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Lung function, airway remodeling, and inflammation in infants: outcome at 8 years

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ABSTRACT

Background: Associations between early deficits of lung function, infant airway disease, and outcome at school age in symptomatic infants are still unclear.

Objective: To report follow-up data on a unique cohort of children investigated invasively in infancy to determine predictive value of airway disease for school-aged respiratory outcomes.

Methods: Fifty-three infants previously studied using bronchoscopy and airway conductance were approached at 8 years of age. Symptoms, lung volumes, and airway responsiveness were reassessed. Data on lifetime purchase of asthma medication were obtained. Lung function was compared with that of 63 healthy nonasthmatic children.

Results: Forty-seven children were reevaluated. Physician-diagnosed asthma was present in 39 children (83%). Twenty-five children (53%) had current and 14 children (30%) had past asthma. No pathologic feature in infancy correlated with any outcome parameter. As expected, study children had significantly reduced lung function and increased airway responsiveness compared with healthy controls, and very early symptoms were risk factors for reduced lung function. Current asthma was associated with reduced infant lung function and parental asthma. Reduced lung function in infancy was associated with purchase of inhaled corticosteroids when 6 to 8 and 0 to 8 years of age.

Conclusion: The lack of predictive value of any pathologic measure in infancy, reported here for the first time to our knowledge, demonstrates that pathologic processes determining the inception of asthma, which are as yet undescribed, are different from the eosinophilic inflammation associated with ongoing disease.

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Introduction

Lower respiratory tract symptoms and wheeze are common during infancy. Clinical characteristics that affect outcome include frequency and severity of wheezing, history of eczema, peripheral

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blood eosinophilia, allergic rhinitis, wheezing without colds, and parental history of asthma.¹ Antenatal and infant factors are important in the long-term airway function. Reduced lung function measured as early as at birth to 3 months of age in healthy children has been associated with an increased risk of physician-diagnosed asthma by 2 years of age,² as well with an increased risk of asthma and severe airway responsiveness (AR) at 10 years of age.³ A birth cohort study revealed that lower respiratory tract illness, i.e. cough in the presence of upper respiratory tract infection, in the first six months was associated with increased AR later in childhood.⁴ Increased AR at 1 year of age has been associated with increased wheeze at 11 years of age, whereas the persistence of AR was associated with male sex, early respiratory illness, and maternal smoking and asthma.^{5–7} However, although the effects of infant

airway obstruction are well studied, nothing is known about whether (if at all) airway disease in highly symptomatic infants relates to school-aged asthma.

Thickening of the bronchial epithelial reticular basement membrane (RBM), increased airway smooth muscle (ASM), and eosinophilic inflammation are characteristic pathologic findings in adult asthma,⁸ in school-aged children with severe asthma,^{9–11} and in preschool-aged children with severe, recurrent wheeze.¹² Thickened RBM, increased epithelial loss, and angiogenesis, changes typical of remodeling, have even been found in 4- to 8-year-old children with asthma regardless of bronchial eosinophilia.¹³ Increased ASM mass in severe preschool wheezers has recently been found to be greater in those children who have asthma at school age.¹⁴ Previously, we reported the airway disease in a unique cohort of 53 corticosteroid-naïve infants with recurrent lower respiratory tract symptoms of whom 38 (72%) had reduced lung function but surprisingly no significantly thickened RBM or tissue eosinophilia.¹⁵ In a follow-up study, significant associations between RBM thickness and the density of bronchial mast cells in infancy and the amount of purchased inhaled corticosteroids (ICSs) at the age of 3 years were detected.¹⁶ However, we did not then know which of the infants who underwent bronchoscopy would actually develop school-aged asthma.

In the present study, we rephenotyped the cohort by determining current symptoms, lung function, AR, and the purchase of asthma medication at 8 years of age and related the findings to previous airway disease and infant lung function. Given the previously reported association between ASM at a mean age of 3 years and subsequent asthma,¹⁴ we report ASM area fraction in the infant biopsy specimens also for the first time to our knowledge. We tested the hypothesis that those study children who had developed asthma by 8 years of age were more likely at 1 year of age, when originally studied, to have more airway eosinophilia and remodeling and lower lung function and increased AR compared with those infants who were asymptomatic at 8 years of age. We also compared our patients with a new group of healthy age-matched controls.

Methods

Study Population

The original study population of 53 full-term white infants (mean age, 1.0 year; range, 0.28–2.16 years) referred to a tertiary center for investigation of recurrent lower respiratory tract symptoms, including dyspnea, cough, and wheeze.¹⁵ Children underwent whole-body plethysmography and bronchoscopy. Reduced baseline lung function was defined as specific airway conductance below the fifth percentile (z score < -1.65) of the reference range.¹⁷ Bronchodilator responsiveness was measured¹⁸ (eAppendix).

The carinal biopsy specimens were analyzed for RBM thickness, inflammatory cells, human rhinovirus, and dendritic cells as described previously.^{15,19,20} The individual data were withheld from the pediatricians treating the children. ASM was assessed in evaluable biopsy specimens stained with toluidine blue using computer-aided analysis, and the ASM area was represented as a fraction of subepithelial area as described previously¹⁴ (eAppendix).

Control Population at School Age

We used lung function data from healthy children of the same age from a population-based cohort of 6209 full-term newborn infants recruited to determine the prevalence of cow's milk allergy.²¹ The control population consisted of 63 of 80 recruited individuals who did not have cow's milk allergy or physician-diagnosed asthma and were able to perform spirometry.

Study Design

At 8 years of age, all 53 children were invited to participate in a follow-up study to investigate their respiratory symptoms, atopic manifestations, and lung function at the Skin and Allergy Hospital in Helsinki, Finland. The children were instructed to not take ICSs and oral leukotriene receptor antagonists (LTRAs) for the previous 5 weeks, inhaled long-acting β -agonists for 24 hours, and short-acting β -agonists for 12 hours. The child's parent or guardian filled in a standardized questionnaire on asthma symptoms and medication. Written informed consent was obtained from the children and from one of the parents before the study. The Ethics Committee of Diseases of Children and Adolescents at the Helsinki University Central Hospital approved the study protocol.

Definitions at 8 Years of Age

Children with recurrent wheezing, coughing, mucus production, or dyspnea during the previous year were classified as having ongoing asthma symptoms. Current asthma was defined as physician-diagnosed asthma ever and current regular or intermittent use of ICSs or LTRAs during the past 2 years. Past asthma was defined as physician-diagnosed asthma ever and use of ICSs or LTRAs but not in the last 2 years. No asthma was defined as no physician-diagnosed asthma ever and no use of ICSs or LTRAs. Atopy was defined by a positive skin prick test result to food or aeroallergens.¹⁵ Frequent wheeze was defined as wheeze on at least 3 separate occasions before infant lung function tests were performed.

Lung Function Tests at 8 Years of Age

Lung function and bronchodilator responsiveness were measured using a pneumotachograph-based flow-volume spirometer (Masterscreen Pneumo, Jaeger GmbH, Würzburg, Germany) (eAppendix). AR was evaluated with a dosimetric histamine challenge test on the second study day.²² The provocation dose of inhaled histamine producing a decrease of 15% in forced expiratory volume in 1 second (FEV₁) (PD₁₅) was determined, and increased AR was defined as a PD₁₅ of 0.4 mg or less. The AR to histamine was expressed in terms of the slope of the dose-response curve as a continuous and log-normally distributed index²³ (eAppendix). Exhaled nitric oxide was assessed according to the American Thoracic Society recommendations,²⁴ and the height-adjusted results were expressed as z scores (eAppendix).

Purchase of Inhaled Asthma Medication

Individual lifetime data on the purchase of asthma medication were collected (eAppendix).

Statistical Analysis

There are no data to inform a power calculation, and the sample size was opportunistic. The Kruskal-Wallis test and Mann-Whitney test were used for comparisons of the use and purchase of asthma medication, questionnaire data, and lung function results at 8 years of age between the patient and control groups. Comparison of means with normally distributed variables was performed with t test. The associations between findings at infancy and at 8 years and the use of asthma medication during lifetime and from 6 to 8 years of age were evaluated with Spearman correlations and χ^2 tests. The association in 2-by-2 tables was tested with the Fisher exact test (eAppendix).

Results

A total of 47 of 53 children (89%) completed follow-up at a median age of 8.1 years. There was a statistically significant but clinically trivial age difference between the study children and the healthy control children. Details of the children are given in Table 1.

Table 1

Outcome at 8 years of age according to LF at infancy

Characteristic	Infant LF reduced (n = 36) ^a	Infant LF normal (n = 11)	P value ^b
Age, median (range), y	8.1 (7.7–9.3)	8.1 (7.6–8.9)	.83 (Mann-Whitney test)
Male, No. (%)	25 (69)	7 (64)	.72 (Fisher exact test)
TGV at infancy, median (IQR)	0.59 (1.3)	0.06 (1.0)	.06 (Mann-Whitney test)
sGaw at infancy, median (IQR)	–3.3 (1.9)	–0.36 (2.3)	<.001 (Mann-Whitney test)
Parental smoking at infancy, No. (%)	12 (33)	3 (27)	>.99 (Fisher exact test)
Parental smoking at present, No. (%)	14 (39)	4 (36)	>.99 (Fisher exact test)
Parental asthma ever, No. (%)	17 (47)	3 (27)	.31 (Fisher exact test)
Physician-diagnosed disease ever, No. (%)			
Asthma	33 (92)	6 (55)	.04 (Fisher exact test)
Atopic eczema	19 (33)	8 (73)	.31 (Fisher exact test)
Rhinoconjunctivitis	17 (47)	7 (64)	.49 (Fisher exact test)
Food allergy	19 (53)	4 (36)	.49 (Fisher exact test)
Ongoing asthma symptoms, No. (%)	21 (58)	6 (55)	>.99 (Fisher exact test)
Current asthma, No. (%) ^c	22 (61)	3 (27)	.01 (Fisher exact test)
Past asthma, No. (%) ^d	11 (31)	3 (27)	.08 (Fisher exact test)
Skin prick test positive, No. (%) (n = 41)	13/31 (42)	6/10 (60)	.47 (Fisher exact test)
Atopy (SPT positive, blood eosinophil count >6%, or specific IgE >320 kU/L), No. (%)	20 (56)	8 (80)	.90 (Fisher exact test)

Abbreviations: IQR, interquartile range; LF, lung function; sGaw, specific airway conductance; SPT, skin prick test; TGV, thoracic gas volume.

^aReduced LF in infancy indicates reduced LF (specific airway conductance z score <–1.65) with or without bronchodilator reversibility.^bFisher exact test for dichotomous variables and Mann-Whitney test for continuous variables.^cCurrent asthma indicates physician-diagnosed asthma and regular or intermittently inhaled corticosteroids or leukotriene receptor antagonist in the past 2 years.^dPast asthma indicates physician-diagnosed asthma and earlier use of inhaled asthma medication or leukotriene receptor antagonist beyond 2 years.

For the sake of clearness, the results are presented according to lung function at infancy.

Diagnosis and Symptoms of Asthma and Atopy

Physician-diagnosed asthma ever was present in 39 of 47 children (83%), current asthma in 25 (53%), and past asthma in 14 (30%). Ongoing asthma symptoms were present in 27 children (57%) of whom 7 (15%) had ongoing wheeze. The ongoing symptoms were triggered by multiple factors in 26 children (96%) and only by viruses in 1 child (4%). Current asthma was associated with decreased infant lung function and parental asthma ($P = .007$ and $P = .009$, respectively; χ^2 test).

Skin prick testing was available for 41 children in infancy. Atopy, defined as positive skin prick test result in infancy, was present in 19 of 41 children (46%). Atopic status was unchanged in 28 children (68%), sensitization had developed in 8 children, and sensitization had disappeared in 5 children. Atopy, defined as a positive skin prick test result, blood eosinophil count greater than 6%, or serum IgE level greater than 320 kU/L, was present in 27 of 41 children (66%). The number of wheezing episodes before infant lung function tests were performed did not correlate with atopy or the number of positive skin prick test results.

Current Lung Function and AR

Spirometry was performed successfully in 42 of the 47 study children (89%). The percentage of predicted forced vital capacity (FVC), FEV₁, and FEV₁/FVC ratio in the children with reduced infant lung function were significantly lower compared with those in the age-matched, healthy, nonasthmatic children (Table 2). In contrast, in the children with normal lung function at infancy, only the percentage of predicted FEV₁/FVC was significantly lower than in controls. Individual specific airway conductance or thoracic gas volume z scores was not associated with any of the lung function parameters at 8 years of age.

Histamine provocation was performed successfully in 38 study children (90%) and in 58 healthy, nonasthmatic control children (92%). The AR was significantly increased in children with reduced and normal infant lung function compared with the healthy, nonasthmatic control children ($P < .001$ and $P = .005$, respectively; Fisher exact test) (Fig 1 and Table 2). Increased AR was found in 10

children with current asthma, 6 children with past asthma, and 1 child with no asthma.

Associations of reduced lung function and increased AR at school age, in addition to infant recurrent lower respiratory tract symptoms and reduced lung function, were sought. These associations were thickness of RBM, fraction of ASM area, number of inflammatory cells, presence of human rhinovirus messenger RNA, wheeze ever, frequent wheeze, number of wheezing episodes, atopy, exposure for smoking, and age at the onset of symptoms. Infant exposure to smoking was associated significantly with lower percentage of predicted FEV₁/FVC ($P = .03$; Mann-Whitney test) but not AR. The age at the onset of symptoms was 0 to 1.3 years (median, 0.42 years). The lower age at the onset of symptoms was associated significantly with lower FVC and percentage of predicted FEV₁ ($r = 0.315$, $P = .04$, and $r = 0.467$, $P = .002$, respectively; Spearman test) (Fig 2A and B) and lower percentage of predicted FEV₁/FVC ($r = 0.325$, $P = .04$; Spearman test) (Fig 2C) but not AR. In addition, when analyzing only children with wheezing in infancy ($n = 24$), the number of wheezing episodes was associated only with lower percentage of predicted FEV₁/FVC ($r = -0.515$, $P = .01$; Spearman test). Among the wheezing infants, a younger age at the onset of symptoms was also associated with a significantly lower percentage of predicted FEV₁ and FEV₁/FVC ($r = 0.577$, $P = .003$, and $r = 0.506$, $P = .01$; Spearman test).

Airway Smooth Muscle

The ASM was evaluable from 29 infants with 1 to 3 available biopsy sections (mean, 1.3 sections per child). The median ASM proportion did not differ between the infant lung function groups: children with reduced lung function had a mean ASM of 0.092 (interquartile range, 0.12), and infants with normal lung function had a median ASM of 0.10 (interquartile range, 0.13) (Fig 3A). There was no difference in age ($P = .45$; Fisher exact test) or atopic status ($P = .65$; Fisher exact test) between children with ASM and without ASM on endobronchial biopsy.

Association With Airway Disease in Infancy

Children with current asthma at school age did not differ in the thickness of RBM or proportion of ASM in infancy from those children with past or no asthma (Fig 3B, C, and D). The thickness of

Table 2

LF at 8 years of age according to LF at infancy compared with LF of healthy children at 8 years of age

Characteristic	Healthy children (n = 63)	Infant LF reduced (n = 32) ^a	Infant LF normal (n = 10)	P value ^b	P value ^c
Age, median (range), y	8.7 (8–9.3)	8.1 (7.1–9.0)	8.2 (7.6–8.9)	<.001	.004 (Mann-Whitney test)
Height, median (IQR), cm	133.9 (7.0)	128.5 (7.8)	127.0 (12.3)	<.001	.04 (Mann-Whitney test)
Height, median (IQR), %	0.25 (5.0)	–0.10 (3.4)	–0.35 (4.0)	.004	.22 (t test)
Weight, median (IQR), kg	29.2 (7.8)	27.3 (6.6)	28.0 (8.3)	.12	.31 (Mann-Whitney test)
Weight, median (IQR), %	3.5 (7.2)	6 (50)	3.5 (24)	.32	.96 (t test)
Body mass index, mean (SD)	17.0 (2.6)	17.2 (2.0)	16.8 (1.7)	.76	.82 (t test)
Spirometry					
FVC, median (IQR), L	1.95 (0.3)	1.79 (0.4)	1.87 (0.6)	.02	.56 (Mann-Whitney test)
FVC, median (IQR), % of predicted	92.2 (12.9)	97.0 (14.3)	100.0 (15.3)	.03	.11 (Mann-Whitney test)
FEV ₁ , median (IQR), L	1.85 (0.31)	1.53 (0.32)	1.62 (0.64)	<.001	.06 (Mann-Whitney test)
FEV ₁ , median (IQR), % of predicted	97.4 (15.8)	90.5 (14.8)	92.0 (23.8)	.02	.06 (Mann-Whitney test)
FEV ₁ /FVC, median (IQR)	94.4 (7.9)	82.4 (15)	86.0 (12.8)	<.001	.003 (Mann-Whitney test)
FEV ₁ /FVC, median (IQR), % of predicted	104.8 (8.5)	91.5 (16.5)	96.0 (14.1)	<.001	.002 (Mann-Whitney test)
Histamine provocation ^d					
PD ₁₅ , median (IQR), mg	1.4 (2.5)	0.47 (0.8)	0.33 (1.0)	<.001	.005 (Mann-Whitney test)
Log dose response slope	0.011 (0.016)	0.037 (0.043)	0.042 (0.037)	<.001	.01 (Mann-Whitney test)
Increased airway responsiveness, No. (%) ^e	4 (7)	12 (40)	5 (63)	<.001	.001 (Fisher exact test)
Fraction of exhaled nitric oxide					
Fraction of exhaled nitric oxide, median (IQR), ppb	10.5 (7.0)	8.2 (4.8)	9.9 (6.1)	.03	.68 (Mann-Whitney test)
z score, median (IQR)	0.60 (1.6)	0.0 (1.7)	0.5 (1.7)	.04	>.99 (Mann-Whitney test)

Abbreviations: FEV₁ forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range; LF, lung function; PD₁₅, provocation doses causing a 15% decrease in FEV₁.

^aReduced LF in infancy indicates reduced LF (specific airway conductance sGaw z score <–1.65) with or without bronchodilator reversibility.

^bChildren with reduced LF at infancy vs healthy children. Fisher exact test for dichotomous variables, Mann-Whitney test for continuous variables, and t test for equality of means. P values are not corrected for multiple comparisons.

^cChildren with normal LF at infancy vs healthy children.

^dHistamine provocation was successful for 30 infants with reduced LF at infancy, for 8 children with normal LF at infancy, and for 58 healthy children.

^eIncreased airway responsiveness indicates PD₁₅ less than 0.4 mg.

epithelial RBM did not correlate significantly with AR ($r = 0.017$, $P = .92$; Spearman test) or with the purchase of ICSs during 6 to 8 years of age or 0 to 8 years of age ($r = 0.05$, $P = .72$, and $r = 0.056$, $P = .68$; Spearman test). The proportion of ASM had a positive correlation only with PD₁₅ ($r = 0.664$, $P = .002$; Spearman test), but this was mainly due to a few outliers. Neither endobronchial inflammatory cells nor endobronchial biopsy specimens positive for human rhinovirus correlated significantly with any outcome parameters. Only a weak association with human rhinovirus in the endobronchial biopsy specimens of infants with reduced lung function and increased AR expressed as physician-diagnosed asthma at school age was found ($r = 0.351$, $P = .062$; Spearman test). A significant

correlation was found between the number of CD1aLP and CD1a dendritic cells with percentage of predicted FEV₁ ($r = 0.463$, $P = .008$, and $r = 0.371$, $P = .04$; Spearman test), but this was mainly due to a few outliers.

Association With Reduced Lung Function at Infancy

Reduced infant lung function was associated with physician-diagnosed asthma ($P = .04$; Fisher exact test) and current asthma ($P = .01$; Fisher exact test) but not with the presence of ongoing asthma symptoms or current atopy (Table 1) or with current lung function, AR, or fraction of exhaled nitric oxide (Table 2). Reduced infant lung function was significantly associated with duration (in years) of regular ICSs use reported by parents ($P < .001$; Mann-Whitney) (Table 3) and with the purchased amount of ICSs during 6 to 8 years of age ($P = .02$; Mann-Whitney test) and 0 to 8 years of age ($P < .001$; Mann-Whitney test) (Table 3 and Fig 4).

Reported Use and Purchase of Asthma Medication at 8 Years of Age

Reported use and purchase of asthma medication are presented at Table 3. Purchase of ICSs was registered for 24 of 47 children (51%) 6 to 8 years of age and 43 of 47 children (91%) 0 to 8 years of age. Purchase of LTRAs was registered for 9 of 47 children (19%) 6 to 8 years of age and 0 to 8 years of age, whereas purchase of long-acting β -agonists was registered for 3 of 47 children (6%) and 9 of 47 children (19%), respectively. Only 1 child was solely taking LTRAs. The cumulative amount of purchased ICS in milligrams by 8 years of age did not correlate with airway responsiveness. The number of wheezing episodes was associated with the purchased amount of ICSs from 6 to 8 years of age and 0 to 8 years of age ($r = 0.457$, $P = .001$, and $r = 0.564$, $P < .001$; Spearman test).

Discussion

We report unique follow-up outcome data at school age from the only cohort of infants systematically and invasively investigated for severe wheeze. We found, for the first time to our knowledge,

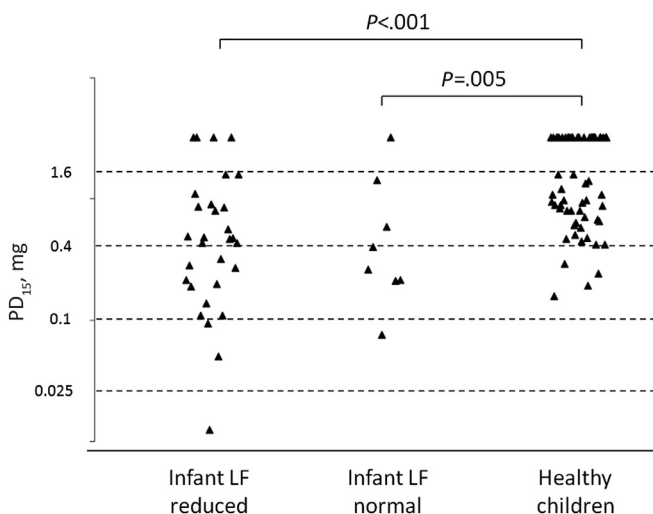


Figure 1. Scatterplot of the provocation dose of histamine producing a decrease of 15% in forced expiratory volume in 1 second (FEV₁) (PD₁₅) in the children with reduced infant lung function (LF), normal infant LF, and healthy control children. P values refer to the Mann-Whitney test by airway responsiveness ranks.

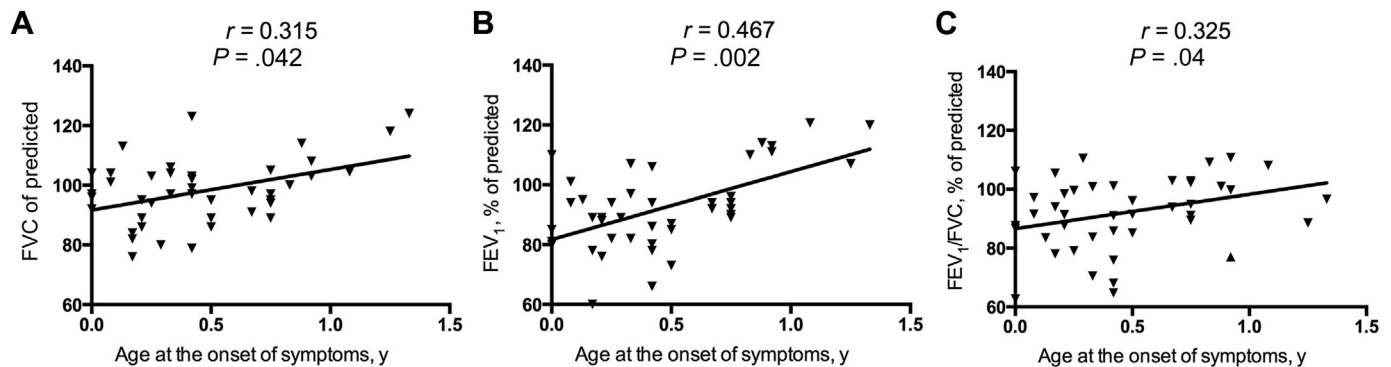


Figure 2. Age at the onset of symptoms and its association with percentage of predicted forced vital capacity (FVC) (A), percentage of predicted forced expiratory volume in 1 second (FEV₁) (B), and percentage of predicted FEV₁/FVC (C). *P* values refer to the Spearman test.

that the thickness of RBM, ASM fraction, or endobronchial inflammatory cell counts in severely symptomatic infants did not correlate significantly with any asthma outcome parameter at 8 years of age. As expected and as a useful positive control implying that our findings did not relate to lack of power, children with recurrent lower respiratory tract symptoms and impaired lung function in infancy had significantly lower lung function and increased AR compared with healthy controls at school age. Reduced infant lung function was associated with higher prevalence of physician-diagnosed asthma, current asthma, and greater treatment needs. The very early onset of symptoms and infant exposure to smoking were risk factors for reduced lung function at school age.

Association Between Infant Airway Disease and Current Lung Function

Increased ASM mass in severe preschool wheezers has recently been reported to be associated with current asthma at school age.¹⁴ We report, for the first time to our knowledge, the fraction of ASM area in infancy and found that it did not relate to infant lung function. Because of the size and depth of the biopsy specimens, we only had sufficient material to quantify ASM in 29 of 53 children. There is no evidence that this subset were different from the group as a whole. The fraction of the ASM area (median, 9%–10%) was similar in preschool wheezers not developing (6%) and developing (12.7%)

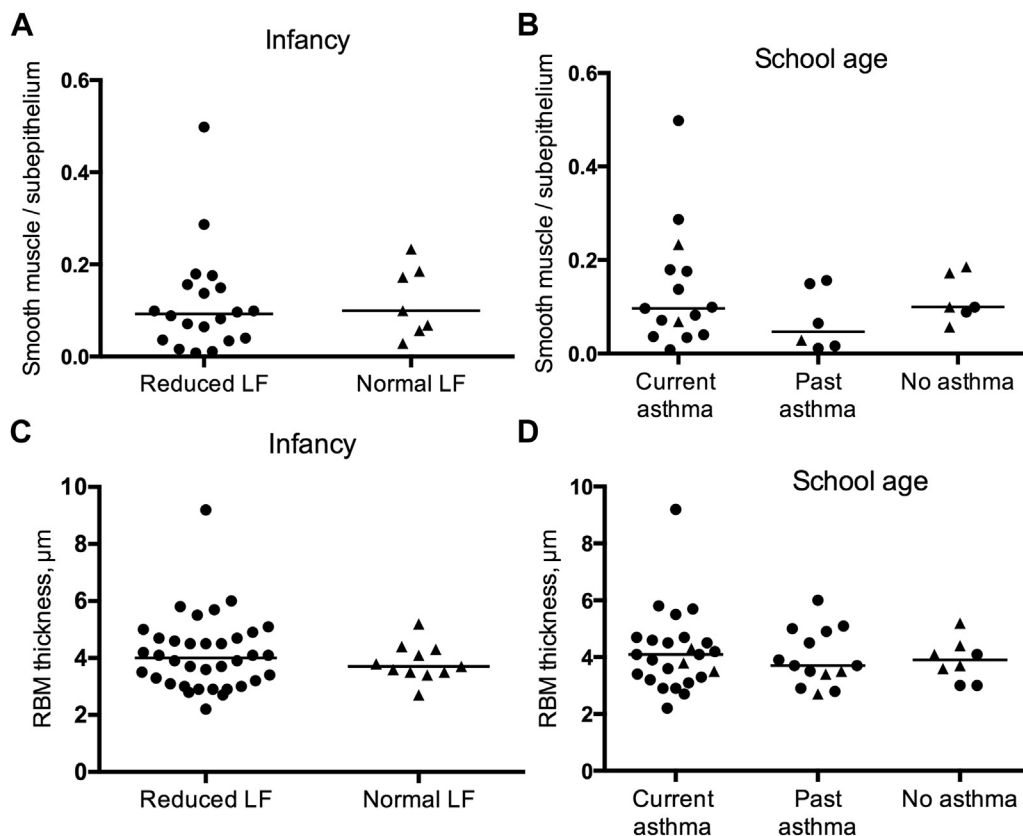


Figure 3. A, Infant proportion of airway smooth muscle area and the association with infant lung function (LF). No significant differences were found between the infant groups ($P > .99$; χ^2 test). B, Infant proportion of airway smooth muscle and the pattern of asthma at school age. No differences were found between the groups ($P = .26$; χ^2 test). C, Infant thickness of reticular basement membrane (RBM) and the association with infant LF. D, Infant RBM and the pattern of asthma at school age. No differences were found between the groups ($P = .93$; χ^2 test). Current asthma indicates physician-diagnosed asthma ever plus current regular or intermittent use of inhaled corticosteroids (ICSs) or leukotriene receptor agonists (LTRAs) during the past 2 years. Past asthma indicates physician-diagnosed asthma ever plus use of inhaled asthma medication or LTRAs beyond 2 years. No asthma indicates no physician-diagnosed asthma ever and no use of ICSs or LTRAs.

Table 3
Use and purchase of asthma medication

Characteristic	Infant LF reduced (n = 36) ^a	Infant LF normal (n = 11)	P value ^b
Reported use of ICS during the past year, No. (%)			
Regular	14 (39)	1 (9)	.08 (Fisher exact test)
Periodic	7 (19)	1 (9)	.13 (Fisher exact test)
Reported regular use of LABA during the past year, No. (%)	3 (8)	0	>.99 (Fisher exact test)
Reported regular use of LTRA during the past year, No. (%)	7 (19)	0	.18 (Fisher exact test)
Purchase of asthma medication during 6–8 years of age			
ICS, median (IQR), mg	40 (97)	0 (0)	.02 (Mann-Whitney test)
SABA, median (IQR), mg	20 (40)	0 (20)	.11 (Mann-Whitney test)
LABA, median (IQR), mg	0 (0)	0 (0)	.33 (Mann-Whitney test)
LTRA, median (IQR), mg	0 (105)	0 (0)	.07 (Mann-Whitney test)
Purchase of asthma medication during 0–8 years of age			
ICS, median (IQR), mg	372 (413)	40 (156)	<.001 (Mann-Whitney test)
SABA, median (IQR), mg	220 (235)	12 (120)	<.001 (Mann-Whitney test)
LABA, median (IQR), mg	0 (0)	0 (0)	.30 (Mann-Whitney test)
LTRA, median (IQR), mg	0 (543)	0 (0)	.05 (Mann-Whitney test)
Reported regular use of ICS, median (IQR), y	3.1 (5.0)	0.2 (1.4)	<.001 (Mann-Whitney test)

Abbreviations: ICS, inhaled corticosteroid; IQR, interquartile range; LABA, long-acting α -agonist; LF, lung function; LTRA, leukotriene receptor antagonist; SABA, short-acting α -agonist.

^aReduced LF in infancy indicates reduced LF (specific airway conductance z score <−1.65) with or without bronchodilator reversibility.

^bFisher exact test for dichotomous variables and Mann-Whitney test for continuous variables.

asthma by school age.¹⁴ The only correlation of the fraction ASM area and the outcome parameters was found with AR (ie, PD₁₅), indicating paradoxically less hyperreactivity in those with a thicker ASM. However, both measurements were successful in only 19 patients, and the association may be spurious and certainly requires confirmation. Because the amount of ASM was similarly distributed in children with reversible obstruction and irreversible obstruction and normal lung function, it seems that ASM quantity is not the driving factor for bronchodilator responsiveness in this age group. We speculate that bronchodilator responsiveness may relate to altered ASM function, reduced airway compliance,²⁵ or loss of alveolar attachments,²⁶ which we could not measure. Because AR was not measured in infancy, the role of ASM in AR in this age group is still unclear. In addition, there is less ASM at the bifurcation of the trachea than in more distal airways; therefore, the carinal site of biopsy for ASM analysis may not be optimal.²⁷ The proportion of ASM may also vary according to the depth of the biopsy specimen, again introducing noise in the measurements. However, this limitation was minimized by assessing ASM as the proportion or fraction of the biopsy. As the biopsy specimens were small, only one section per biopsy was available for ASM quantification. Ideally, ASM assessments from several different depths within the biopsy would have been made to completely eliminate the bias that resulted from biopsy depth.

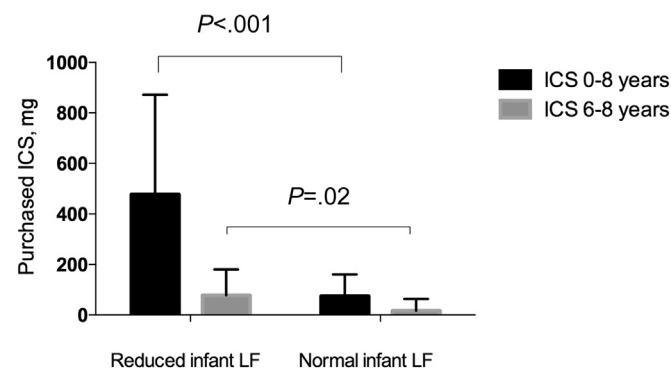


Figure 4. Purchase of inhaled corticosteroids (ICSs) in milligrams of budesonide during 0 to 8 and 6 to 8 years of age according to infant lung function (LF). Reduced infant LF was significantly associated with the purchased amount of ICSs during 6 to 8 years and 0 to 8 years of age.

No association was found between infant RBM thickness and current asthma symptoms, lung function, or AR. There was no difference in infant ASM area fraction or RBM thickness between the patients with current or past asthma or with no asthma (Fig 3B and D). The only significant correlation between histologic findings and lung function was between the density of the bronchial dendritic cells CD1aLP and CD1a and percentage of predicted FEV₁, although this was mainly due to a few outliers and is likely spurious. The bronchial densities of antigen-presenting cell subsets did not differ in relation to clinical presentation of the study population at infancy.²⁰ There was no association between any other inflammatory cell counts and any asthma-related outcomes.

Association Between Infant Lung Function and Subsequent Lung Function

Reduced lung function in early infancy is known to be related to outcomes in later childhood, including reduced lung function,²⁸ increased airway hyperresponsiveness,^{3,6} asthma,³ and greater amount of reported use³ or purchased¹⁶ ICSs, confirmed by our present results. This measure serves as a useful positive control, demonstrating that our study is powered to have expected results. Possible effects of the disease itself on the development of lung function are additional, and the present findings are not fully comparable with birth cohort study findings. In the present study, the children with reduced infant lung function had significantly higher purchased amount of ICSs (Fig 4), but there is no evidence that ICSs affect the evolution of lung function.

Although all the study children had onset of the lower respiratory tract symptoms during early life (0–1.3 years of age), we were still able to find a correlation between the age at the onset of symptoms and lung function at school age (Fig 2A, B, and C). This finding means that very early symptoms are associated with later reduced lung function, percentage of predicted FVC, FEV₁, and FEV₁/FVC. These children may have had a genetically or antenatally lower lung function that remained low. The same was found in the subgroup of children with wheeze. In addition, infant exposure to smoking determined on history was associated significantly with lower percentage of predicted FEV₁/FVC at school age. Similarly, maternal smoking was associated with increased exhaled nitric oxide and poorer lung function in children with multiple-trigger wheeze at preschool age.²⁹

Reported Use and Purchase of Asthma Medication

We have a reliable register for purchases of medication in Finland. However, we acknowledge that purchase of medication does not mean it was used appropriately or even used at all. Purchase of medication is a better surrogate than asking parents what medications were used, but it is not ideal.³⁰ We have previously reported a significant correlation between both RBM thickness and reduced lung function in infancy and subsequent purchase of ICSs at the 3 years of age.¹⁶ The correlation among RBM thickness, ASM fraction, and purchase of ICSs was no longer present at school age. Instead, reduced infant lung function and the number of wheezing episodes correlated with treatment needs during the 6 to 8 years of age. Similarly, children from a birth cohort study with infant lung function at or below median, measured by tidal breathing flow-volume loops, had significantly more reported use of ICSs at 10 years of age.³ Similarly, there was no correlation between RBM thickness or bronchial eosinophilia reported at a median age of 2 years and lung function by the multiple-breath washout method at 5 years of age,³¹ but there was a positive association between preschool airway disease and current exhaled nitric oxide.

In conclusion, recurrent lower respiratory tract symptoms in infancy are associated with decreased lung function and increased AR at 8 years of age. Reduced infant lung function but not thickness of RBM or proportion of ASM in infancy is associated with physician-diagnosed asthma, current asthma, and treatment needs in this study population. The very early onset of symptoms and infant exposure to smoking were risk factors for reduced lung function at school age. These data, which indicate for the first time to our knowledge the signal failure of very early airway disease to predict asthma outcomes in childhood in severe, infant wheezers, underscore that the early pathologic processes that initiate asthma, which are as yet undescribed, are very different from the eosinophilic inflammation associated with ongoing disease and that very different approaches will be needed if asthma is to be prevented rather than treated once it has developed.

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Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.09.019>.

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eAppendix

eMethods

Study Population

Children underwent whole-body plethysmography and bronchoscopy. Reduced baseline lung function was defined as specific airway conductance below the fifth centile (z score <-1.65) of reference range.¹ Bronchodilator responsiveness (BDR) was diagnosed if specific airway conductance increased by more than 30% and at least 2 SDs of baseline variation after administration of 0.6 mg of salbutamol via a nebulizer (Nebunette; AstraZeneca, Södertälje, Sweden).² The infants were grouped according to lung function: group A ($n = 16$), reduced specific airway conductance with BDR; group B ($n = 22$), reduced specific airway conductance without BDR; and group C ($n = 15$), normal airway conductance. The term *reduced infant lung function* is used for groups A and B.

Quantification of Airway Smooth Muscle

Endobronchial biopsy specimens were taken at infancy during clinically indicated bronchoscopy using a rigid bronchoscope (3.0 mm) from the main carina. Sections (1 μ m) were cut and stained with toluidine blue. Evaluable biopsy specimens were those fulfilling the following criteria: (1) presence of epithelium, reticular basement membrane (RBM), and associated subepithelial tissue; and (2) minimal crush artefact, edema, or blood within the biopsy. There was 53 evaluable biopsy specimens: 29 with airway smooth muscle (ASM) and 24 without ASM. The ASM area and total subepithelial area were measured at an original magnification $\times 100$ by drawing around the outer limits of the smooth muscle bundles and the subepithelial area of the biopsy in evaluable biopsy specimens stained with toluidine blue.^{3,4} The subepithelial and ASM area was then calculated using computer-aided image analysis. ASM was then expressed as a fraction of the total subepithelial area. Results are reported as the mean of all tissue sections measured for an individual child. Median biopsy area measured was 0.85 mm² (range, 0.21–1.71 mm²). Intraobserver repeatability was assessed by measuring 3 sections chosen randomly on 3 occasions 1 week and 1 month apart. The mean coefficient of variation for the 3 biopsy specimens was 4% (1%, 4%, and 7%). Measurements were made masked to lung function in infancy and outcome data.

Lung Function Tests

Spirometry was measured using a pneumotachograph-based flow volume spirometer (Masterscreen Pneumo; Jaeger GmbH, Würzburg, Germany). Of 3 successful expirations, the highest forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were recorded. On the first study visit day, the BDR was measured with a spirometer after administration of 0.3 mg of salbutamol via a spacer device (Volumatic; GlaxoSmithKline, Middlesex, England).⁵

Airway hyperreactivity was evaluated with a dosimetric histamine challenge test on the second study day.⁶ An inhalation synchronized dosimeter with controlled tidal breathing (Spira Elektro 2; Respiratory Health Care Centre, Hämeenlinna, Finland) was used to nebulize increasing inhaled doses of 0.025, 0.1, 0.4, and 1.6 mg of buffered histamine diphosphate. By using the dose-response curve, the provocation dose of inhaled histamine producing a decrease of 15% in FEV₁ (PD₁₅) was determined, and increased airway hyperreactivity was defined as PD₁₅ of 0.4 mg or less. The airway responsiveness to histamine was expressed in terms of the slope of the dose-response curve, as a continuous and log-normally distributed index,⁷ by dividing the percentage decrease in FEV₁ after the last dose by the last dose of histamine.

Fraction of exhaled nitric oxide was assessed according to the American Thoracic Society recommendations⁸ using a

chemiluminescence analyzer (Niox; Aerocrine AB, Solna, Sweden), which was calibrated with a certified nitric oxide calibration gas mixture. Children were seated, without a nose clip, and asked to fill their lungs completely with nitric oxide-free air and thereafter to exhale with a mean and instantaneous flow of 50 (5) mL/s. Exhalation time of 10 seconds was used as default, but exhalation time was allowed to be reduced to 6 seconds, if needed. Three reproducible nitric oxide values within 10% were requested, and the mean fraction of exhaled nitric oxide was recorded. The height-adjusted reference values were used,⁹ and the results were expressed as z scores.

Purchase of Inhaled Asthma Medication

Individual data on the purchase of asthma medication were collected from the central register of the Social Insurance Institution for the time from birth to the follow-up visit. The total amounts of ICSs, short-acting and long-acting β -agonists, and oral leukotriene antagonists were calculated from the number of packages, doses per package, and strength of the molecule. The ICSs was calculated in milligrams of budesonide, and because the dose equivalence between fluticasone and budesonide is approximately half 1:2,^{10,11} the fluticasone dose was doubled to give the budesonide equivalence. The unit of inhaled short-acting β -agonist was determined as milligrams of salbutamol, and terbutaline was considered to be 40% of salbutamol. The unit of inhaled long-acting β -agonist was determined as milligrams of salmeterol, and because the dose equivalence between formoterol and salmeterol is approximately 9:50,¹² the formoterol dose was multiplied with 50/9 to give the salmeterol equivalence. Leukotriene receptor antagonists were determined as milligrams of montelukast.

Statistical Analysis

Normality was assessed with the Shapiro-Wilk test. Dose response slope data were log-transformed before analysis. The Kruskal-Wallis test and Mann-Whitney test were used for comparisons of the use and purchase of asthma medication, questionnaire data, and lung function results at 8 years of age between the patient and control groups. Groups A and B were pooled to represent infants with reduced lung function. Comparison of means with normally distributed variables was performed with the t test. The associations between findings at infancy and at 8 years of age and the use of asthma medication during lifetime and during 6 to 8 years of age were evaluated with Spearman correlation coefficients and χ^2 tests. The association in 2×2 tables was tested with the Fisher exact test. Two-sided $P < .05$ was considered statistically significant. Statistical analyses were performed with SPSS statistical software for Windows version 17.0 (SPSS Inc, Chicago, Illinois).

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