

Reduced breath condensate pH in asymptomatic children with prior wheezing as a risk factor for asthma

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Background: Early noninvasive detection of increased risk of asthma with exhaled breath condensate (EBC) pH measurement has not been applied to preschool children.

Objective: We sought to evaluate the ability of EBC pH measurement to identify young asymptomatic children at risk of asthma using the combination of recurrent wheezing and atopic sensitization as a proxy for a high risk of asthma.

Methods: pH values were measured in deaerated EBC from 191 children (median age, 4.4 years [interquartile range, 2.2 years]). Children were divided into one of 5 groups: asymptomatic children with recurrent wheezy bronchitis with (group 1, n = 34) or without (group 2, n = 64) allergic sensitization, acute wheezy bronchitis (group 3, n = 18), allergic rhinoconjunctivitis without recurrent wheezy bronchitis (group 4, n = 15), and healthy control subjects (group 5, n = 60). The Asthma Predictive Index score was calculated for groups 1 and 2. Statistical significance was evaluated with the appropriate nonparametric tests, and the discriminatory accuracy was evaluated with receiver operating characteristic analysis.

Results: Deaerated EBC pH values were significantly lower in groups 1 and 3 than in groups 2, 4, and 5 (median, 7.49 [interquartile range, 0.94] and 7.44 [interquartile range, 0.70] vs 7.93 [interquartile range, 0.23], 8.02 [interquartile range, 0.17], and 7.96 [interquartile range, 0.25], respectively; $P < .001$ and area under the receiver operating characteristic curve ≥ 0.80 in all comparisons). The area under the curve for the differentiation between groups 1 and 2 improved from 0.80 to 0.94 (sensitivity, 0.94; specificity, 0.84; positive predictive value, 0.76) when breath condensate pH values and Asthma Predictive Index scores were combined.

Conclusion: A reduced deaerated EBC pH value might help identify young asymptomatic children at high risk of asthma. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Asthma, allergic sensitization, Asthma Predictive Index, early childhood asthma, exhaled breath condensate pH, recurrent wheezy bronchitis

Asthma is the leading chronic disease among children in most industrialized countries.¹ Approximately 80% of children with asthma have symptoms before the fifth birthday.² Wheezing preschool children at high risk of asthma might benefit from regular inhaled corticosteroid (ICS) therapy more than those at low risk.^{3,4} Therefore it is critical to classify wheezing preschool children according to their presumed risk for the progression to chronic childhood asthma.

Various studies that focused on this classification, namely the Tucson Children's Respiratory Study, the National Asthma Campaign Manchester Asthma and Allergy Study, and the Multicenter Allergy Study, identified atopic sensitization in early life and a family history of atopy or asthma as major risk factors for the subsequent development of asthma.⁵⁻⁷ Infants with an atopic family history have recurrent wheezing independently of respiratory syncytial virus prophylaxis according to the latest findings of the Palivizumab Long-Term Respiratory Outcomes Study.⁸ On the other hand, the Childhood Origins of Asthma cohort study and the Childhood Asthma Study suggested that the interaction between viral infections and atopy in infancy promotes later asthma.^{9,10} Wheezing in response to viral triggers but also to allergen exposure, tobacco smoke, mist, crying, laughing, or exercise has been classified as multiple-trigger wheezing.¹¹ According to the latest report of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology, persistence of wheezing during the preceding year might be the key differentiator of the asthma phenotype in preschool children.² Appropriately, the Asthma Predictive Index (API), based on the Tucson Children's Respiratory Study, includes the following criteria: wheezing persistence apart from colds, wheezing frequency, atopic eczema or rhinitis, blood eosinophilia, and parental asthma.¹²

Subclinical airway inflammation in the clinically asymptomatic state is typical of multiple-trigger wheezing, and objective measures, such as lung function or bronchoscopy, might help in its diagnosis.^{11,13,14} However, pulmonary function testing cannot be performed in all preschool children under routine conditions because of the need for cooperation with forced expiration maneuvers. Bronchoscopy is too invasive to be used widely in young children. Likewise, induced sputum analysis is not appropriate as a noninvasive measure of airway inflammation in preschool

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Abbreviations used

API:	Asthma Predictive Index
AR:	Allergic rhinitis
AUC:	Area under the curve
dEBC pH:	Deaerated exhaled breath condensate pH
EBC:	Exhaled breath condensate
FA:	Family asthma
FAT:	Family atopy
FENO:	Fraction of exhaled nitric oxide
ICS:	Inhaled corticosteroid
MEF ₂₅ :	Maximal expiratory flow at 25% of forced vital capacity
ROC:	Receiver operating characteristic
RWB:	Recurrent wheezy bronchitis

children.² Therefore the differentiation of children with transient nonallergic wheeze from those who will progress to persistent asthma remains a clinical dilemma.¹⁵

The analysis of exhaled biomarkers is a promising new technique for the noninvasive assessment of airway inflammation, particularly because it has been proven to be suitable in infants and preschool children even without sedation.¹⁶⁻¹⁸ In 2005, a task force of the American Thoracic Society and the European Respiratory Society published recommendations on the collection and measurement of exhaled biomarkers.¹⁹ Apart from fraction of exhaled nitric oxide (FENO), the pH of deaerated exhaled breath condensate pH (dEBC pH) was proposed as the most valid and reliable exhaled biomarker, producing reproducible results even in samples collected with different methods, such as EcoScreen and Rtube.^{20,21} Mean and median dEBC pH values in healthy adults are 7.83 and 8.0, respectively, and similar values have been measured in children.^{22,23} Importantly, dEBC pH values are not affected by age, sex, time of day collected, volume of exhaled breath condensate (EBC) collected, hyperventilation or hypoventilation, acute use of albuterol, oral ammonia, methacholine-induced airway obstruction, temperature or duration of sample storage, or choice of CO₂-free gas used for deaeration (oxygen vs argon).^{22,24} A reduced dEBC pH value (<7.4) has been found to reflect acidification of the epithelial lining fluid and has been reported in patients with acute lung injury, mechanically ventilated patients, and patients with chronic obstructive pulmonary disease, bronchiectasis, chronic cough, cystic fibrosis, and acute and asymptomatic asthma. Furthermore, it is related to eosinophilic and neutrophilic airway inflammation and to treatment modalities.^{16,19}

Consistent with the notion that a reduced dEBC pH value reflects some forms of airway pathology, we have previously shown that these values are reduced in recurrently wheezing preschool children, even in the clinically asymptomatic state.²⁵ However, the study design did not allow us to identify subgroups with different risk levels for the development of asthma by means of dEBC pH alone. We therefore hypothesized that additional risk stratification would be necessary to reveal any value of dEBC pH in the prediction of chronic asthma. Considering that long-term follow up data are not available in this cross-sectional study, the combination of atopic sensitization and recurrent wheezing was therefore used as a proxy for a high risk of a later diagnosis of chronic asthma. Hence the aims of the present study were (1) to evaluate the ability of dEBC pH measurement to differentiate among subgroups of recurrently wheezing children at differential

risk for asthma, as reflected by allergic sensitization, and (2) to test whether combining dEBC pH values with data from the patient's history and medication use might improve this discriminatory ability.

METHODS

Study population

The study was approved by the Ethics Committee of the University Medical Center of the Technical University Dresden, Germany. Written informed consent was obtained from all parents, and assent was obtained from the children. Between November 2007 and November 2008, 190 preschool children were recruited from the University Children's Hospital Dresden and 26 from a neighboring nursery school. Exclusion criteria were (1) a diagnosis of gastroesophageal reflux disease; (2) a specific respiratory disease, such as cystic fibrosis, primary ciliary dyskinesia, interstitial lung disease, pneumonia, or tuberculosis; and (3) recurrent fever of unknown origin, systemic prednisolone therapy during acute wheezy bronchitis, or inability to assess atopy status.

Procedures

The study participants were separated into 5 groups on the basis of history and clinical and laboratory findings (Table I): asymptomatic children with recurrent wheezy bronchitis (RWB; defined as ≥ 3 episodes of wheeze within the preceding 12 months) with (group 1) or without (group 2) allergic sensitization; children with acute wheezy bronchitis defined as cough, normal or slightly increased body temperature (<38°C), and wheezy lung sounds on auscultation lasting less than 3 weeks (group 3); children with allergic rhinitis (AR) diagnosed according to the Allergic Rhinitis and Its Impact on Asthma criteria²⁶ without a history of RWB (group 4); and healthy control subjects without parentally or clinically reported wheeze or asthma, symptoms of airway inflammation in the last 8 weeks, allergy, AR, or atopic eczema (group 5, consisting of 34 children recruited from the hospital and the 26 children from the neighboring nursery school).

Therapy with ICSs was documented, and the inhaled beclomethasone equivalent (in micrograms) was calculated. A detailed history of family atopic disease or asthma was documented and used to generate scores expressing the presumed genetic risks for the incidence of atopic disease (family atopy [FAT]) in 7-year-old children according to the method of Kjellman²⁷ and according to an asthma score (family asthma [FA]) developed by the authors (Table II). The API was calculated according to the method of Castro-Rodriguez et al.¹²

EBC was collected without sedation according to American Thoracic Society/European Respiratory Society recommendations by using a modified, commercially available breath condensate sampler (EcoScreen; Jaeger, Höchberg, Germany). The design of these collection devices prevents salivary contamination of the breath condensate.¹⁹ Children had not ingested solids or liquids for at least 1 hour, rinsed the mouth with water before EBC collection, and did not wear nose clips during the procedure. Seated on the legs of their parents and distracted by an animated film, children were asked to breathe through the EcoScreen's mouthpiece for 5 minutes and to temporarily discontinue collection if they needed to swallow saliva. The exhaled air was cooled to -20°C in the condensing chamber, where the condensate was then collected in an autoclaved polypropylene container. The samples were then thawed, transferred to sterile tubes (Eppendorf, Hamburg, Germany), and stored at -30°C until further analysis.

pH was measured after a median storage time of 2.5 (interquartile range, 15) days. pH was always measured with the same equipment (PHM240 Meter Lab; Radiometer Analytical, Lyon, France) with a pH-sensitive electrode (Orion 9103APWP AquaPro pH-Electrode; Thermo Scientific, Waltham, Mass). The pH meter was calibrated to pH 1.68, 4.01, 7.00, and 10.01 before each series of assays. A stable dEBC pH value was achieved after deaeration with argon (350 mL/min) for 10 to 12 minutes.

Atopic sensitization was defined as a RAST class of greater than 2, a positive skin prick test response to at least 1 common airborne allergen, or both. Skin prick tests (Allergopharma, Reinbek, Germany) were performed

TABLE I. Demographic and laboratory characteristics of the subject groups*

	Group 1	Group 2	Group 3	Group 4	Group 5	
	Asymptomatic RWB		Acute wheezy bronchitis	AR	Healthy	P value
Characteristic	Atopic	Nonatopic				
No.	34	64	18	15	60	
Male sex (%)	68	62	56	53	52	.561‡
Age (mo)	64.5 (15)	41.5 (27)	50 (21.5)	68 (19.5)	52.5 (26)	<.001†
EBC volume (mL)	0.51 (0.37)	0.38 (0.23)	0.40 (0.24)	0.51 (0.22)	0.39 (0.15)	.020†
History of AR (%)	68	0	33	100	0	<.001‡
History of atopic eczema (%)	56	0	22	60	0	.001‡
Family history of asthma (FA ≥3)¶	38	22	22	27	3	<.001†
API score, positive (%)	97	22	ND	ND	ND	<.001§
Eosinophils (%)	6.7 (6.0)	3.0 (3.0)	3.8 (4.0)	7.0 (5.5)	Not tested	.001†
Total IgE (kU/L)	289.0 (534.0)	25.0 (42.7)	116.0 (430.0)	282.0 (331.0)	Not tested	<.001†
Serum ECP (μg/L)	50.5 (35.0)	30.0 (26.8)	33.4 (30.0)	58.0 (41.9)	Not tested	<.001†
FEV ₁ (% predicted); no.	105.3 (22.0); 22	101.6 (14.3); 23	98.3 (18.1); 6	112.6 (24.3); 8	Not tested	.122†
MEF ₂₅ (% predicted); no.	92.3 (42); 22	89.3 (15.6); 23	58.1 (16.8); 6	110.3 (33.4); 8	Not tested	.011†
FENO (ppb); no.	13.4 (15.6); 21	5.4 (4.0); 18	7.7 (9.8); 4	12.4 (11.3); 7	Not tested	.001†
ICS treatment (%)	16 (47)	28 (44)	6 (33)	3 (20)	0 (0)	.75‡
≤200 μg	12 (35)	21 (33)	6 (33)	3 (20)	0 (0)	.85§
>200 μg	4 (12)	7 (11)	0 (0)	0 (0)	0 (0)	1.0§
dEBC pH	7.49 (0.94)	7.93 (0.23)	7.44 (0.70)	8.02 (0.17)	7.96 (0.25)	<.001†

ECP, Eosinophil cationic protein; ND, not determined.

*Results are expressed as medians (interquartile ranges) unless stated otherwise.

†Kruskal-Wallis analysis.

‡ χ^2 Test (P value for trend of dose = .27), groups 1 and 2 only.

§Fisher exact test.

||Inhaled beclomethasone equivalent per day.

¶According to Table II.

against *Penicillium* species, *Cladosporium* species, *Aspergillus* species, *Mucor* species, *Alternaria* species, mugwort, ribwort, grasses, alder, hazelnut, birch, rye, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*; sensitization was defined as a wheal at least 3 mm greater in diameter than that elicited by the negative control. Plasma total and specific IgE concentrations to common airborne and food allergens (cow's milk protein, egg protein, *D pteronyssinus*, *D farinae*, grass, rye, birch, *Alternaria* species, cat, and dog) were assessed by means of RAST, and total IgE and eosinophil cationic protein levels were assessed by means of fluorescence immunoassay (all with UniCAP 205; Phadia, Freiburg, Germany). The time interval between RAST and EBC collection was kept at a median of 2 (interquartile range, 8) months.

Peripheral blood eosinophils were quantified in a venous blood sample with an automated blood cell counter (XE 2100; Sysmex, Norderstedt, Germany) and expressed as the percentage of the total leukocyte count. Blood samples were not obtained from the healthy children (group 5) for ethical reasons.

FEV₁ and maximal expiratory flow at 25% of forced vital capacity (MEF₂₅) values were measured in children 5 years of age and older with the pneumotach function of a body plethysmograph (MasterLab, Jaeger). Parameters were expressed as a percentage of predicted value. FENO was measured in participants 5 years of age or older with a chemiluminescence analyzer (CLD 88 sp; Eco Medics, Duernten, Switzerland), according to the American Thoracic Society/European Respiratory Society²⁸ criteria, and expressed in parts per billion.

Statistical analysis

SPSS for Windows Version 15.0 (SPSS, Inc, Chicago, Ill) and SAS for Windows Version 9.1 (SAS Institute, Inc, Cary, NC) were used for statistical analyses. The data were not normally distributed and were thus expressed as medians and interquartile ranges. Differences among the groups were tested with the Kruskal-Wallis test, followed, if appropriate, by the Mann-Whitney U test for between-group comparisons. The χ^2 test was used to evaluate the differences in categorical variables between groups. Spearman rank correlation was used to assess correlations between metric data and dEBC pH values. Significance was defined as a P value of less than .05. The ability of dEBC pH,

FAT, FA, ICS use, API score, and their combinations to discriminate between any 2 groups was quantified with receiver operating characteristic (ROC)^{29,30} and multiple logistic regression analysis.

RESULTS

Of 216 children screened, 7 could not produce sufficient EBC volume for pH analysis, and 18 failed the inclusion criteria. Demographic, clinical, and atopic characteristics and dEBC pH values of the remaining 191 study subjects (median age, 4.4 years [interquartile range, 2.2 years]) are shown in Table I and Fig 1. Children from group 1 were older than those from groups 2 and 5 ($P < .001$ and $P = .035$), whereas children from group 2 were younger than those from group 4 ($P < .001$). The median EBC volume of all children was 0.41 mL (interquartile range, 0.22 mL) and correlated positively with the age of the subjects ($r = 0.38$, $P < .001$). There was no significant correlation between dEBC pH values and sex ($P = .12$), age ($P = .11$), or sample volume ($P = .096$).

Pulmonary function tests were performed successfully in 45% and FENO measurement in 38% of the study participants. Groups 1 and 2 did not differ significantly in median ICS dose or the percentage of ICS-treated patients, not even when the patients were stratified according to ICS dose (>200 μg/day vs <200 μg/d). Deaerated EBC pH values did not correlate significantly with FEV₁ ($r = 0.11$, $P = .41$), MEF₂₅ ($r = 0.20$, $P = .10$), or inhaled beclomethasone equivalent ($r = 0.029$, $P = .74$) values. Children from group 3 with acidic dEBC pH had significantly lower MEF₂₅ values than children from group 2 with less acidic dEBC pH ($P = .001$), suggesting a simultaneous occurrence of airway acidification and asthmatic obstruction of the small airways during

TABLE II. Genetic risk scores

FAT*	Percentage	FA†	Score
No family member with any kind of atopic disease	10	No family member with asthma	0
One parent with any kind of atopic disease	20	One grandparent or 1 uncle or aunt with asthma	1
One brother or sister with any kind of atopic disease	35	Two grandparents with asthma (maternal and paternal)	2
One brother or sister and 1 parent with any kind of atopic disease	35	One parent with asthma	3
Two parents with any kind of atopic disease	42	Two parents with asthma	4
Two parents with identical manifestation of atopic disease	75	One or 2 siblings with asthma	5

*Percent prevalence of atopic disease in 7-year-old children according to Kjellman.²⁷

†Risk score generated by the authors.

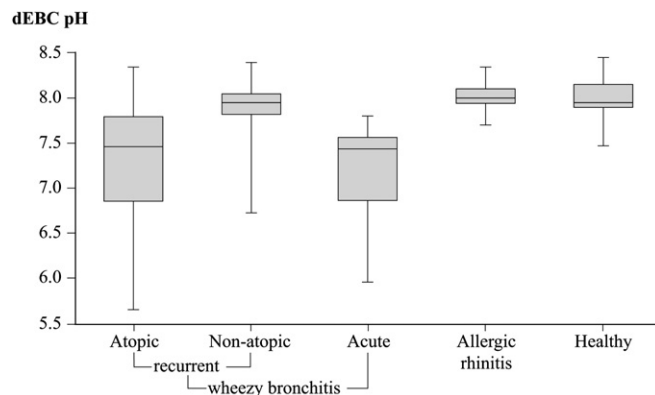


FIG 1. dEBC pH values in the 4 patient groups and healthy control subjects. dEBC pH values were significantly lower in the atopic recurrent (group 1) and acute wheezy bronchitis (group 3) groups compared with those seen in the nonatopic RWB (group 2), AR (group 4), and healthy control (group 5) groups. $P < .001$ for each of the 6 comparisons.

acute wheezy bronchitis. There was a marginally significant negative correlation between dEBC pH and FENO values ($r = -0.27$, $P = .062$).

Between-group differences in dEBC pH values

There were no significant differences in dEBC pH values between groups 1 and 3 ($P = .34$), 2 and 4 ($P = .13$), 2 and 5 ($P = .056$), or 4 and 5 ($P = .73$, Fig 1). In contrast, dEBC pH values were significantly lower in groups 1 and 3 than in groups 2, 4, or 5 ($P < .001$ for each of the 6 comparisons), even when the analysis was restricted to age 2 to 4 years (group 1 vs 2, $P = .001$; group 1 vs 4, $P = .046$; group 1 vs 5, $P = .0002$). These findings confirmed previous reports that dEBC pH values are reduced in patients with acute wheezy bronchitis (compare groups 3 and 5).²⁵ Moreover, dEBC pH values were normal in the children with AR without a history of wheezing (compare groups 4 and 5), demonstrating that atopic disease alone did not lead to reduced dEBC pH values. Most importantly, these data suggested that a reduced pH might be useful in the diagnostic differentiation between atopic and nonatopic recurrent asymptomatic wheezers (compare groups 1 and 2).

The potential discriminatory ability of dEBC pH, API score, FAT, FA, and ICS use was therefore quantified with ROC analysis (results for the discrimination between groups 1 and 2 are shown in Table III). Consistent with the results shown in Fig 1, the areas under the curve (AUCs) for dEBC pH values in the differentiation between groups 1 and 3 and between group 2 and groups 4 or 5 were nondiscriminatory, but it differentiated accurately between group 1 and groups 4 or 5 (AUC, 0.86 and 0.85, respectively).

Remarkably, dEBC pH values also discriminated well between groups 1 and 2, although the discriminatory ability of the API score was even better (Table III, rows 1 and 2). In a logistic regression model combining dEBC pH values with API scores improved the ability to differentiate between groups 1 and 2 further compared with either test alone. The subsequent addition of FAT, FA, and ICS use resulted in further stepwise improvement, yielding a final AUC of 0.98 (Table III, row 9).

DISCUSSION

Diagnostic value of dEBC pH

We evaluated the ability of dEBC pH measurement to identify asymptomatic children with a history of wheezing who are at increased risk of chronic asthma. To our best knowledge, our findings constitute the first report that dEBC pH values are significantly reduced in asymptomatic atopic children with a history of recurrent wheezing, and thus their measurement might potentially serve as a noninvasive, cost-effective prognostic test that can be performed during an asymptomatic interval. The discriminatory power of dEBC pH measurement was quantified with ROC analysis, an objective measure for evaluating the discriminatory power of clinical tests,²⁹ biomarkers of disease,^{31,32} and disease scores.³³ The AUC of 0.80 for the discrimination between atopic and nonatopic recurrent wheezers corresponds to “excellent discrimination” in a commonly accepted ROC nomenclature.³⁰ Notably, the addition of a simple, well-validated risk score, the API score, improved the discriminatory ability of dEBC pH values further, with the resulting AUC of 0.94 (“outstanding discrimination”³⁰) falling within the range of AUCs of many tests used in clinical practice. dEBC pH values differentiated not only between atopic and nonatopic preschool wheezers but also between those at high or low genetic risk for asthma and atopy according to family history (Table II). Moreover, they discriminated accurately between children with or without a positive API score and correlated inversely with FENO, a well-validated marker for allergic airway obstruction. Taken together, the present study extends previous results demonstrating that dEBC pH measurement can classify recurrently wheezing preschool children into those at high (“atopic”) or low (“nonatopic”) risk for asthma. Future studies should aim to identify additional biomarkers or elements of the patient’s history that can improve the discriminatory ability of dEBC pH values and API scores further. For instance, FENO levels were significantly higher in group 1 than in group 2 and represent an easily measured noninvasive marker that promises to refine the risk stratification of children at risk of asthma in conjunction with dEBC pH values and API scores. However, values were only available for subsets of participants, and FENO levels were

TABLE III. Ability of the 5 variables to differentiate between atopic and nonatopic children with a history of recurrent wheezing (groups 1 and 2)

	AUC	95% CI	P value	Sensitivity*	Specificity*	PPV*	Accuracy†
pH‡	0.80	0.69-0.90	1.5×10^{-6}	0.79	0.75	0.63	0.77
API score‡	0.88	0.81-0.95	1.0×10^{-9}	0.97	0.78	0.70	0.88
FAT‡	0.66	0.55-0.77	.008	0.94	0.30	0.42	0.62
FA‡	0.60	0.48-0.72	.12	0.38	0.78	0.48	0.58
ICS use‡	0.51	0.39-0.63	.86	0.41	0.73	0.45	0.57
pH + API score§	0.94	0.90-0.98	7.6×10^{-13}	0.94	0.84	0.76	0.89
pH + API score + FAT§	0.95	0.92-0.99	1.7×10^{-13}	0.88	0.91	0.83	0.90
pH + API score + FAT + FA§	0.97	0.94-1.00	4.3×10^{-14}	1.00	0.83	0.76	0.92
pH + API score + FA + FAT + ICS use§	0.98	0.96-1.00	7.9×10^{-15}	0.97	0.92	0.87	0.95

PPV, Positive predictive value.

*Values obtained with ROC analysis.

†Values obtained with multiple logistic regression analysis.

‡At the tradeoff value: the value of the ROC curve coordinate yielding the maximal sum of sensitivity plus specificity (tradeoff value for pH = 7.83).

§Sensitivity[fraction] + Specificity[fraction]/2 at the tradeoff value.

therefore not investigated further in the present study. Future studies are needed to define their role as an adjunct parameter in combination with dEBC pH values and API scores.

dEBC pH values in patients with AR and those with acute wheezing

In the present study dEBC pH values were normal in children with AR without a history of RWB. In agreement with this finding, Profita et al³⁴ reported normal oral but decreased nasal dEBC pH values in children. Together, these results support the view that the dEBC pH value is more likely a marker of inflammatory processes in general and not of atopic processes within the airways *per se* such as FENO.³⁵ In contrast, Brunetti et al³⁶ reported decreased oral dEBC pH values in children with atopic dermatitis and AR without clinical signs of airway inflammation. They hypothesized therefore that dEBC pH values might be an early predictor of the progression of the atopic march toward the development of asthma. However, a history of RWB was not excluded explicitly in that study, and it is therefore possible that the children were clinically asymptomatic at the time of EBC collection but actually might have wheezed in the past. Further studies are needed for a final assessment.

We have previously shown that dEBC pH values are reduced in patients with acute wheezing.²⁵ The reduced values in the present new cohort of 18 acute wheezers (group 3) validate these results and underscore the robustness of our approach to dEBC pH measurement.

Possible mechanisms of reduced dEBC pH values

The decreased dEBC pH values in asymptomatic atopic children with RWB at high risk for asthma (group 1) were found to be comparable with published values from 10-year-old children with active moderate asthma.^{34,37} dEBC pH values might reflect endogenous airway acidification resulting from altered pH homeostasis in infectious and inflammatory disease processes. Mechanisms capable of acidifying the airways are then activated, whereas mechanisms capable of neutralizing the airways appear to be turned off.³⁸ On the other hand, dEBC pH values might also reflect exogenous airway acidification, such as by means of inhalation of fog or gaseous pollutants or microaspiration of gastric acids. Airway acidification impairs ciliary motility and causes destruction of bronchial epithelium. It evokes cough,

bronchoconstriction, airway hyperreactivity, microvascular leakage, and heightened production of mucus, fluid, and nitric oxide.³⁷ In children who wheeze in the absence of common colds but in response to multiple triggers, it is plausible to hypothesize a constant subclinical eosinophilic and neutrophilic inflammation of the airways.^{2,11} Bronchoscopic findings and the measurement of FENO levels have confirmed this hypothesis in preschool children.^{14,17}

Influence of other variables on dEBC pH values

Most studies show that dEBC pH values tend to normalize after anti-inflammatory treatment, even in children.^{39,40} Unexpectedly, dEBC pH values did not correlate with ICS treatment in our study, and dEBC pH values were not higher in ICS-treated than in ICS-naïve children. However, we acknowledge that the design of the present study did not allow us to control for important aspects, such as compliance with inhalation therapy or therapy effectiveness, or to assess whether the prescribed doses were adequate.^{41,42} Although the overall correlation between dEBC pH and FENO values was not significant, group 1 did have significantly higher FENO levels than group 2, which agreed well with the lower dEBC pH values in group 1. This is consistent with the insight that, at low pH, nitrite is protonated and forms nitric oxide.⁴³ This finding also lends further support to the notion that low dEBC pH values are pathogenetically relevant.

Potential limitations

First, microaspiration of gastric contents should be considered a potential cause of airways acidification, particularly in infants and patients with obstructive lung disease.³⁷ Because of ethical reasons, gastroesophageal reflux was excluded by means of parental report of symptoms and not by means of diagnostic testing.

Second, the children in group 1 were older than those in group 2, likely because atopic children with RWB are more likely to be persistent or late-onset wheezers, who are usually older than transient wheezers.^{2,15} However, we do not believe that this age difference affected the measured dEBC pH values significantly because it is known that age does not influence the dEBC pH value appreciably,²² and the dEBC pH differences between groups 1 and 2 remained significant when the analysis was limited to patients 2 to 4 years of age.

Third, treatment duration was not assessed in the children receiving ICSs, which makes it difficult to judge its influence on

dEBC pH values. Finally, the results of the present study will have to be compared with the diagnostic gold standard (ie, the formal diagnosis of asthma according to established criteria when the children have reached school age) to determine the reliability of dEBC pH measurement in predicting the development of chronic asthma. For this purpose, we are planning to reevaluate all study participants after the sixth birthday. At that time, we will also be able to evaluate whether combining the data for dEBC pH values and API scores with those for allergic sensitization (all from the present study) will improve the predictive value further.

In summary, the results of the present study suggest that dEBC pH measurement, which has been proposed as a simple, noninvasive, and reproducible measure of airway inflammation,²⁰ might serve as an additional marker for the early recognition of childhood asthma, particularly when combined with additional information, such as the API score.

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Clinical implications: Our results suggest that a low dEBC pH value might serve as an additional parameter for the detection of preschool children at high risk of asthma.

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