tIgE or sIgE at ages 10 (63.6% vs 60.6%), 13 (56.6% vs 50.9%) and 18 years (49.4% vs 55.3%), and a slightly diverging overlap at age 7 years (34.9% vs 25.6%). Additionally, there was a similar number of children with asthma, who had isolated elevation of either tIgE or sIgE at 7 years (2.3% vs 4.7%), 10 years (15.2% vs 12.1%), 13 years (11.3% vs 17.0%), and 18 years (10.6% vs 12.9%).

Cross-sectional analyses of tIgE and sIgE vs T2-high/low asthma

Logistic regression was performed on the cross-sectional association between elevated tIgE and

Cohort	COPSAC2000 (N = 411)			COPSAC2010 (N = 700)
Time point	7 years (%)	13 years (%)	18 years (%)	10 years (%)
Sex (Male)	169/336 (50.3)	180/362 (49.7)	183/371 (49.3)	330/640 (51.6)
Asthma (Yes)	47/336 (14.0)	62/362 (17.1)	94/371 (25.4)	42/640 (6.6)
T2-high asthma (Yes)[a]	34/334 (10.2)	33/353 (9.3)	55/362 (15.2)	23/631 (3.6)
b-eos (Elevated)	204/311 (65.6)	106/308 (34.4)	92/337 (27.3)	208/511 (40.7)
FeNO (Elevated)	27/317 (8.5)	77/330 (23.3)	150/365 (41.1)	91/598 (15.2)
tIgE (Elevated)	122/296 (41.2)	129/315 (41.0)	173/355 (48.7)	248/532 (46.6)
sIgE (Elevated)	76/296 (25.7)	146/315 (46.3)	187/355 (52.7)	187/532 (35.2)
SPT (Elevated)	24/290 (8.3)	115/316 (36.4)	175/358 (48.9)	148/532 (27.8)
Rhinitis symptoms (Yes)	33/214 (15.4)	133/357 (37.3)	163/370 (44.1)	112/636 (17.7)

Table 1. Distribution of children with asthma and elevated type-2 biomarkers for each clinical visit during school-age. Elevated blood eosinophil count (b-eos) was defined as $\geq$0.30 10^9/L, fractional exhaled nitric oxide (FeNO) as $\geq$20 ppb, total immunoglobulin E (tIgE) as $\geq$ age-specific cutoff values, specific immunoglobulin E (sIgE) as $\geq$0.35 kU/L and skin prick test (SPT) as positive for tested aeroallergens.
[a]*Missing data for T2-high asthma was 2/9/9/9 children at 7/10/13/18 years with either b-eos or FeNO missing.*

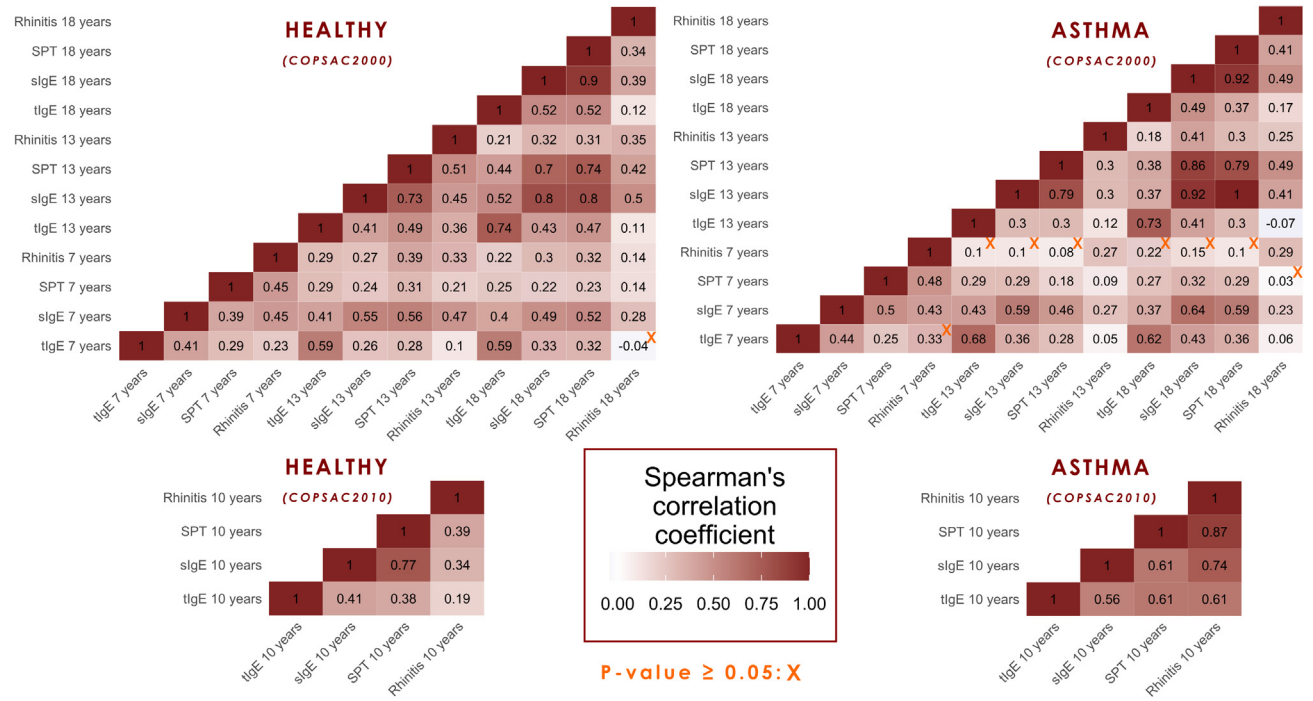

Fig. 1 Heatmap of correlation between type-2 biomarkers in COPSAC2000 and COPSAC2010 at ages 7, 10, 13 and 18 years. Elevated total immunoglobulin E (tIgE ≥ age-specific cutoff values), specific immunoglobulin E (sIgE ≥0.35 kU/L), positive aeroallergen skin prick test (SPT) and rhinitis symptoms (Rhinitis) were compared. P-values are ≤0.05 unless stated otherwise

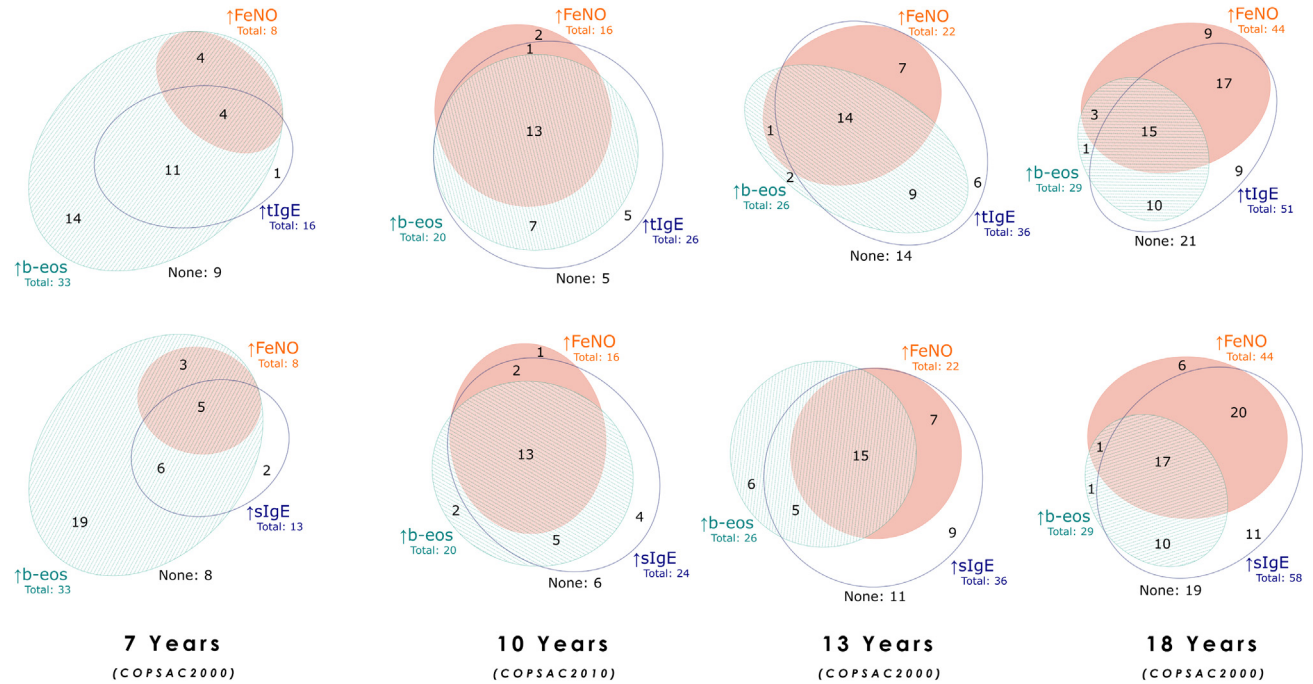

Fig. 2 Venn diagram of children with asthma diagnosis at school-age time points in relation to elevated blood eosinophil count (b-eos) ≥0.30 10^9/L, fractional exhaled nitric oxide (FeNO) ≥20 ppb and either total immunoglobulin E (tIgE) ≥63 kU/L at 7 years, and ≥85 kU/L at 10, 13, and 18 years or specific immunoglobulin E (sIgE) ≥0.35 kU/L

-	Elevated tIgE, sIgE, SPT or rhinitis symptoms and concurrent T2-high vs T2-low asthma				Elevated tIgE, sIgE, SPT or rhinitis symptoms at early age and later T2-high vs T2-low asthma			
-	N (T2-high	Odds ratio	Confidence interval	P-value	N (T2-high)	Odds ratio	Confidence interval	P-value
7 Years								
tIgE	33	7.50	[1.20–146.49]	0.03	29	5.73	[1.14–43.37]	0.03
sIgE	33	2.00	[0.41–14.77]	0.43	30	0.73	[0.15–4.08]	0.70
SPT	31	3.27	[0.49–65.28]	0.24	27	2.53	[0.35–51.78]	0.42
Rhinitis	14	1.50	[0.19–14.25]	0.70	13	1.71	[0.24–15.75]	0.60
10 Years								
tIgE	23	10.50	[1.74–90.78]	0.03	–	–	–	–
sIgE	23	10.00	[1.88–67.27]	0.007	–	–	–	–
SPT	23	15.56	[2.83–115.51]	0.001	–	–	–	–
Rhinitis	23	7.13	[1.43–42.08]	0.02	23	3.67	[0.72–27.96]	0.12
13 Years								
tIgE	33	23.33	[5.70–127.87]	<0.001	21	5.67	[1.31–28.62]	0.02
sIgE	33	5.50	[1.64–20.39]	0.005	22	1.67	[0.44–6.88]	0.46
SPT	30	3.00	[0.94–10.08]	0.94	23	4.23	[0.87–31.52]	0.10
Rhinitis	33	3.26	[1.03–10.86]	0.04	21	2.46	[0.46–19.12]	0.32
18 Years								
tIgE	55	7.54	[2.87–21.40]	<0.001	40	2.91	[1.04–8.52]	0.04
sIgE	55	10.15	[3.67–30.75]	<0.001	40	6.00	[1.72–28.28]	0.004
SPT	54	10.00	[3.66–29.86]	<0.001	42	1.41	[0.36–7.05]	0.63
Rhinitis	55	4.85	[1.89–13.04]	<0.001	24	10.15	[1.60–200.12]	0.01

Table 2. Logistic regression models assessing association between T2-high asthma and elevated total immunoglobulin E (tIgE ≥ age-specific cutoff values), specific immunoglobulin E (sIgE ≥0.35 kU/L), aeroallergen skin prick test (SPT) or rhinitis symptoms (Rhinitis). Model 1 examines cross-sectional associations at ages 7–18 years. Model 2 explores early-life risk factors (0–4 years for IgE/SPT, 0–6 years for rhinitis symptoms) for later T2-high asthma

sIgE and T2-high asthma defined by either elevated b-eos and/or FeNO at ages 7, 10, 13, and 18 years (Table 2). Using age-specific cut-off values, elevated tIgE was significantly associated with an increased risk of T2-high asthma at ages 7 years (OR = 7.5, 95% CI = 1.2–146.5, P = 0.03), 10 years (10.5, 1.7–90.8, P = 0.03), 13 years (23.3, 5.7–127.9, P < 0.01) and 18 years (7.5, 2.9–21.4, P < 0.01). Elevated sIgE was also significantly associated with an increased risk of T2-high asthma at ages 10 years (10.0, 1.9–67.3, P < 0.01), 13 years (5.5, 1.6–20.4, P < 0.01), and 18 years (10.2, 3.7–30.8, P < 0.01), but not at age 7 years (2.0, 0.4–14.8, P = 0.43).

Similarly, a positive SPT was significantly associated with an increased risk of T2-high asthma at ages 10 years (15.6, 2.8–115.5, P < 0.01) and 18 years (10.0, 3.7–29.9, P < 0.01), but not at ages 7 years (3.3, 0.5–65.3, P = 0.24) or 13 years (3.0, 0.9–

10.1, P = 0.94). Rhinitis symptoms were also significantly associated with an increased risk of T2-high asthma at ages 10 years (7.1, 1.4–42.1, P = 0.02), 13 years (3.3, 1.0–10.9, P = 0.04), and 18 years (4.9, 1.9–13.0, P < 0.01), but not at age 7 years (1.5, 0.2–14.3, P = 0.70) (Table 2).

To compare whether associations with T2-high asthma were stronger for tIgE, sIgE, or a combination of both, Vuong's LRT and AIC/BIC calculations were performed for model selection. This was done for either elevated tIgE or sIgE in relation to concurrent T2-high asthma in comparison to using a combination model of both tIgE and sIgE. These analyses showed that at age 13 years there was a significant fit preference for using a combination model of both tIgE and sIgE instead of sIgE alone (P < 0.01), but there were no significant preferences at ages 7, 10, or 18 years (p-values>0.10). Further, there was no fit preference for using a combination model of both tIgE and sIgE instead of tIgE alone (p = 0.61) (Supplementary Table 4).

ROC analyses for the cross-sectional performance of elevated tIgE and sIgE for determining risk of T2-high asthma are shown in Supplementary Table 5. Using DeLong's test there was a significant difference between the AUC values for tIgE and sIgE at age 13 years (P = 0.04) but not at ages 7, 10, or 18 years (p-values>0.09).

Early life tIgE and sIgE vs later T2-high/low asthma

Logistic regression was performed to investigate the association between elevated tIgE and sIgE in early childhood and the later risk of developing T2-high asthma (Table 2). Elevated tIgE at ages 0–4 years was significantly associated with an increased risk of having T2-high asthma at ages 7 years (5.7, 1.1–43.4, P = 0.03), 13 years (5.7, 1.3–28.6, P = 0.02), and 18 years (2.9, 1.0–8.5, P = 0.04). Elevated sIgE at ages 0–4 years was also significantly associated with an increased risk of T2-high asthma at age 18 years (6.0, 1.7–28.3, P < 0.01), but not at ages 7 years (0.7, 0.2–4.1, P = 0.70) or 13 years (1.7, 0.4–6.9, P = 0.46).

Conversely, an early positive SPT was not significantly associated with the risk of T2-high asthma at ages 7 years (2.5, 0.4–51.8, P = 0.42), 13 years (4.2, 0.9–31.5, P = 0.10) or 18 years (1.4, 0.4–7.1, P = 0.63). Parentally reported rhinitis symptoms at ages 0–6 years was significantly associated with the risk of having T2-high asthma at age 18 years (10.2, 1.6–200.1, P = 0.01) but not at ages 7 years (1.7, 0.2–15.8, P = 0.60), 10 years

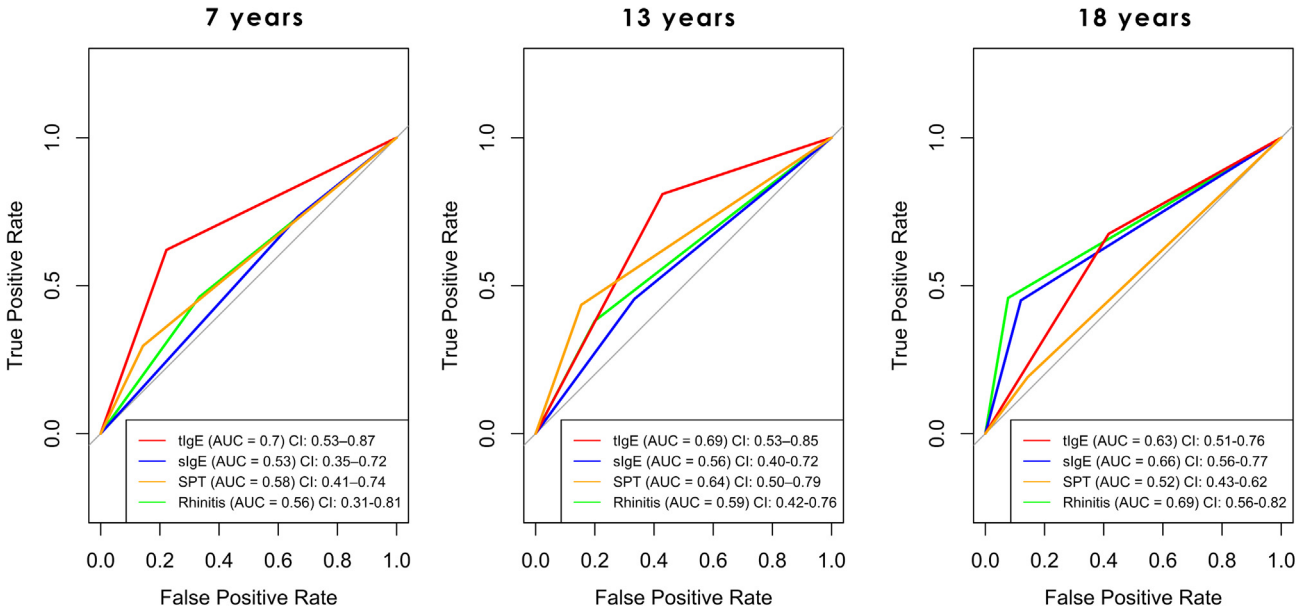

Fig. 3 Coupled Receiver operating characteristic (ROC) curves of logistic models for elevated total immunoglobulin E (tIgE), specific immunoglobulin E (sIgE), positive aeroallergen skin prick test (SPT) and rhinitis symptoms (yes/no) between age 0–6 years in relation to T2-high asthma at later time points. Corresponding area under curve (AUC) and confidence interval (CI) values are shown

(3.7, 0.7–28.0, P = 0.12) or 13 years (2.5, 0.5–19.1, P = 0.32) (Table 2).

ROC analyses and corresponding ROC curves for the performance of early childhood elevated tIgE and sIgE for predicting risk of developing T2-high asthma after age 7 years is shown in Fig. 3 and Supplementary Table 6. At ages 7, 13, and 18 years, the AUC values [95% CI] for tIgE were 0.70 [0.53–0.87], 0.69 [0.53–0.85], and 0.63 [0.51–0.76], respectively, while those for sIgE were 0.53 [0.35–0.72], 0.56 [0.40–0.72], and 0.66 [0.56–0.77]. Likewise, the positive predictive value (PPV) and negative predictive value (NPV) were calculated for tIgE at 7 years (PPV/NPV: 90%/39%), 13 years (74%/67%), and 18 years (73%/52%), and for sIgE at 7 years (79%/27%), 13 years (67%/45%), and 18 years (86%/50%). Using DeLong's test there were no significant differences between the AUC values for tIgE and sIgE at ages 7, 13, or 18 years (p-values>0.20). Sensitivity and specificity for tIgE were 0.62/0.78 at 7 years, 0.81/0.57 at 13 years, and 0.68/0.58 at 18 years, while for sIgE, they were 0.73/0.33 at 7 years, 0.45/0.67 at 13 years, and 0.45/0.88 at 18 years.

For SPT, the AUC values were 0.58 [0.41–0.74], 0.64 [0.50–0.79], and 0.52 [0.43–0.62] for ages 7, 13, and 18 years, with corresponding PPV/NPV values of 89%/24%, 83%/46%, and 73%/35%. Likewise, for rhinitis symptoms, the AUC values were 0.56 [0.31–0.81], 0.64 [0.47–0.81], 0.59 [0.42–0.76], and 0.69 [0.56–0.82] for ages 7, 10, 13, and 18 years, with corresponding PPV/NPV values of 75%/36%, 85%/40%, 80%/38%, and 92%/48% (Fig. 3 and Supplementary Table 6).

Sensitivity analyses

Exploration of a higher cut-off value for b-eos ($\geq 0.5 \times 10^9$/L) and a uniform not age-specific cut-off value for elevated tIgE (≥50 kU/L) yielded similar results for both cross-sectional analyses and for early life elevated tIgE and sIgE in relation to risk of developing T2-high asthma (Supplementary Tables 7–8). Likewise, comparing T2-high asthma to healthy children instead of T2-low yielded similar associations (Supplementary Table 9). Additionally, sensitivity analyses adjusting for confounders such as sex, atopic dermatitis, seasonal allergies, and parental predisposition to asthma yielded no significant changes from the unadjusted model (Supplementary Table 10).

DISCUSSION

We found that elevated tIgE and sIgE had similar cross-sectional associations with T2-high asthma from age 10 years and above, whereas elevated tIgE was also associated with T2-high asthma at age 7 years unlike sIgE. There were almost interchangeable overlapping areas of elevated tIgE and sIgE with T2-high asthma from age 7. Importantly, either tIgE or sIgE were solely elevated for a considerable number of children with asthma during adolescence that otherwise would not be defined as having T2-high asthma using only elevated b-eos and/or FeNO. Further, elevated levels of both tIgE and sIgE in early childhood was associated with an increased risk of developing T2-high asthma though this was only significant at 18 years for sIgE, whereas it was significant across all time points in childhood for tIgE. However, there were no significant differences in AUC values for tIgE vs sIgE. These findings suggest that elevated tIgE and sIgE are equally useful stand-alone biomarkers for defining and predicting risk of T2-high asthma in childhood.

We found that up to 17% of the children with asthma had elevated tIgE and/or sIgE without accompanying elevated b-eos and/or FeNO. These children could be defined as having T2-high asthma, but it is uncertain whether T2-high asthma should be defined solely by elevated tIgE or sIgE levels or whether combining both would be preferential.[22,23] A previous study has shown that the risk of developing asthma in childhood is greatest when multiple type-2 biomarkers are elevated in combination as opposed to when only 1 biomarker is elevated.[24] In contrast, we found no benefit using a combination model of both elevated tIgE and sIgE in relation to the risk of T2-high asthma compared to using either elevated tIgE or sIgE alone. However, children with asthma with an isolated elevated IgE without other elevated type-2 biomarkers could also be children, who do not have the conventional T2-low asthma phenotype but instead a similar phenotype of T2-low with elevated IgE as reported in adults.[25]

Other studies have shown that both elevated tIgE and sIgE are associated with increased

severity of asthma.[26,27] Being able to correctly identify and predict risk of T2-high asthma could lead to improved personalized treatment, better asthma control, and lessen the burden of asthma exacerbations in refractory cases, where there is a need for specific treatments with biologics and ultimately lead the way for precision medicine.[7,10,28,29] As such, the possibility of categorizing asthma early in life into T2-high and T2-low groups and predicting the inflammatory phenotype later in childhood with a higher accuracy has a clinical perspective for clinicians treating childhood asthma. Our findings suggested that both tIgE and sIgE are equally useful biomarkers during childhood and adolescence to define risk of T2-high asthma. However, elevated tIgE exhibited a more consistent association with T2-high asthma at every time point from age 7 years, whereas elevated sIgE was associated with risk of T2-high asthma from age 10 years.

Our findings suggest that both tIgE and sIgE could serve as valuable biomarkers for predicting risk of T2-high asthma later in life. Elevated tIgE performed slightly better than elevated sIgE with a significant association to T2-high asthma at all time points and overall showed higher AUC values, but these were not significantly different than the sIgE AUC values. These findings are consistent with previous studies, which have shown that elevated tIgE and sIgE levels during childhood were both significant predictors for the development of wheeze and asthma,[24,30,31] with our study notably contextualizing these associations in children with T2-high asthma. Generally, the overall predictive performance of both tIgE and sIgE, as indicated by their AUC, were relatively low ($\leq$0.70), with sIgE only being significantly better than random at 18 years of age. In our study, we observed age-related variations in the predictive power of tIgE and sIgE. Notably, tIgE demonstrated superior accuracy to sIgE in identifying T2-high children and adolescents, with the best PPV observed at 13 years (tIgE vs sIgE, 90% vs 79%). In contrast, sIgE surpassed tIgE in terms of PPV at 18 years (86% vs 73%). However, for NPV, tIgE appeared to outperform sIgE at all time points (39–67% vs 27–50%).

There is an ongoing debate whether sIgE or SPT is the most informative tool for identifying clinically significant sensitization,[32–34] particularly as type-2 biomarkers, with limited consensus in the existing literature. Though SPT is associated with pain and anxiety in children,[35,36] it is seen as minimally invasive in comparison to venipuncture for sIgE measurements.[37] We observed similar cross-sectional risks of T2-high asthma for sIgE vs SPT, whereas early childhood sIgE was a stronger predictor than SPT for development of T2-high asthma.

Allergic rhinitis frequently coexists with asthma in children.[38] Our analysis of parentally reported rhinitis symptoms independent of allergic sensitization testing showed that this was non-inferior to elevated tIgE and sIgE for defining and predicting T2-high asthma. This could prove useful in a clinical setting, as evaluating symptoms of rhinitis is straightforward and does not require any form of blood testing, making it a completely non-invasive approach. However, reported rhinitis symptoms may encompass several other causes than allergy in a general practice or asthma clinic unlike our closely monitored cohorts, where the parents and children where prospectively trained in recognizing asthma and allergy symptoms.

One important limitation of this study is the small number of children with asthma in the cohorts, which hampers the ability to study T2-high asthma subtypes and may have caused lack of association specifically for elevated sIgE, positive SPT, and reported rhinitis symptoms at early ages, where sensitization to aeroallergens is not very prevalent compared to elevated tIgE. We also observed a difference in the prevalence of T2-high asthma between the 2 cohorts. The prevalence was lower in the COPSAC2010 cohort at 10 years compared to the COPSAC2000 cohort at 13 years (3.6% vs 9.3%), likely due to cohort differences, such as the asthma at-risk status in COPSAC2000. Also, children from an asthma at-risk cohort are more likely to have a genetic predisposition to asthma and atopy, which may influence the association between tIgE and sIgE levels and the development of T2-high asthma. Specifically, an asthma at-risk cohort may have higher baseline levels of tIgE and sIgE, making it difficult to determine if elevated IgE is related to the development of T2-high asthma or simply a reflection of the genetic background.[27] However, we found similar results at age 10 years in the population-based COPSAC2010 cohort, suggesting that our findings are representative of the general

population. Additionally, exploring a higher cut-off value for b-eos and a uniform not age-specific cut-off for tIgE did not alter our findings. Previous studies have examined age-related changes in tIgE levels, and while several reference intervals have been suggested, there is still variability in the definition of normal range for different age groups.[39,40] Comparison between children with T2-high asthma and healthy children was similar to that between children with T2-high and T2-low asthma children. Finally, it is a limitation that we did not include regular assessment of asthma severity or control using, e.g., the Asthma Control Test (ACT) or Asthma Control Questionnaire (ACQ) tools.

CONCLUSION

Elevated tIgE and sIgE are equally relevant stand-alone biomarkers for defining and predicting risk of T2-high asthma in childhood, particularly during adolescence, with SPT performing similarly. The consistent utility of both tIgE and sIgE as T2-high biomarkers across age groups underscores their role for improving diagnostic accuracy of T2-high asthma, providing clinicians with valuable tools for asthma phenotyping. Parent-reported rhinitis symptoms were also useful in our closely monitored birth cohorts for defining and predicting risk of T2-high asthma without any need for blood samples or skin prick tests. These findings are useful for clinicians working with childhood asthma to develop personalized care and precision medicine.

Abbreviations

ACQ: Asthma Control Questionnaire; **ACT**: Asthma Control Test; **AIC**: Akaike information criterion; **AUC**: Area under the curve; **BIC**: Bayesian Information Criterion; **b-eos**: Blood eosinophil count; **CI**: Confidence Interval; **COPSAC2000**: Copenhagen Prospective Studies on Asthma in Childhood 2000; **COPSAC2010**: Copenhagen Prospective Studies on Asthma in Childhood 2010; **FeNO**: Fractional exhaled nitric oxide; **GINA**: The Global Initiative for Asthma; **IL**: Interleukin; **IgE**: Immunoglobulin E; **LRT**: Likelihood-Ratio-Test; **OR**: Odds ratio; **P**: P-value; **Ppb**: Parts per billion; **RAST**: Radioallergosorbent test; **ROC**: Receiver operating characteristic; **sIgE**: Specific immunoglobulin E; **tIgE**: Total immunoglobulin E

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Author's contributions

The guarantor of the study is BC, from conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. TS has written the first draft of the manuscript. All co-authors have provided important intellectual input and contributed considerably to the analyses and interpretation of the data. All authors guarantee that the accuracy and integrity of any part of the work have been appropriately investigated and resolved and all have approved the final version of the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit for publication. No honorarium, grant, or other form of payment was given to any of the authors to produce this manuscript.

Ethics approval

The studies were approved by the Copenhagen Ethics Committee (COPSAC2000: KF 01–289/96, COPSAC2010: H–B-2008-093) and the Danish Data Protection Agency (COPSAC2000 & COPSAC2010: 2015-41-3696). Parental and participant consent was obtained.

Author's consent for publication

All the authors reviewed the final draft and provided consent for publication.

Governance

We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) good clinical practice guidelines and the Helsinki Declaration. Privacy is important to us which is why we follow national and international legislation on General Data Protection Regulation (GDPR), the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

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Declaration of competing interest

All authors declare no potential, perceived, or real conflict of interest regarding the content of this manuscript. The funding agencies did not have any role in design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript. No pharmaceutical company was involved in the study.

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Appendix A. Supplementary data
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