

ORIGINAL ARTICLE

AIRWAY DISEASES

Exhaled nitric oxide in symptomatic children at preschool age predicts later asthma

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Abstract

Background: Prediction of asthma in young children with respiratory symptoms is hampered by the lack of objective measures applicable in clinical routine. In this prospective study in a preschool children cohort, we assessed whether the fraction of exhaled nitric oxide (FeNO), a biomarker of airway inflammation, is associated with asthma at school age.

Methods: At baseline, IgE and eosinophils were measured in the blood, and FeNO was measured offline in 391 children aged 3–47 months with lower airway symptoms. We developed an asthma predictive index (API) including high FeNO as major criterion. At follow-up, primary outcome was physician-diagnosed asthma based on standardized interviews in those children reaching school age ($n = 166$).

Results: FeNO was significantly elevated in those children with later asthma (68/166) as compared to children not developing asthma. Median (IQR) FeNO was 10.5 (6.6–17.2) vs 7.4 (5.3–10.3) ppb. Per 5 ppb FeNO increase, the odds ratio (95% CI) for asthma increased by 2.44 (1.61–3.70) without changing when adjusting for confounders. Using the new API, children scored at risk had 58.0% probability for later asthma, whereas the negative predictive value was 78.2%, which was comparable to the classical API.

Conclusions: In this cohort of high-risk preschool children, elevated FeNO is associated with increased risk for school-age asthma. The new API including FeNO identifies children at risk of later asthma comparably to the classical API, but does not require blood sampling.

Asthma is a complex dynamic disease characterized by fluctuations of bronchial hyper-responsiveness, airway obstruction, and airway inflammation over time (1–3). These variable pathophysiological features hamper the prediction of respiratory disease outcomes. In preschool children, prediction of asthma is especially challenging as their clinical presentation, that is, wheezing, is unspecific and triggers for wheezing are heterogeneous (4–6). Further, objective measures of lung physiology are limited. Clinical scores based on historical atopic features and blood eosinophils for wheezy infants may predict the risk for asthma later in life. However, cross-validation in other study populations was less encouraging for routine use (5, 6).

There is scanty evidence to estimate the risk of later asthma in young children. The fraction of exhaled nitric oxide (FeNO) is a noninvasive biomarker associated with

airway inflammation and can be measured across all ages (7–11). Atopic infants at risk for asthma have increased FeNO prior to respiratory symptoms (12). This association of FeNO with later symptoms in children seems further enhanced in mothers who smoke (13). In a cohort of preschool children with respiratory symptoms, we have recently shown that elevated FeNO characterizes an asthma phenotype with predominantly wheezy symptoms and helps to distinguish from those children with recurrent coughing (10).

We hypothesize that elevated FeNO early in life is associated with asthmatic symptoms at school age. In this prospective cohort study, we assessed whether elevated FeNO at preschool age is associated with asthma at school age. Secondly, we compared two clinical asthma predictive indices (APIs) using eosinophilic counts in the blood or FeNO as biomarkers for airway inflammation.

Methods

Study design

A total of 391 preschool children were consecutively recruited to this cohort at the University Children's Hospital Zurich, Switzerland, between 2000 and 2004 (Fig. 1). Inclusion criteria were age between 3 months and 4 years and presence of recurrent coughing or wheezing, and ability to perform FeNO measurements. Exclusion criteria were prescribed oral corticosteroids at least 4 weeks prior to the study and respiratory diseases as described previously (10), for example, pneumonia or upper respiratory tract infection. At baseline, data on respiratory health, physical examination, FeNO measurements, and allergic sensitization were obtained. At follow-up, we performed telephone interviews on respiratory health in those children aged between 6 and 10 years. For internal validation, we randomly assigned half of the school-aged children to a nested cohort additionally performing FeNO measurement, spirometry, and sensitization assessment.

The primary outcome was physician-diagnosed asthma at school age. Secondary school-age outcomes were (i) current asthma symptoms (at least one episode of wheezing 12 months prior to follow-up and/or recurrent exercise-induced wheeze or dry cough and/or recurrent dry cough during sleep without upper or lower respiratory tract infection), (ii) prescribed current asthma medication, and (iii) current atopy (atopic dermatitis and/or rhinoconjunctivitis and/or allergic sensitization, but the absence of a physician-diagnosed asthma). The study was approved by the Ethics Committee of the University Children's Hospital Zurich. The children's assent was obtained and parents or caregivers provided written informed consent for this study.

Assessments

Two respiratory physicians (IL and NK) performed standardized interviews on asthma (14) including pre- and post-natal passive smoke exposure and medication use at baseline and follow-up visits.

FeNO was measured offline at baseline and online at follow-up according to ATS/ERS recommendations and as described previously (10, 15). Inhaled corticosteroids (ICS)

and short-acting beta-agonists were withheld for 24 h prior to measurements. For the reservoir-offline technique, children were sitting on the legs of the mother while tidally breathing NO-free air for ten breaths. A reusable inert collection bag (750 ml; Quintron, WI, USA) was attached to the expiratory side of a non-rebreathing valve with a resistor in place. Five breaths during relaxed tidal breathing were sampled, and FeNO was analyzed within 15 min using a chemiluminescence analyzer (CLD 77 AM; Eco Medics AG, Durnten, Switzerland). Crying, coughing, leaks, or otherwise unsteady breathing leads to rejection of the measurement. Preschool FeNO thus reflects the average NO content of five tidal exhalations. The same device (CLD 77 AM) was applied for the single-breath on-line FeNO measurement at follow-up. After ten breaths of NO-free air, children were exhaling against an expiratory resistance with a constant flow of 50 ml/s for at least 6 s.

At baseline, blood was sampled from children for eosinophil count and aero- and food-allergen IgE levels were measured by a radioallergosorbent test (RAST; Unicap, Pharmacia, Switzerland). Children were considered sensitized if any allergen RAST class is two or greater (≥ 0.7 kU/l) (10). At follow-up, a skin prick test was applied as described previously (10). Allergens assessed were birch, hazel, six-grass mix, cat and dog dander, and house dust mite. After 15 min, the test was scored positive if the wheal diameter was at least 3 mm greater than the saline wheal given a positive histamine reaction. Categories were negative, monosensitization (positive for one allergen), and polysensitization (positive for two or more allergens). Spirometry at follow-up was performed using the Masterlab (Jaeger, Wurzburg, Germany) according to current recommendations (16).

Two API based on respiratory and atopic symptoms and two different measures of eosinophilic inflammation in preschool children were compared: (i) the classical API according to Castro-Rodriguez et al. (5), and (ii) the new API with elevated FeNO replacing blood eosinophilia (Table 1).

Statistics

FeNO data were right-skewed and thus log-transformed or described as median and interquartile range (IQR) and compared using nonparametric tests. FeNO was also broken

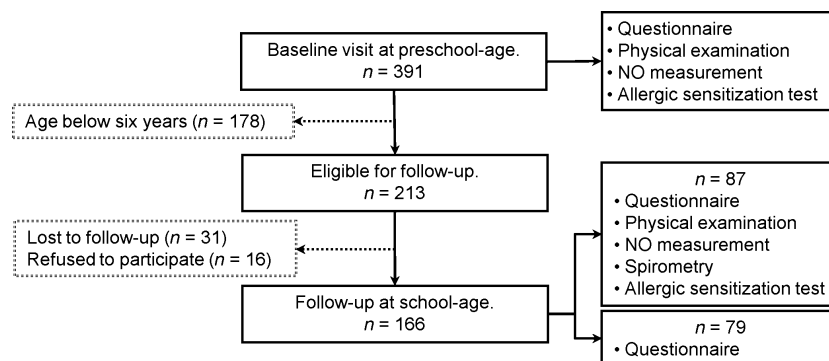


Figure 1 Flow chart of the study population.

Table 1 Asthma predictive indices

Wheeze phenotypes		
Early wheezer (EW): 1–2 attacks (last 12 months); early frequent wheezer (EFW): ≥ 3 attacks (last 12 months)		
Asthma predictive criteria		
Major		
(A) Parental physician-diagnosed asthma		
(B) Physician-diagnosed atopic dermatitis		
(C) Fraction of exhaled nitric oxide >10 ppb		
Minor		
(a) Wheezing apart from colds		
(b) Physician-diagnosed allergic rhinitis		
(c) Eosinophils $\geq 4\%$ of white blood cells		

	Classical asthma predictive index	New asthma predictive index
Loose index	EW + 1/2 major (A, B) or 2/3 minor (a–c)	EW + 1/3 major (A–C) or 2 minor (a, b)
Stringent index	EFW + 1/2 major (A, B) or 2/3 minor (a–c)	EFW + 1/3 major (A–C) or 2 minor (a, b)

Definitions of wheeze and historical criteria were used from previous reports (5, 10). In the new asthma predictive index, the major criterion (C) fraction of exhaled nitric oxide replaced the minor criterion (c) eosinophils.

down into tertiles ensuring equal subject distribution. We used several measures to estimate risk and predictive value for school-age asthma. We applied logistic regression analysis with school-age asthma as binary outcome and pre-school-age log FeNO, FeNO tertiles, and the two API (three categories each) as explanatory variables. In multivariable logistic regression analysis, we adjusted for potential confounders of FeNO measurement at baseline: age at FeNO measurement, gender, use of ICS, allergic sensitization, and passive smoke exposure. Risk estimates were crude and adjusted odds ratio (OR) with 95% confidence intervals (95% CI). We plotted receiver-operating characteristics (ROC) curves for FeNO and determined positive and negative predictive values (PPV and NPV). Internal validation

was applied using (i) current asthma symptoms or prescribed asthma medication as alternative definitions of school-age asthma, and (ii) current atopy as alternative outcome. We further applied stratification according to asthma control medication and age to assess the association of FeNO and the new API with school-age asthma in these groups. *P*-values <0.05 were considered statistically significant, and all analyses were carried out using Stata™ (Stata Statistical Software: Release 11. StataCorp LP, College Station, TX, USA).

Results

Study population

At baseline, 391 children aged between 3 and 47 months were studied (Fig. 1, Table 2). At the time of the follow-up study, 213 children were eligible (older than 6 years). Of these, 47 children were lost to follow-up and 166 school-aged children were studied. FeNO and respiratory health status at baseline were comparable between children participating in the follow-up study and those who did not (Table 2). However, children lost to follow-up were on average younger and prevalence of allergic sensitization was lower compared to children studied both at baseline and at follow-up. From the 166 children studied at follow-up, 68 (41.0%) children had asthma. Of these, 34 were atopic and 36 were currently on ICS treatment (Table 3).

FeNO at preschool age and asthma at school age

FeNO measured at preschool age was elevated in those children with later asthma compared to children without later asthma (Fig. 2). Median (IQR) FeNO was 10.5 (6.0–17.2) ppb at baseline in children with later asthma, whereas in children without later asthma it was 6.2 (3.9–9.3) ppb, $P < 0.001$. This effect was influenced by ICS treatment at preschool age (Fig. 2). Compared to preschool children without later asthma, ICS-naïve preschool children with later asthma had even higher FeNO 12.5 [7.8–20.0] ppb at baseline,

Table 2 Characteristics of the cohort

	Baseline study <i>n</i> = 391	Follow-up study <i>n</i> = 166	Too young for follow-up* <i>n</i> = 178	Lost to follow-up <i>n</i> = 47	<i>P</i> -value
Sex (girls/boys)	154/237	73/93	63/115	19/28	0.43
Age (months)	22.5 [12–37]	36 [24–42]	14 [9–22.5]	20 [15–39]	<0.001
Allergic sensitization	79 (19.2%)	48 (28.9%)	27 (15.2%)	4 (8.5%)	0.003
ICS – use	121 (30.9%)	47 (28.3%)	56 (31.5%)	18 (38.3)	0.60
LTRA – use	18 (4.6%)	10 (6.0%)	4 (2.3%)	4 (8.5%)	0.12
Passive smoke exposure	120 (30.7%)	55 (33.1%)	52 (29.2%)	13 (27.7%)	0.70
Blood eosinophils (%)	2.8 [1.5–4.0]	2.8 [1.0–4.3]	2.7 [1.5–4.0]	2.7 [1.9–4.2]	0.90
FeNO (ppb)	7.3 [4.3–13.0]	7.2 [4.3–13.0]	7.3 [4.3–12.3]	8.5 [4.7–15.5]	0.92

ICS, Inhaled corticosteroids; LTRA, Leukotriene receptor antagonists; FeNO, fraction of exhaled nitric oxide.

Data are given as median and [interquartile ranges] or absolute numbers (%), respectively. Data were compared group by group using Mann–Whitney *U*-test and Chi-squared tests, respectively, and lowest *P*-values are displayed.

*Children younger than 6 years were not enrolled for school-age follow-up.

Table 3 Characteristics of the cohort assessed at baseline and follow-up

	Asthma <i>n</i> = 68	No asthma <i>n</i> = 98	<i>P</i> -value
Sex (girls/boys)	33/35	38/60	0.105
Age at follow-up (years)	7.5 (0.9)	7.5 (0.8)	0.688
Family history of asthma	35 (52.2%)	19 (19.4%)	<0.001
Passive smoke exposure			
Preschool age	23 (33.8%)	33 (33.7%)	0.876
School age	25 (36.8%)	34 (34.7%)	0.677
Atopy			
Preschool age	34 (50.0%)	13 (13.3%)	<0.001
School age	34 (50.0%)	26 (26.5%)	0.002
FeNO (ppb)			
Preschool age	10.5 [6.6–17.2]	7.4 [5.3–10.3]	<0.001
School age	6.9 [4.2–8.9]	6.3 [4.1–8.4]	0.213
Lung function at school age			
FEV ₁ %pred	101.8 (10.5)	97.5 (14.5)	0.125
MEF ₅₀ %pred	74.2 (22.5)	88.4 (17.8)	0.002
ICS treatment			
Preschool age	31 (45.6%)	20 (20.4%)	0.001
School age	38 (55.6%)	0 (0%)	<0.001

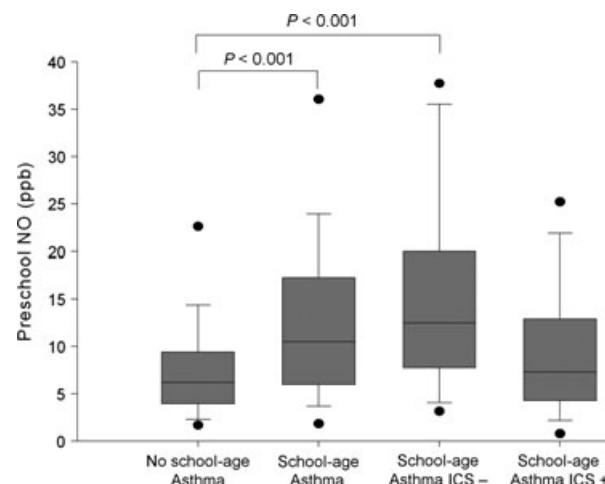
FEV₁, Forced expiratory volume in 1 s and MEF₅₀, Mean expiratory flow at 50% of expired volume are given as percent predicted (37). ICS, Inhaled corticosteroids.

Data are given as median [IQR], mean (standard deviation), and absolute numbers (%). Data were compared using Mann–Whitney *U*-test, unpaired *t*-tests, and Chi-squared test, respectively. At school-age follow-up, fraction of exhaled nitric oxide (FeNO) was measured using spirometry in 87 children (48 with asthma) within the nested cohort study. Atopy is defined as a physician-diagnosed atopic dermatitis and/or a physician-diagnosed rhinoconjunctivitis and/or allergic sensitization.

while ICS-treated preschool children with later asthma had similar FeNO (7.0 [3.6–9.6]) ppb.

Elevated FeNO at preschool age was associated with an increased risk for asthma at school age. Crude and adjusted OR (95% CI) were 2.27 (1.44–3.57) and 2.54 (1.50–4.33) per one log ppb FeNO increase, respectively. To report more intuitive FeNO categories than the log scale, we categorized children into FeNO tertiles. FeNO tertile ranges were (i) 0.5–5.2 ppb, (ii) 5.3–10.0 ppb, and (iii) >10.1 ppb with 55 to 56 children per group. Per one FeNO-category increase, the risk for asthma at school age tripled on average: Adjusted OR was 2.99 (1.78–5.05). Stratifying children into two age groups of 6–24 months (*n* = 43, 14 [33%] with later asthma) and 25–47 months (*n* = 123, 54 [44%] with later asthma), elevated preschool FeNO was associated with an increased risk to develop school-age asthma in both groups. Per FeNO-category increase, the adjusted OR was 4.39 (1.23–15.73) in younger children and 2.95 (1.61–5.41) in older children.

Individual FeNO measured at preschool age was a predictive estimate for asthma at school age. Mean area under the ROC curve (95% CI) was 0.69 (0.61–0.77) for diagnosing

**Figure 2** Comparison of exhaled nitric oxide in preschool children with and without later asthma. Box-and-whiskers plots of exhaled nitric oxide fractions in 166 preschool children, of whom 47 (28.3%) were atopic, 51 (30.7%) received inhaled corticosteroids (ICS), and 68 (41.0%) developed asthma at school age. Groups were compared by Mann–Whitney *U*-tests.**Table 4** Association of baseline nitric oxide with school-age outcomes

School-age outcomes (<i>n</i> / <i>N</i>)	Univariable Model		Adjusted Model	
	OR	95% CI	OR	95% CI
Physician-diagnosed asthma (68/166)				
log ppb	2.27	1.44–3.57*	2.54	1.50–4.33*
5 ppb	2.44	1.61–3.70*	2.99	1.78–5.05*
Current asthma symptoms (66/166)				
log ppb	2.38	1.50–3.77*	2.66	1.54–4.59*
5 ppb	2.58	1.69–3.94*	3.23	1.88–5.54*
Current asthma medication (46/166)				
log ppb	1.46	0.93–2.30§	1.40	0.82–2.41§
5 ppb	1.65	1.07–2.54†	1.73	1.00–3.00§
Current atopy (52/166)				
log ppb	0.89	0.59–1.34§	0.77	0.48–1.25§
5 ppb	1.06	0.71–1.58§	1.02	0.64–1.62§

Odds ratios (OR; 95% confidence intervals) for school-age outcomes per one log ppb or 5 ppb (tertile) increase in the fraction of exhaled nitric oxide (FeNO) were determined by logistic regression. FeNO tertiles (FeNO range, subjects) were (i) 0.5–5.2 ppb, *n* = 56, (ii) 5.3–10.0 ppb, *n* = 55, and (iii) >10.0 ppb, *n* = 55. The adjusted logistic regression model accounts OR for the following baseline (preschool) confounders: age, gender, allergic sensitization, inhaled corticosteroid treatment, and passive smoke exposure. *P*-values: * ≤ 0.001; †0.024, ‡0.049, § ≥ 0.050.

asthma at school age. Comparing the areas under the ROC in children being ICS naïve at baseline (*n* = 115, 37 with later asthma) and children on ICS treatment (*n* = 51, 31 with later asthma), the ROC areas were 0.76 (0.67–0.85) and 0.59 (0.44–0.75), *P* = 0.076. Preschool FeNO was not predictive for atopy in nonasthmatic children (Table 4).

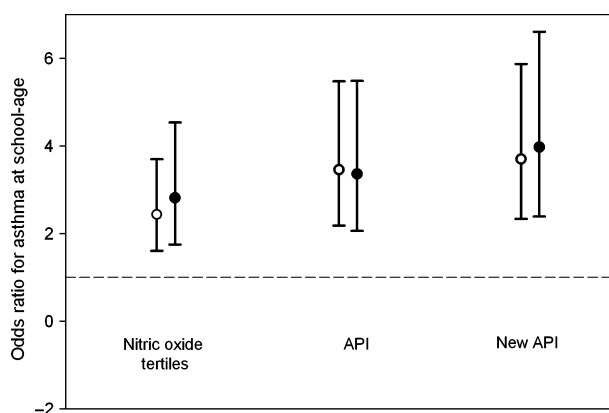


Figure 3 Odds ratios for school-age asthma determined by exhaled nitric oxide and clinical scores. The crude (open circles) and adjusted odds ratios (closed circles) with 95% confidence intervals are given in preschool children ($n = 166$, 68 with school-age asthma) for school-age asthma. The adjusted model accounts odds ratios for the following baseline (preschool) confounders: age, gender, allergic sensitization, inhaled corticosteroid treatment, and passive smoke exposure. Odds ratios rise per unit increase of (i) five ppb in fraction of exhaled nitric oxide (FeNO), (ii) the classical asthma predictive index (API) (5), and (iii) the new API based on the same clinical criteria but using FeNO as major criterion (Table 1). P -values for odds ratios were all <0.001 .

The new asthma predictive index including FeNO

The new API including FeNO and the classical API including blood eosinophilia (5) (Table 1) performed comparably in estimating the risk for later asthma. Comparing the risk for later asthma per category increase in the new API and the classical API, the OR (95% CI) was 3.70 (2.33–5.87) and 3.46 (2.18–5.48), respectively (Fig. 3). Preschool children within the 'nonrisk' category of both scores had a probability (NPV) of remaining free of asthma symptoms of 78% and 73%, respectively (Table 5). Children within the stringent index of both scores had a probability (PPV) of developing school-age asthma of 69% and 72%. PPV of children in either loose or stringent indices of both scores was 58% and 60%, respectively. In ICS-naïve children ($n = 115$), the new API performed better: The PPV was 68% and the NPV was 86%. After age stratification, both API performed comparably in younger children (6–24 months) as in the whole study population: The PPV derived from the classical and new API in young children was 59% and 60%, respectively. The NPV was 84% and 79%, respectively. In older children, prediction of school-age asthma was best: In children older than 2 years (25–47 months, $n = 123$), the PPV derived from the classical and new API in older children was 73% and 76%, respectively. The NPV was 92% and 93%, respectively. In children older than 3 years (37–47 months, $n = 79$), NPV from both scores was 100% and PPV was 72% and 76%, respectively.

Eighty-seven children participated in the nested cohort study; of them, 48 (55.2%) children had school-age asthma. Preschool log FeNO weakly correlated with log FeNO

Table 5 Performance of two asthma predictive indices

	Classical asthma predictive index	New asthma predictive index
Loose index [n/N (%)]	40/166 (24.1%)	50/166 (30.1%)
Stringent index [n/N (%)]	32/166 (19.3%)	38/166 (22.9%)
Crude odds ratio (95% CI)	3.46 (2.18–5.48)	3.70 (2.33–5.87)
Adjusted odds ratio (95% CI)†	3.36 (2.06–5.49)	3.98 (2.39–6.61)
Sensitivity	63.2%	75.0%
Specificity	70.4%	62.3%
Positive predictive value	59.7%	58.0%
Negative predictive value	73.4%	78.2%

Preschool children ($n = 166$, 68 with school-age asthma) classified by two asthma predictive indices taking the same clinical but different biomarkers into account.

†Crude and adjusted odds ratios (95% confidence intervals) for asthma at school age for each FeNO unit increase (5 ppb) are determined by logistic regression. The adjusted model accounts odds ratios for the following baseline (preschool) confounders: age, gender, allergic sensitization, inhaled corticosteroid treatment, and passive smoke exposure.

P -values for crude and adjusted odds ratios were <0.001 .

measured at school age, Pearson's $r = 0.22$, $P = 0.045$. Mean expiratory flow at 50% of expired volume (MEF₅₀), but not FeNO and forced expiratory volume in 1 s (FEV₁) measured at school age, distinguished between asthmatic and healthy children (Table 3).

Discussion

Summary

This is the first prospective study to assess the predictive value of FeNO measured in young children aged between 6 months and 4 years. Elevated FeNO in preschool children with a history of frequent wheeze is a risk factor for asthma at school age. Per 5 ppb increase in FeNO at baseline, the risk for later asthma triples. The combined information of clinical and FeNO data enables a new API. Probability for later asthma is 58% for preschool children identified at risk. Children not at risk had 78% probability of staying free of asthma. In ICS-naïve children or children older than 3 years, the new API was more clinically relevant (PPV 68–76%, NPV 86–100%). Compared to the classical API (5), this new API has similar diagnostic performance but includes the biomarker FeNO, which is noninvasive and easily measureable across all age groups.

The biomarker FeNO in young children

This study sheds light on the complex dynamics of asthma in early life. We have recently shown that preschool children with at stringent index for asthma have higher FeNO values than their peers despite similar phenotypes (10). The follow-up of this cohort now allows a definite analysis of the

predictive value of FeNO measurements in infants and preschool children. Per 5 ppb FeNO increase, the risk for asthma at school age triples on average. In other words, a preschool child with a FeNO value of 10.1 ppb or higher had a ninefold higher risk for school-age asthma as compared to a child with low FeNO between 0.5 and 5.2 ppb.

Previous cohort studies support our data suggesting that FeNO has predictive information on subsequent respiratory symptoms. Latzin et al. (12) have shown that FeNO in asymptomatic infants is positively correlated with subsequent respiratory symptoms in the first year of life. Chawes et al. (17) similarly showed that increased FeNO in asymptomatic infants was associated with recurrent wheeze within the first year of life. However, the predictive value of FeNO seemed restricted to 1 year. In the PIAMA cohort (8), FeNO was associated with risk for school-age asthma. However, sensitivity of FeNO seemed lower. FeNO categories of more than 40 ppb were applied to estimate asthma risk, children were older (4 years at baseline), and time spans between FeNO and asthma assessments were shorter. Many other studies have shown associations between FeNO and environmental exposures, recurrent wheezing, atopy, and airway inflammation and response to anti-inflammatory treatment (18–24).

While single FeNO measurements may be regarded as snapshot only, it is rather not only atopy but asthma smiling from the picture. In the multivariable model adjusting for allergic sensitization in preschool age, FeNO was a significant risk factor for school-age asthma in both children younger and older than 24 months. Elevated FeNO seems to be a sensitive biomarker in the early march of asthma development (25–27). Although airway inflammation and FeNO significantly fluctuate in asthma, they are high on average (28). To disentangle underlying mechanisms, one would need to measure, for example, FeNO and NO synthase enzyme expression over time (29, 30).

We measured FeNO according to the ATS/ERS recommendations (15). The tidal offline technique was feasible in the youngest children of our study. The rather loose association between preschool- and school-age FeNO ($r = 0.22$) could be explained by the fluctuating nature of FeNO (31, 32). Prior ICS use at baseline FeNO measurements seems to impact upon the association with school-age asthma. This may be due to the frequently observed decrease in FeNO following ICS therapy (9, 33). However, the frequency of prior ICS use was comparable between baseline and follow-up FeNO measurements (Table 2). Technical differences, for example, uncontrolled tidal flow during offline FeNO measurements vs controlled flow during online measurements, and loss of power within the nested cohort may have also weakened the association between preschool- and school-age FeNO. Given the rather large age range (6–47 months) studied, longitudinal FeNO measurements could theoretically better identify those children who do not 'convert' from low FeNO levels to higher FeNO levels. School-age FeNO marginally differed between asthmatic children and nonasthmatic children possibly due to population characteristics, for example, 26.5% of nonasthmatics were atopic and 55.6% of asth-

matics were on ICS, and the smaller sample size compared to baseline.

The new asthma predictive index

This new API using FeNO may prove useful in both clinical and epidemiological settings; especially in ICS-naïve children (PPV = 68%, NPV = 86%), in children older than 2 years (PPV = 76%, NPV = 93%) or older than 3 years (PPV = 76%, NPV = 100%), the new API seems clinically significant for predicting school-age asthma. In the whole population, the predictive values (PPV = 58%, NPV = 78%) of the new API were comparable to those of the classical API (PPV = 60%, NPV = 73%), but both API may be less clinically relevant.

FeNO measurement is standardized, samples are obtained noninvasively, the results are available within minutes, and measurements can be repeatedly applied although experienced teams are required for FeNO measurement in infants. The strength of FeNO measurement lies within the combination of clinical and historical data and not as a standalone biomarker. Normative FeNO studies using classical reference equations explained <20% of FeNO variability in large populations (34, 35). We thus added FeNO as major criterion into the context of clinical and historical variables from individuals as also recommended for FeNO-based trials (9, 33). Compared to the new API, the NPV of the classical API was higher (92–94%), but the PPV was lower (26–42%) in both the Tucson (5) and Leicester (6) cohorts. This may be explained by the higher sensitivity of the biomarker FeNO but also by different exposures, asthma prevalence, and age ranges in study populations.

Our study population seems representative for, for example, primary care as physicians are usually not confronted with the prediction of asthma in healthy children. However, the API including FeNO may be less specific in other populations, for example, in infants or children on ICS therapy. External validation is required prior to widespread clinical application. Internal validation revealed good prediction of alternative school-age asthma outcomes, for example, asthmatic symptoms, but not atopy alone.

The new API may help to specifically target anti-inflammatory treatment (33). It is expected that some children in the current study had previously undetected eosinophilic airway inflammation and may benefit from ICS. ICS interventions are well established for disease control although disease-modifying effects seem less likely (36).

Conclusions

The new API including FeNO identifies risk for later asthma comparably to the classical API (5), but does not require blood sampling. In ICS-naïve children or children older than 2 years, preschool FeNO measurement is clinically relevant for the prediction of school-age asthma. FeNO in preschool age may help to more reliably identify those children at risk for later asthma who would directly benefit from anti-inflammatory disease control.

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Author contributions

Florian Singer: Dr Singer carried out the analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted. Dr Singer and Dr Luchsinger equally contributed to the manuscript. Isabelle Luchsinger: Dr Luchsinger collected data, carried out the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted. Dr Singer and Dr Luchsinger equally contributed to the manuscript. Demet Inci: Dr Inci collected data,

drafted the initial manuscript, and approved the final manuscript as submitted. Nicola Knauer: Dr Knauer collected data, drafted the initial manuscript, and approved the final manuscript as submitted. Philipp Latzin: Dr Latzin carried out the analyses, drafted the initial manuscript, and approved the final manuscript as submitted. Johannes H. Wildhaber: Dr Wildhaber conceptualized the study, coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted. Dr Moeller and Dr Wildhaber equally contributed to the manuscript. Alexander Moeller: Dr Moeller designed the study, coordinated and supervised data collection, drafted the initial manuscript, and approved the final manuscript as submitted. Dr Moeller and Dr Wildhaber equally contributed to the manuscript.

Conflict of interest

Dr Singer, Dr Luchsinger, Dr Inci, Dr Knauer, Dr Latzin, Dr Wildhaber, and Dr Moeller have no competing interests to disclose.

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