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Exhaled breath condensate pH for the identification of asymptomatic children at risk for asthma

Marie von Jagwitz¹, Frank Pessler, MD, PhD^{1,2}, Manas Akmatov, DrPH²,
Jialiang Li, PhD³, Ursula Range⁴, and Christian Vogelberg, MD, PhD¹.

¹Division of Pediatric Pulmonology and Allergology, University Children's Hospital, Dresden, Germany,

²Department of Infection Genetics, Helmholtz Center for Infection Research,

Braunschweig, Germany, ³Department of Statistics and Applied Probability, National University of

Singapore, and ⁴Department of Medical Computer Science and Biometry, University Hospital, Dresden.

Reprint requests:

Christian Vogelberg, MD, PhD,

Division of Pediatric Pulmonology and Allergology,

University Children's Hospital,

Fetscherstraße 74, 01307 Dresden, Germany.

Tel.: +49 (0)351 458-5699

Fax: +49 (0)351 458-4334.

E-mail: christian.vogelberg@uniklinikum-dresden.de

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Abstract

Background: Early noninvasive detection of increased risk of asthma by means of exhaled breath condensate pH has not been applied to preschool children.

Objective: To evaluate the ability of exhaled breath condensate pH to identify young asymptomatic children at risk for asthma.

Methods: pH was measured in deaerated exhaled breath condensate from 191 children (median age [interquartile range]: 4.4 years [2.2]). Children were divided into 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 but no recurrent wheezy bronchitis (group 4; n=15), and healthy controls (group 5; n=60). The asthma predictive index was calculated in children with recurrent wheezy bronchitis. Statistical significance was evaluated with the appropriate nonparametric tests and the discriminatory accuracy with binary and multcategory receiver operating characteristic (ROC) analysis.

Results: Deaerated exhaled breath condensate pH was significantly lower in groups 1 and 3 than in groups 2, 4 and 5 (median [interquartile range]: 7.49 [0.94] and 7.44 [0.70] versus 7.93 [0.23], 8.02 [0.17] and 7.96 [0.25]; $P < .001$ and $AUC \geq 0.80$ in all comparisons). The differentiation between groups 1 and 2 improved further when exhaled breath condensate pH was combined with the asthma predictive index (AUC, 0.89). Multcategory ROC analysis identified exhaled breath condensate pH as the test with the best overall discriminatory ability.

Conclusion: A reduced deaerated exhaled breath condensate pH might help identify young asymptomatic children at high risk for asthma.

Clinical Implications: Our results suggest that a low pH in deaerated exhaled breath condensate may serve as an additional parameter for the detection of preschool children at high risk for asthma.

49 **Capsule summary:** The differentiation of children with transient recurrent wheeze from those who will
50 progress to persistent asthma is a clinical challenge. A reduced deaerated exhaled breath condensate pH
51 might help the clinician identify future asthmatics.

52

53 **Key words:** *asthma, allergic sensitization, asthma predictive index, early childhood asthma, exhaled*
54 *breath condensate pH, recurrent wheezy bronchitis.*

55

56 **Abbreviations used**

57	API	Asthma Predictive Index
58	AR	Allergic rhinitis
59	AUC	Area under the curve
60	dEBC pH	Deaerated exhaled breath condensate pH
61	EBC	Exhaled breath condensate
62	FA	Family Asthma
63	FAT	Family Atopy
64	FeNO	Fractional exhaled nitric oxide
65	HUM	Hypervolume under the manifold
66	ICS	Inhaled corticosteroid
67	RAST	Radioallergosorbent test
68	ROC	Receiver operating characteristic
69	RWB	Recurrent wheezy bronchitis

Introduction

Asthma is the leading chronic disease among children in most industrialized countries¹. Approximately 80% of children with asthma develop symptoms prior to the fifth birthday². Wheezing preschool children at high risk for asthma may benefit from regular inhaled corticosteroid (ICS) therapy more than those at low risk^{3,4}. Therefore, it is of utmost importance 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 (NACMAAS), and the Multicenter Allergy Study (MAS), identified atopic sensitization in early life and a family history of atopy or asthma as major risk factors for the subsequent development of asthma⁵⁻⁷. Infants with an atopic family history develop recurrent wheezing independently of respiratory syncytial virus prophylaxis according to the latest findings of the Palivizumab Long-Term Respiratory Outcomes Study⁸. On the other hand, the Childhood Origins of ASThma (COAST) cohort study and the Childhood Asthma Study (CAS) suggested that the interaction between viral infections and atopy in infancy promotes asthma later on^{9,10}. Wheezing in response to viral triggers, but also to allergen exposure, tobacco smoke, mist, crying, laughter or exercise has been classified as multiple-trigger wheezing¹¹. According to the latest report of the American Academy of Allergy, Asthma, and Immunology and the European Academy of Allergy and Clinical Immunology, persistence of wheezing during the preceding year may be the key differentiator of the asthma phenotype in preschool children². 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 asthma¹². Sub-clinical airway inflammation in the clinically asymptomatic state is typical of multiple-trigger wheezing, and objective measures such as lung function or bronchoscopy may help to diagnose it^{11,13,14}. However, pulmonary function testing cannot be performed in all preschool children under routine

conditions due to 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 children². Therefore, the differentiation of children with transient nonallergic wheeze from those who will progress to persistent asthma remains a clinical dilemma¹⁵.

The analysis of exhaled biomarkers is a promising new technique for the noninvasive assessment of airway inflammation, particularly since it has been proven to be suitable in infants and preschool children even without sedation¹⁶⁻¹⁸. In 2005, a task force of the American Thoracic Society and the European Respiratory Society published recommendations on the collection and measurement of exhaled biomarkers¹⁹. Apart from FeNO, the pH of deaerated exhaled breath condensate (dEBC pH) was proposed as the most valid and reliable exhaled biomarker, giving reproducible results even in samples collected with different methods, such as EcoScreen and Rtube^{20, 21}. Mean and median dEBC pH in healthy adults are 7.83 and 8.0, respectively, and similar values have been measured in children^{22, 23}. Importantly, dEBC pH is not affected by age, sex, time of day collected, volume of EBC collected, hyper- or hypoventilation of the subject, acute use of albuterol by the patient, oral ammonia, or methacholine-induced airway obstruction, temperature and duration of sample storage, or choice of CO₂-free gas used for deaeration (oxygen vs. argon)^{22,24}. A reduced dEBC pH (<7.4) has been found to reflect acidification of the epithelial lining fluid and has been reported in acute lung injury, mechanically ventilated patients, COPD, bronchiectasis, chronic cough, cystic fibrosis, and acute and asymptomatic asthma. Furthermore, it is related to eosinophilic and neutrophilic airway inflammation and to treatment modalities (reviewed in^{16, 19}).

Consistent with the notion that a reduced dEBC pH reflects some forms of airway pathology, we have previously shown that it is reduced in recurrently wheezing preschool children, even in the clinically asymptomatic state²⁵. However, the study design did not allow to identify subgroups with different risk levels for the development of asthma by means of dEBC pH alone. We therefore hypothesized that

120 additional risk stratification would be necessary in order to reveal any value of dEBC pH in the prediction
121 of chronic asthma. Hence, the aims of the present study were (1) to evaluate the ability of dEBC pH to
122 differentiate among subgroups of recurrently wheezing children with different risk levels for the
123 development of asthma and (2) to test whether combining dEBC pH with data from the patients' history
124 and medication usage might improve this discriminatory ability.

125 **Methods**

126 **Study population**

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

135 **Procedures**

136 The study participants were separated into 5 groups on the basis of history and clinical and laboratory
 137 findings (Table I): asymptomatic children with recurrent wheezy bronchitis (RWB) with (group 1) or
 138 without (group 2) allergic sensitization; acute wheezy bronchitis defined as cough, normal or slightly
 139 elevated body temperature ($<38^{\circ}\text{C}$), and wheezy lung sounds on auscultation, lasting <3 weeks (group 3);
 140 allergic rhinitis diagnosed according to ARIA criteria²⁶ (AR) without a history of RWB (group 4); healthy
 141 controls excluding children with parentally or clinically reported wheeze or asthma, symptoms of airway
 142 inflammation in the last eight weeks, allergy, AR or atopic eczema (group 5). Therapy with ICS was
 143 documented, and the inhaled beclomethasone equivalent (in μg) was calculated. A detailed history of
 144 family atopic disease or asthma was documented and used to generate scores expressing the presumed
 145 genetic risks for the incidence of atopic disease (FAT) in 7-year-old children according to Kjellmann²⁷
 146 and according to an asthma score (FA) developed by the authors (Table II). The API was calculated
 147 according to Castro-Rodriguez et al.¹²
 148 EBC was collected without sedation according to American Thoracic Society/European Respiratory
 149 Society recommendations 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 (see review¹⁹). 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 min and to temporarily discontinue collection if they needed to swallow saliva. The exhaled air was cooled to -20°C inside of 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 (interquartile range) of 2.5 (15) days. pH was always measured using the same equipment (PHM240 Meter Lab, Radiometer Analytical, Lyon, France) with a pH sensitive electrode (Orion 9103APWP AquaPro pH-Electrode, Thermo Scientific, USA). The pH meter was calibrated to pH 1.68, 4.01, 7.00 and 10.01 prior to each series of assays. A stable dEBC pH was achieved after deaeration with argon (350 ml/min) for 10-12 min. Atopic sensitization was defined as a radioallergosorbent test (RAST) class >2 and/or a positive skin prick test (SPT) for at least one common airborne allergen. SPT (Allergopharma, Reinbek, Germany) were performed against penicillium, cladosporium, aspergillus, mucor, alternaria, mugwort, ribwort, grasses, alder, hazelnut, birch, rye, Dermatophagoides pteronyssinus and farinae; sensitization was defined as a wheal at least 3 mm greater in diameter than the negative control. Plasma total and specific IgE concentrations to common airborne and food allergens (cow milk protein, egg protein, Dermatophagoides pteronyssinus and farinae, grass, rye, birch, alternaria, cat, dog) were assessed by RAST, and total IgE and eosinophil cationic protein (ECP) with fluorescence immuno assay (all with UniCAP 205, Phadia, Freiburg, Germany). The time interval between RAST and EBC collection was kept at a median (interquartile range) of two (eight) 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 healthy children for ethical reasons.

FEV₁ and MEF₂₅ were measured in children ≥ 5 yrs of age with body plethysmography (MasterLab, JAEGER, Höchberg, Germany). Parameters were expressed as % of predicted value. FeNO was measured in participants ≥ 5 yrs of age using a chemiluminescence analyzer (CLD 88 sp, Eco Medics, Duernten, Switzerland) according to the American Thoracic Society/European Respiratory Society²⁸ criteria and expressed as parts per billion (ppb).

Statistical analysis

The data were not normally distributed and were thus expressed as median and interquartile range. 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 Chi-square test was used to evaluate the distribution of categorical variables (number of occurrences) in the five groups. Spearman's rank correlation coefficients were used to assess the degree of association between metric data and dEBC pH. Significance was defined as $P < .05$. The abilities of dEBC pH, FAT, FA, ICS and the API and of their combinations to discriminate between any two subgroups were quantified with binary receiver operating characteristic (ROC) curve and logistic regression analysis, using SPSS for Windows (SPSS Inc., Version 11.5; Illinois, USA). Multicategory ROC analysis is a recently developed method to measure the performance of a test in the simultaneous discrimination among several diagnoses^{29, 30}. Using code written in the R software environment for biostatistical computing (<http://www.r-project.org/>), we used it to rank dEBC pH, FAT, FA and ICS according to their overall abilities to differentiate among the five sample groups.

195 Results

196 Of 216 children screened, seven could not produce sufficient EBC volume for pH analysis, and 18 failed
 197 the inclusion criteria. Demographic, clinical, and atopic characteristics and dEBC pH results of the
 198 remaining 191 study subjects with a median age (interquartile range) of 4.4 years (2.2) are shown in Table
 199 I and Fig 1. Children from group 1 were older than groups 2 and 5 ($P < .001$ and $P = .035$), whereas
 200 group 2 was younger than group 4 ($P < .001$). The median EBC volume (interquartile range) of all
 201 children was 0.41 ml (0.22) and correlated positively with the age of the subjects ($r = 0.38$, $P < .001$).
 202 There was no significant correlation between dEBC pH and sex ($P = .12$), age ($P = .11$) or sample volume
 203 ($P = .096$). Pulmonary function tests were performed successfully in 45% and FeNO measurement in
 204 38% of the study participants. Deaerated EBC pH did not correlate significantly with FEV₁ ($r = 0.11$, $P =$
 205 $.41$), MEF₂₅ ($r = 0.20$, $P = .010$) or inhaled beclomethasone equivalent ($r = 0.029$, $P = .74$). There was a
 206 marginally significant negative correlation with FENO ($r = -0.27$, $P = .062$).

207 ***Between group differences in dEBC pH.*** There were no significant differences in dEBC pH between
 208 groups 1 and 3 ($P = .34$), groups 2 and 4 ($P = .13$), 2 and 5 ($P = .056$), or 4 and 5 ($P = .73$) (Fig. 1).
 209 Notably, dEBC pH was significantly lower in groups 1 and 3 than in groups 2, 4 or 5 ($P < .001$ for each
 210 of the 6 comparisons). These findings confirmed previous reports that dEBC pH is reduced in acute
 211 wheezy bronchitis²⁵ (compare groups 3 and 5). Moreover, oral dEBC pH was normal in these children
 212 with AR without a history of wheezing (compare groups 4 and 5), demonstrating that atopic disease alone
 213 did not lead to reduced dEBC pH. Most importantly, these data suggested that a reduced pH might be
 214 useful in the diagnostic differentiation between atopic and non-atopic recurrent asymptomatic wheezers
 215 (compare groups 1 and 2). The potential discriminatory ability of dEBC pH was therefore quantified with
 216 ROC analysis. Multicategory ROC analysis identified dEBC pH as, by far, the best test in the
 217 simultaneous differentiation among all 5 groups, followed by FAT, ICS and FA (Table III; the API could
 218 not be included in this analysis since it had been determined in groups 1 and 2 only). In binary ROC
 219 analysis, dEBC pH differentiated accurately between group 1 and groups 4 or 5 (AUC, 0.86 and 0.85,

220 respectively). Remarkably, it also discriminated well between groups 1 and 2 (AUC, 0.80; sensitivity =
221 0.76, specificity = 0.77, positive predictive value = 0.63, all at the trade-off value of pH 7.79). In contrast,
222 the AUCs for the differentiation between groups 1 and 3 and between group 2 and groups 4 or 5 were
223 nondiscriminatory. In a logistic regression model, combining dEBC pH with the API improved the ability
224 to differentiate between groups 1 and 2 further (AUC, 0.89), with a sensitivity and specificity of 0.79 and
225 0.86, respectively, and a PPV of 0.75 at the trade-off value of pH 7.83. The subsequent addition of FAT,
226 FA and ICS resulted only in marginal further improvement (AUC, 0.90).

227 Discussion

228 **Diagnostic value of dEBC pH.** We evaluated the ability of dEBC pH to identify asymptomatic
 229 children with a history of wheezing who are at increased risk of developing chronic asthma. To our best
 230 knowledge, our findings constitute the first report that dEBC pH is significantly reduced in asymptomatic
 231 atopic children with a history of recurrent wheezing and thus may potentially serve as a noninvasive, cost-
 232 effective prognostic test that can be performed during an asymptomatic interval. The discriminatory
 233 power of dEBC pH was quantified with ROC analysis, an objective measure for the evaluation of clinical
 234 tests. The AUC of 0.80 for the discrimination between atopic and non-atopic recurrent wheezers
 235 corresponds to “excellent discrimination” in a commonly accepted ROC nomenclature³¹. Notably, the
 236 addition of a simple, well validated risk score, the API, improved the discriminatory ability of dEBC pH
 237 further, the resulting AUC of 0.89 falling within the range of the AUCs of many tests used in clinical
 238 practice. dEBC pH differentiated not only between atopic and non-atopic preschool wheezers but also
 239 between those at high or low genetic risk for asthma and atopy according to family history (Table II).
 240 Moreover, it discriminated accurately between children with or without a positive API and correlated
 241 inversely with FENO, a well validated marker for allergic airway obstruction. Taken together, the present
 242 study extends previous results demonstrating that dEBC pH can classify recurrently wheezing preschool
 243 children into those at high risk (“atopic”) or low risk (“non-atopic”) for the development of asthma.
 244 Future studies should aim to identify additional biomarkers or elements of patient history that can
 245 improve the prognostic ability of dEBC pH and the API further. For instance, FeNO was significantly
 246 higher in group 1 than in group 2. However, values were only available for subsets of participants, and
 247 FeNO was therefore not investigated further in the present study. Future studies are needed to define its
 248 role as an adjunct parameter in combination with dEBC pH and API.

249 **dEBC pH in allergic rhinitis.** In the present study, dEBC pH was normal in children with AR
 250 without a history of RWB. In agreement with this finding, Profita et al.³² reported normal oral, but
 251 decreased nasal, dEBC pH in children. Together, these results support the view that dEBC pH is more

likely a marker of inflammatory processes in general and not of atopic processes within the airways *per se*, such as FeNO³³. In contrast, Brunetti et al.³⁴ reported decreased oral dEBC pH levels in children with atopic dermatitis and allergic rhinitis without clinical signs of airway inflammation. They hypothesized therefore that dEBC pH might be an early predictor of the progression of the atopic march towards 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, may have wheezed in the past. Further studies are needed for a final assessment.

Possible mechanisms of reduced dEBC pH. The decreased dEBC pH values in asymptomatic atopic children with RWB at high risk for asthma (group 1) were found to be comparable to dEBC pH of children with acute wheezy bronchitis (group 3) and published values from ten-year-old children with active moderate asthma³². dEBC pH may reflect endogenous airway acidification resulting from altered pH homeostasis in infectious and inflammatory disease processes, which may sometimes be allowed for host defense purposes, e.g. in response to rhinovirus infection³⁵. Mechanisms capable of acidifying the airways are then activated, such as the Na⁺-H⁺-exchange protein 1, the reduced nicotinamide adenine dinucleotide phosphate-dependent airway epithelial (lumenally acidifying) proton pump, and the release of vacuolar ATPase acidified granules. Simultaneously, mechanisms capable of neutralizing the airway, such as the provision of airway lining fluid proteins, including albumin, activate bicarbonate secretion into the lumen, and the glutaminase-induced production of ammonia and bicarbonate appears to be turned off³⁶. On the other hand, dEBC pH may also reflect exogenous airway acidification, which occurs during recurrent or accidental inhalation of fog or gaseous pollutants or during 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 oxide³⁵. In children who wheeze in the absence of common colds but in response to multiple triggers, it is plausible to hypothesize a constant sub-clinical eosinophilic and neutrophilic

inflammation of the airways^{2, 11}. Bronchoscopic findings and the measurement of FeNO have confirmed this hypothesis in preschool children^{14, 17}.

Influence of other variables on dEBC pH. Most studies show that dEBC pH tends to normalize after anti-inflammatory treatment, even in children^{35, 37}. Unexpectedly, the dEBC pH did not correlate with the inhaled beclomethasone equivalent in our study, and ICS-treated children did not have higher dEBC pH values than ICS-naïve children. However, we acknowledge that the design of the present study did not allow to control for important aspects such as compliance with inhalation therapy, therapy effectiveness, or judgment of the suitable dose^{38, 39}. Although the overall correlation between the dEBC pH and FeNO was not significant, group 1 had significantly higher FeNO values than group 2, in concordance with lowered dEBC pH results. This is consistent with the insight that, at low pH, nitrite is protonated and forms nitric oxide⁴⁰. This finding also lends further support to the notion that low dEBC pH values are pathogenetically relevant. Similarly, 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 acute wheezy bronchitis. This seems obvious with regard to the underlying pathophysiological processes during airway inflammation, including acidification³⁶.

Potential limitations. There are certain limitations to this study. Firstly, microaspiration of gastric contents should be considered as a potential cause of airways acidification, particularly in infants and patients with obstructive lung disease³⁵. Due to ethical reasons, gastroesophageal reflux was excluded by parental report of symptoms and not by diagnostic testing. Secondly, the children in group 1 were older than those in group 2, likely due to the fact that 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 since it is known that age does not influence dEBC pH appreciably²². Thirdly, treatment duration was not assessed in the children receiving corticosteroid inhalation therapy, which makes it difficult to judge its influence on dEBC pH.

Finally, in order to determine the reliability of the dEBC pH in predicting the development of chronic asthma, the results of the present study will have to be compared to the diagnostic gold standard, i.e. the formal diagnosis of asthma according to established criteria when the children have reached school age. For this purpose, we are planning to re-evaluate the study participants of groups 1 and 2 after the sixth birthday. At that time, we will also be able to evaluate whether combining the data for dEBC pH and API with those for CAP class (all from the present study) will improve the predictive value further.

In summary, the results of the present study suggest that dEBC pH, which has been proposed as a simple, noninvasive, and reproducible measure of airway inflammation²⁰, may serve as an additional marker for the early recognition of childhood asthma, particularly when combined with additional information such as the API.

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315 **References**

- 316 1. Weinmayr G, Weiland SK, Björkstén B, Brunekreef B, Büchele G, Cookson WO, et al. Atopic
 317 sensitization and the international variation of asthma symptom prevalence in children. *Am J Respir Crit*
 318 *Care Med* 2007;176(6):565-74.
- 319 2. Bacharier LB, Boner A, Carlsen KH, Eigenmann PA, Frischer T, Götz M, et al. Diagnosis and
 320 treatment of asthma in childhood: a PRACTALL consensus report. *Allergy* 2008;63(1):5-34.
- 321 3. Bacharier LB, Guilbert TW, Zeiger RS, Strunk RC, Morgan WJ, Lemanske RF Jr, et al. Patient
 322 characteristics associated with improved outcomes with use of an inhaled corticosteroid in preschool
 323 children at risk for asthma. *J Allergy Clin Immunol* 2009;123(5):1077-82.
- 324 4. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szeffler SJ, et al. Long-term
 325 inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med* 2006;354:1985-97.
- 326 5. Morgan WJ, Stern DA, Sherrill DL, Guerra S, Holberg CJ, Guilbert TW, et al. Outcome of asthma
 327 and wheezing in the first 6 years of life: follow-up through adolescence. *Am J Respir Crit Care Med*
 328 2005;172:1253–8.
- 329 6. Lowe L, Murray CS, Custovic A, Simpson BM, Kissen PM, Woodcock A; NAC Manchester
 330 Asthma and Allergy Study Group. Specific airway resistance in 3-year-old children: a prospective cohort
 331 study. *Lancet* 2002;359(9321):1904-8.
- 332 7. Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U; Multicentre Allergy Study (MAS)
 333 group. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study.
 334 *Lancet* 2006;368(9537):763-70.
- 335 8. Simões EA, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick L, Groothuis JR. The effect of
 336 respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. *J Allergy*
 337 *Clin Immunol*. 2010 Aug;126(2):256-62.

- 338 9. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing
 339 rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care*
 340 *Med* 2008;178(7):667-72.
- 341 10. Kusel MM, de Klerk NH, Keadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life
 342 respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma.
 343 *J Allergy Clin Immunol* 2007;119(5):1105-10.
- 344 11. Brand PLP, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition,
 345 assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur*
 346 *Respir J* 2008;32:1096-110.
- 347 12. Castro-Rodríguez JA. The Asthma Predictive Index: A very useful tool for predicting asthma in
 348 young children. *J Allergy Clin Immunol* 2010;126(2):212-6.
- 349 13. Sonnappa S, Bastardo CM, Wade A, Saglani S, McKenzie SA, Bush A, et al. Symptom-pattern
 350 phenotype and pulmonary function in preschool wheezers. *J Allergy Clin Immunol*. 2010 Jun 23 [Epub
 351 ahead of print].
- 352 14. Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, et al. Nonatopic children
 353 with multitrigger wheezing have airway pathology comparable to atopic asthma. *Am J Respir Crit Care*
 354 *Med* 2008;178:476-82.
- 355 15. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and
 356 wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med*
 357 1995;332(3):133-8.
- 358 16. Kharitonov SA, Barnes PJ. Exhaled biomarkers. *Chest* 2006;130:1541-6.
- 359 17. Moeller A, Diefenbacher C, Lehmann A, Rochat M, Brooks-Wildhaber J, Hall GL, et al. Exhaled
 360 nitric oxide distinguishes between subgroups of preschool children with respiratory symptoms. *J Allergy*
 361 *Clin Immunol* 2008;121(3):705-9.

18. Rosias PPR, Robroeks CM, van de Kant KD, Rijkers GT, Zimmermann LJ, van Schayck CP, et al. Feasibility of a new method to collect exhaled breath condensate in pre-school children. *Pediatr Allergy Immunol* 2010;21:e235-44.
19. Horváth I, Hunt J, Barnes PJ. ERS/ATS Taskforce. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005;26:523-48.
20. Vaughan J, Ngamtrakulpanit L, Pajewski T, Turner R, Nguyen TA, Smith A, et al. Exhaled breath condensate pH is a robust and reproducible assay of airway acidity. *Eur Respir J* 2003;22:889-94.
21. Soyer OU, Dizdar EA, Keskin O, Lilly C, Kalayci O. Comparison of two methods for exhaled breath condensate collection. *Allergy* 2006;61(8):1016-8.
22. Paget-Brown AO, Ngamtrakulpanit L, Smith A, Bunyan D, Hom S, Nguyen A, et al. Normative data for pH of Exhaled Breath Condensate. *Chest* 2006; 129:426-30.
23. Nicolaou NC, Lowe LA, Murray CS, Woodcock A, Simpson A, Custovic A. Exhaled breath condensate pH and childhood asthma: unselected birth cohort study. *Am J Respir Crit Care Med* 2006;174(3):254-9.
24. Wells K, Vaughan J, Pajewski TN, Hom S, Ngamtrakulpanit L, Smith A, et al. Exhaled breath condensate pH assays are not influenced by oral ammonia. *Thorax* 2005;60(1):27-31.
25. Vogelberg C, Würfel C, Knoetzsch A, Kahlert A, Range U, Leupold W. Exhaled breath condensate pH in infants and children with acute and recurrent wheezy bronchitis. *Pediatr Pulmonol* 2007; 42(12):1166-72.
26. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108:S147–S334.
27. Kjellmann M. Atopic disease in 7-year-old children. Incidence in relation to family history. *Acta Paediatr Scand* 1977;66:465-71.
28. Baraldi E, de Jongste JC. ERS/ATS Statement. Measurement of exhaled nitric oxide in children, 2001. *Eur Respir J* 2001;17:1201-7.

29. Li J, Fine JP. ROC analysis with multiple classes and multiple tests: methodology and its application in microarray studies. *Biostatistics* 2008;9(3):566-76.
30. Oddie A, Li J, Dai L, Paessler ME, Yu X, Diaz-Torne C, et al. Identification of broadly discriminatory tissue biomarkers of synovitis with binary and multicategory receiver operating characteristic analysis. *Biomarkers*. 2010 Mar;15(2):183-90.
31. Hosmer DW, Lemeshow S. *Applied logistic regression*. 2nd ed. Amherst (MA), Columbus (O.): Wiley Interscience; 2000.
32. Profita M, LaGrutta S, Carpagnano E, Riccobono L, DiGiorgi R, Bonanno A, et al. Noninvasive methods for the detection of upper and lower airway inflammation in atopic children. *J Allergy Clin Immunol* 2006;118(5):1068-74.
33. Brussee JE, Smit HA, Kerkhof M, Koopman LP, Wijga AH, Postma DS, et al. Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. *Eur Respir J* 2005;25(3):455-61.
34. Brunetti L, Francavilla R, Tesse R, Strippoli A, Polimeno L, Loforese A, et al. Exhaled breath condensate pH measurement in children with asthma, allergic rhinitis and atopic dermatitis. *Pediatr Allergy Immunol* 2006;17(6):422-7.
35. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, et al. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000;161(3 Pt 1):694-699.
36. Ricciardolo FL, Gaston B, Hunt JF. Acid stress in the pathology of asthma. *J Allergy Clin Immunol* 2004;113(4):610-9.
37. Carraro S, Folesani G, Corradi M, Zanconato S, Gaston B, Baraldi E. Acid-base equilibrium in exhaled breath condensate of allergic asthmatic children. *Allergy* 2005;60(4):476-81.
38. Orrell-Valente JK, Jarlsberg LG, Hill LG, Cabana MD. At what age do children start taking daily asthma medicines on their own? *Pediatr* 2008;122:e1186-92.

- 411 39. Schuepp KG, Straub D, Moller A, Wildhaber JH. Deposition of aerosols in infants and children. J
412 Aerosol Med 2004;17:153-6.
- 413 40. Gaston B, Kelly R, Urban P, Liu L, Henderson EM, Doctor A, et al. Buffering airway acid
414 decreases exhaled nitric oxide in asthma. J Allergy Clin Immunol 2006;118(4):817-22.

415 **Table I.** Demographic and laboratory characteristics of the subject groups*

Characteristic	Group 1	Group 2	Group 3	Group 4	Group 5	<i>P</i> value
	Asymptomatic RWB		Acute			
	Atopic	Nonatopic	Wheezy bronchitis	AR	Healthy	
Number	34	64	18	15	60	
Male sex (%)	68	62	56	53	52	.561 [†]
Age (m)	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 [§]
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); n	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); n	92.3 (42); 22	89.3 (15.6); 23	58.1 (16.8); 6	110.3 (33.4); 8	Not tested	.011 [§]
FeNO (ppb); n	13.4 (15.6); 21	5.4 (4.0); 18	7.7 (9.8); 4	12.4 (11.3); 7	Not tested	.001 [§]
dEBC pH	7.49 (0.94)	7.93 (0.23)	7.44 (0.70)	8.02 (0.17)	7.96 (0.25)	<.001 [§]

*Results are expressed as median (interquartile range). [§]Kruskall-Wallis analysis. [†]Chi-square test.

‡according to TABLE II.

416 **Table II.** Genetic risk scores.

FAT¹	%	FA²	
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 one 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 one 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 two siblings with asthma	5

¹Percent prevalence of atopic disease in seven-year-old children according to Kjellmann²⁷. FAT, family atopy.

²Risk score generated by the authors. FA, family asthma.

417 Table III. Overall ability of dEBC pH, FAT, ICS and FA to differentiate among the five subgroups

Rank	Test	HUM ¹
1	dEBC pH	0.0719
2	FAT	0.0011
3	ICS	0.0006
4	FA	0.0002

418 ¹Values represent hypervolumes under the manifold obtained with multicategory ROC analysis according
419 to ref.²⁹

Figure legends

Fig 1. dEBC pH in the four patient groups and healthy controls. dEBC pH was significantly lower in atopic recurrent (group 1) and acute wheezy bronchitis (group 3) compared with non-atopic recurrent wheezy bronchitis (group 2), allergic rhinitis (group 4), and healthy controls (group 5). $P < 0.001$ for each of the six comparisons.