

ORIGINAL ARTICLE

# Childhood Asthma after Bacterial Colonization of the Airway in Neonates

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## ABSTRACT

### BACKGROUND

Pathological features of the airway in young children with severe recurrent wheeze suggest an association between bacterial colonization and the initiating events of early asthma. We conducted a study to investigate a possible association between bacterial colonization of the hypopharynx in asymptomatic neonates and later development of recurrent wheeze, asthma, and allergy during the first 5 years of life.

### METHODS

The subjects were children from the Copenhagen Prospective Study on Asthma in Childhood birth cohort who were born to mothers with asthma. Aspirates from the hypopharyngeal region of asymptomatic 1-month-old infants were cultured for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. Wheeze was monitored prospectively on diary cards during the first 5 years of life. Blood eosinophil count and total IgE and specific IgE were measured at 4 years of age. Lung function was measured and asthma was diagnosed at 5 years of age.

### RESULTS

Hypopharyngeal samples were cultured from 321 neonates at 1 month of age. Twenty-one percent of the infants were colonized with *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms; colonization with one or more of these organisms, but not colonization with *S. aureus*, was significantly associated with persistent wheeze (hazard ratio, 2.40; 95% confidence interval [CI], 1.45 to 3.99), acute severe exacerbation of wheeze (hazard ratio, 2.99; 95% CI, 1.66 to 5.39), and hospitalization for wheeze (hazard ratio, 3.85; 95% CI, 1.90 to 7.79). Blood eosinophil counts and total IgE at 4 years of age were significantly increased in children colonized neonatally with *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms, but specific IgE was not significantly affected. The prevalence of asthma and the reversibility of airway resistance after  $\beta_2$ -agonist administration at 5 years of age were significantly increased in the children colonized neonatally with these organisms as compared with the children without such colonization (33% vs. 10% and 23% vs. 18%, respectively).

### CONCLUSIONS

Neonates colonized in the hypopharyngeal region with *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*, or with a combination of these organisms, are at increased risk for recurrent wheeze and asthma early in life.

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**C**HILDHOOD ASTHMA IS COMMONLY PREceded by recurrent asthma-like symptoms (“recurrent wheeze”).<sup>1</sup> This very common phenotype may be due to early asthma or may represent self-limiting virus-associated symptoms, and there is little to differentiate between the clinical presentations of these two conditions.<sup>2</sup> Biopsy specimens from infants with severe recurrent wheeze and reversible airflow obstruction, even in the presence of atopy, have shown neither thickening of the reticular basal membrane nor eosinophilic inflammation, changes that are characteristic of asthma in later life.<sup>3</sup> Bronchoalveolar lavage in young children with severe recurrent wheeze has demonstrated increased numbers of macrophages and neutrophils but not of eosinophils and mast cells.<sup>4,5</sup> We previously proposed that this pathologic condition of the airway in young children with severe recurrent wheeze suggests an association of bacterial colonization with the initiating events of early asthma.<sup>6</sup> In this report, we examine the association between bacterial airway colonization in asymptomatic neonates and the development of recurrent wheeze and asthma from birth through the first 5 years of life.

#### PATIENTS AND METHODS

The Copenhagen Prospective Study on Asthma in Childhood (COPSAC) is an ongoing clinical, prospective, longitudinal birth-cohort study of 411 infants born to mothers with current or previous asthma (see the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org), for details).<sup>7</sup> The exclusion criteria were severe congenital abnormality, gestational age under 36 weeks at birth, mechanical ventilation required at any time since birth, and the presence of lung symptoms before enrollment.

The study followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee for Copenhagen (KF 01-289/96) and the Danish Data Protection Agency (2002-41-2434), and written informed consent was obtained from the mothers. Data-validation and quality-control procedures followed good clinical practice guidelines. Data were collected online during visits to the COPSAC clinical research unit. This database was double-checked against source data by an external monitor and was subsequently locked. An audit trail was run routinely.

#### AIRWAY BACTERIA

Airway bacteria were investigated in the asymptomatic infants at 1 and 12 months of age. While the 1-month-old infants were sedated to allow lung-function testing,<sup>8</sup> the doctors at the clinical research unit aspirated a sample from the hypopharyngeal region with a soft suction catheter passed through the nose into the hypopharynx (see the Supplementary Appendix for details). Hypopharyngeal sampling was repeated in the 12-month-old awake infants. The samples were transported to the microbiology laboratories within 2 hours after collection and cultured for bacteria with the use of standard methods for identification of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.<sup>9</sup> The identification criteria for the species cultured were chosen before the study. The personnel in the clinical research unit were unaware of the results of the cultures. Because the infants were asymptomatic, the culture results were not reported to the parents and the infants were not treated.

#### CLINICAL END POINTS

The primary end point was the longitudinal analysis of persistent wheeze, defined according to a strict algorithm based on repeated episodes of wheezy symptoms. The survival analysis describes the cumulative risk of persistent wheeze in the past, is not restricted to patients with symptoms at 4 to 5 years of age, and should not be confused with a diagnosis of asthma by age 5. The cross-sectional diagnosis of asthma by the age of 5 was an independent end point defined as having typical asthma symptoms and being treated with inhaled corticosteroids and rescue medication during any part of the year before reaching the age of 5 years.

Respiratory symptoms were recorded by the parents in daily diaries for 5 years. Wheeze was defined to the parents as wheezing or whistling sounds, breathlessness, or persistent troublesome cough severely affecting the well-being of the infant or child; the presence or absence of wheeze was recorded as a composite, dichotomized (yes or no) score, as previously described.<sup>10</sup> The description of symptoms was supported by a book (written for parents, about early childhood wheeze) that was integrated with the diary cards (see the Supplementary Appendix and <http://ipaper.dk/>

copsac/Asthma\_in\_young/). The doctors in the clinical research unit reviewed the definition of symptoms and the diary entries with the parents at clinical sessions every 6 months as well as during acute episodes of wheeze. The infants were given a full physical examination, and a history was obtained by the doctors at the clinical research unit with the use of structured questions and closed response categories focusing on each child's lung symptoms, diagnoses, medication, use of health care, lifestyle, and home environment. The families used the doctors in the clinical research unit (not their family practitioners) for diagnosis and treatment of any respiratory or atopy-related symptoms.

A wheezy episode was defined on the diary card as 3 consecutive days of wheeze, at which point the parents were requested to bring the child to the clinical research unit for examination. Persistent wheeze was defined as five such episodes within 6 months or daily symptoms for 4 consecutive weeks. Differential diagnoses of possible clinical conditions were excluded at that point by chest radiographs and sweat chloride tests. A child was considered to have had an acute severe exacerbation of wheezy symptoms if the diagnosis had been made by the clinical research unit doctor and the child had been treated with oral or high-dose corticosteroids or if the child had been treated at the local hospital with oral or high-dose inhaled corticosteroids for such symptoms. Asthma in 5-year-old children was diagnosed by the clinical research unit doctors according to the Global Initiative for Asthma guidelines<sup>2</sup> on the basis of a history of persistent symptoms (as defined above) that were recorded in diaries and were judged to be typical of asthma (e.g., exercise-induced symptoms, prolonged nocturnal cough, persistent cough not due to common cold, and symptoms causing wakening at night), the response to a 3-month course of inhaled corticosteroids, and a requirement for intermittent use of an inhaled  $\beta_2$ -agonist to relieve dyspnea (see the Supplementary Appendix).

Specific airway resistance was measured at 5 years of age by whole-body plethysmography<sup>11,12</sup> before and after use of an inhaled  $\beta_2$ -agonist.<sup>13</sup> Blood was sampled at the ages of 6 months, 18 months, and 4 years for measurements of eosinophil count (in  $10^9$  cells per liter), total IgE, and

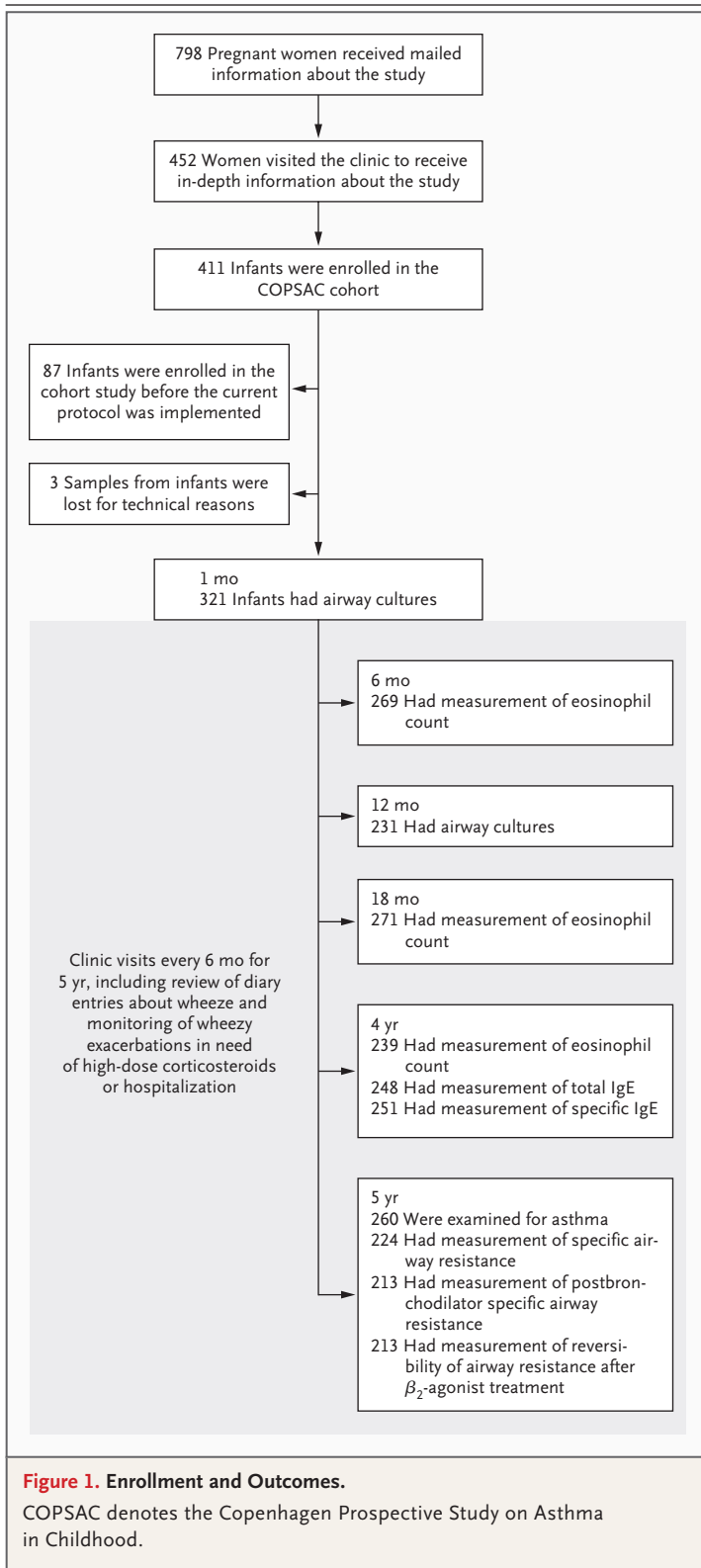
specific IgE. The serum level of total IgE was determined by ImmunoCAP (Phadia),<sup>14</sup> with a detection limit of 2 kU per liter. Specific IgE was determined by a commercial assay for IgE (ImmunoCAP Phadiatop Infant, Phadia) against the most common food and inhalant allergens (hen's eggs, cow's milk, peanuts, shrimp, dust mites, cat dander, dog dander, birch pollen, timothy grass, ragweed, and wall pellitory [nettles]). Values of Phadiatop Infant of 0.35 kU per liter or more were considered indicative of sensitization,<sup>15</sup> and this measure was analyzed as a dichotomized variable (sensitized or not sensitized).

#### TREATMENT ALGORITHMS

Infants with positive cultures were not treated with antibiotics because they were asymptomatic at the time of sampling. All subjects participated in the randomized, controlled clinical trial of intermittent treatment with inhaled budesonide as compared with placebo for 2 weeks during wheezy episodes in the first 3 years of life, which showed no short-term or long-term treatment effects.<sup>10</sup> Wheezy symptoms were treated by the clinical research unit doctors in accordance with the following algorithm: for symptomatic relief, parents were provided with terbutaline (Bricanyl, Astra-Zeneca) in a pressurized, metered-dose inhaler with a spacer<sup>16</sup> to be administered as needed. Persistent wheeze defined the threshold for treatment with 400  $\mu$ g per day of inhaled budesonide administered by a pressurized, metered-dose inhaler with a spacer for 3 months, increasing to 6 and 12 months at subsequent relapses. Montelukast at a dose of 4 mg daily was added for children who had recurrent wheeze despite receiving budesonide maintenance treatment. Acute severe exacerbation of wheeze was treated with 1600  $\mu$ g of budesonide daily for 2 weeks or 1 to 2 mg of oral prednisolone per kilogram of body weight daily for 3 days, at the discretion of the clinical research unit physician. No other treatment for wheezy symptoms was allowed.

#### STATISTICAL ANALYSIS

The primary end points were the indicators of recurrent wheeze: first wheezy episode, persistent wheeze, acute severe exacerbation of wheeze, and hospitalization for wheeze during the 5-year prospective follow-up. Asthma was diagnosed at the age of 5 years. The secondary outcomes were lung



function, blood eosinophil counts, total IgE, and specific IgE.

The cumulative risks of the primary end points stratified according to colonization by bacteria were estimated by the Kaplan–Meier method. Changes in risk due to bacterial colonization were quantified as hazard ratios obtained by Cox regression. Confounder-adjusted hazard ratios were calculated for the subcohort of children who had full records of the confounders (279 of 321 children). The effects of bacterial colonization on the logarithm of postbronchodilator lung function at 5 years of age, untransformed reversibility at 5 years of age, and the logarithm of total IgE at 4 years of age were assessed by analysis of variance. Specific IgE at 4 years and asthma at 5 years were modeled by logistic regression. Log-transformed counts of blood eosinophils at the ages of 6 months, 18 months, and 4 years were modeled by a mixed linear model including a random effect for each child. Estimated measures of colonization effects are presented with 95% confidence intervals.

## RESULTS

A total of 798 pregnant women from greater Copenhagen with a history of physician-diagnosed asthma received mailed written information about the study; 452 of these women attended the clinic to receive in-depth information on the study, and 411 infants were enrolled between August 1998 and December 2001 (Fig. 1). Hypopharyngeal samples were collected from 321 of 324 eligible infants at the 1-month visit (three samples were lost for technical reasons); an additional 87 infants had been enrolled into the COPSAC cohort of 411 infants before this protocol was enacted (see the Supplementary Appendix for details). Of the 321 children with valid samples, 305 (95%) completed 1 year, 287 (89%) completed 2 years, 278 (87%) completed 3 years, 259 (81%) completed 4 years, and 253 (79%) completed 5 years of this clinical study. A second sample was collected from 231 of the 321 infants at the 12-month visit.

## COLONIZATION

At 1 month of age, 30 of the 321 neonates (9%) were colonized with *S. pneumoniae*, 28 (9%) with *H. influenzae*, 27 (8%) with *M. catarrhalis*, and 196 (61%) with *S. aureus*, and 1 (<1%) was colonized with *S. pyogenes*. Sixty-six (21%) were colonized with

*S. pneumoniae*, *M. catarrhalis*, or *H. influenzae* or with more than one of these species. Multiple species were found in 17 infants; 15 had two species and 2 had three species. There were 73 different *fag* gene patterns among *S. aureus*; the most common pattern was found in 14% of *S. aureus* cultures. At 1 year, the prevalence of *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms was 71%, and the prevalence of *S. aureus* was 13%.

#### BASELINE CHARACTERISTICS

Neonates with and those without colonization at 1 month by *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms did not differ with respect to the baseline characteristics of sex, gestational age at birth, maternal smoking

during the third trimester, maternal use of antibiotics during the third trimester, exclusive breast-feeding for at least 4 weeks, lung function (forced expiratory volume in 0.5 second), and bronchial responsiveness (provocative dose estimated from the transcutaneous oxygen tension).<sup>8</sup> Neonates with older siblings at home were more likely to be colonized; 70% of those colonized had older siblings, as compared with 29% of those without colonization (see the Supplementary Appendix for details).

#### COLONIZATION IN RELATION TO WHEEZE

The risk of wheeze increased in neonates colonized with *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms, but not in those colonized with *S. aureus* (Table 1). The

**Table 1. Hazard Ratios for the Presence of Bacteria in Cultures from Airways at 1 Month of Age in Relation to Primary End Points.**

End Point and Bacterial Species	Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)*
First wheezy episode		
<i>Streptococcus pneumoniae</i>	1.54 (1.02–2.31)	1.53 (0.97–2.40)
<i>Haemophilus influenzae</i>	1.49 (1.00–2.22)	1.27 (0.82–1.97)
<i>Moraxella catarrhalis</i>	1.83 (1.20–2.78)	1.76 (1.08–2.85)
<i>Staphylococcus aureus</i>	1.03 (0.80–1.32)	0.97 (0.74–1.26)
At least one of <i>S. pneumoniae</i> , <i>H. influenzae</i> , or <i>M. catarrhalis</i>	1.65 (1.24–2.21)	1.50 (1.08–2.10)
Persistent wheeze		
<i>S. pneumoniae</i>	1.71 (0.85–3.45)	1.41 (0.65–3.07)
<i>H. influenzae</i>	2.85 (1.52–5.33)	2.73 (1.36–5.48)
<i>M. catarrhalis</i>	2.19 (1.12–4.28)	1.53 (0.72–3.25)
<i>S. aureus</i>	1.04 (0.63–1.71)	1.00 (0.59–1.68)
At least one of <i>S. pneumoniae</i> , <i>H. influenzae</i> , or <i>M. catarrhalis</i>	2.40 (1.45–3.99)	2.01 (1.13–3.57)
Acute severe exacerbation of wheeze		
<i>S. pneumoniae</i>	1.80 (0.80–4.02)	2.02 (0.79–5.17)
<i>H. influenzae</i>	3.23 (1.60–6.52)	3.78 (1.70–8.40)
<i>M. catarrhalis</i>	2.72 (1.27–5.84)	2.52 (0.92–5.51)
<i>S. aureus</i>	1.01 (0.56–1.82)	1.09 (0.58–2.05)
At least one of <i>S. pneumoniae</i> , <i>H. influenzae</i> , or <i>M. catarrhalis</i>	2.99 (1.66–5.39)	3.14 (1.57–6.30)
Hospitalization for wheeze		
<i>S. pneumoniae</i>	1.90 (0.73–4.94)	2.33 (0.72–7.54)
<i>H. influenzae</i>	3.81 (1.70–8.51)	4.09 (1.65–10.15)
<i>M. catarrhalis</i>	3.68 (1.58–8.54)	2.93 (1.06–8.11)
<i>S. aureus</i>	1.18 (0.57–2.46)	1.32 (0.58–2.99)
At least one of <i>S. pneumoniae</i> , <i>H. influenzae</i> , or <i>M. catarrhalis</i>	3.85 (1.90–7.79)	3.57 (1.55–8.23)

\* Hazard ratios were adjusted for the following possible confounders: sex, gestational age at birth, maternal smoking during the third trimester, maternal use of antibiotics during the third trimester, breast-feeding, lung function, bronchial responsiveness, and the presence or absence of older children at home.

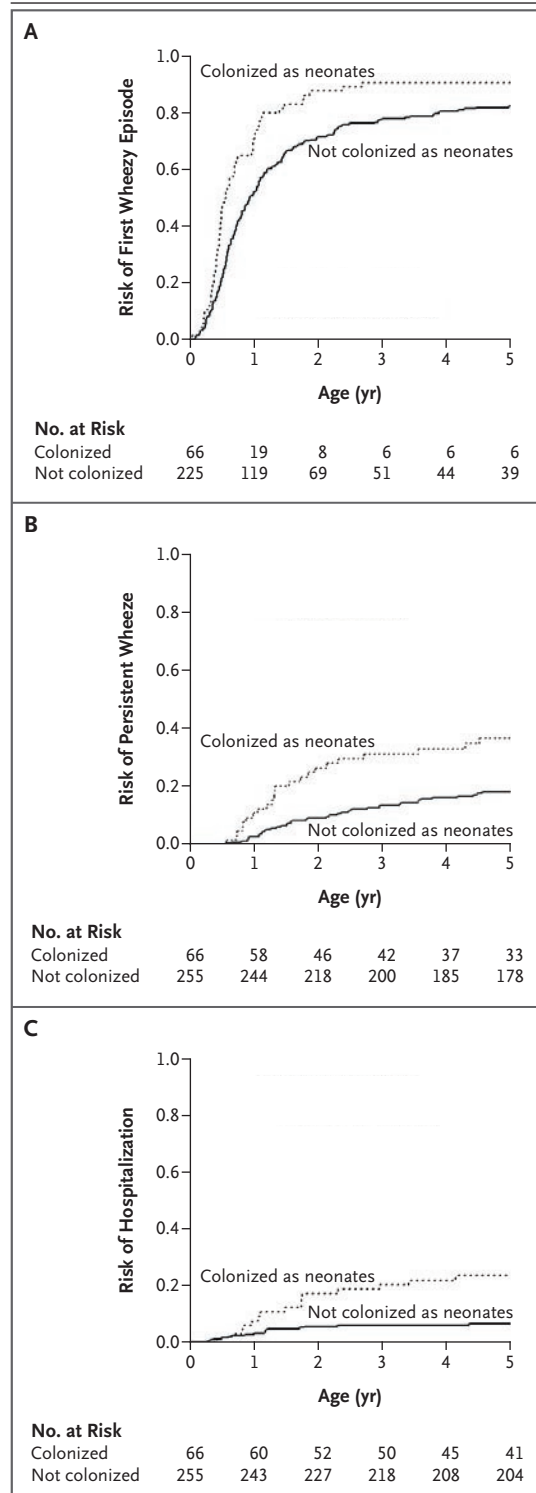
**Figure 2.** Cumulative Risk of First Wheezy Episode (Panel A), Persistent Wheeze (Panel B), and Hospitalization for Wheeze (Panel C) during the First 5 Years of Life for Neonates with and Those without Early Colonization with *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, or a Combination of These Organisms.

hazard ratio for the presence of *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms was 1.65 (95% confidence interval [CI], 1.24 to 2.21) for a first wheezy episode, 2.40 (95% CI, 1.45 to 3.99) for development of persistent wheeze, 2.99 (95% CI, 1.66 to 5.39) for acute severe exacerbation of wheeze, and 3.85 (95% CI, 1.90 to 7.79) for hospitalization for wheeze.

Hazard ratios adjusted for the baseline characteristics did not cause any substantial change in the primary end points (Table 1). Extended Cox regression analyses for primary outcomes found no additional effect of *S. pneumoniae*, *M. catarrhalis*, or *H. influenzae* either individually or in combination.

In a post hoc evaluation of the Kaplan–Meier analysis (Fig. 2), the change in risk of wheeze associated with colonization by *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these species increased during the first 2 years of life and then stabilized. The hazard ratio for persistent wheeze was 3.15 (95% CI, 1.68 to 5.90) for children under 2 years of age and 1.46 (95% CI, 0.59 to 3.64) for children from 2 to 5 years of age; these hazard ratios were not significantly different (P=0.17, Wald test for equality). Similar conclusions were obtained for a first wheezy episode, acute severe exacerbation of wheeze, and hospitalization for wheeze.

Colonization at 12 months was not significantly correlated with neonatal colonization (odds ratio, 1.95; 95% CI, 0.85 to 4.47). To assess the simultaneous effect of colonization at 4 weeks of age and colonization at 12 months of age on outcome, a Cox regression analysis was performed that included an additional 1-to-5-year effect of colonization at 12 months and included only follow-up in the 0-to-1-year age period for children with a missing record of colonization at 12 months. For persistent wheeze, the hazard ratio for colonization at 4 weeks, adjusted for colonization at 12 months, was estimated as 2.63 (95% CI, 1.48 to 4.68), and a Wald test found no significant effect of colonization at 12 months (P=0.39). Similar



conclusions were obtained for a first wheezy episode, acute severe exacerbation of wheeze, and hospitalization for wheeze. There was no confounding effect of the intermittent treatment of

wheezy episodes with inhaled budesonide in the nested, randomized, controlled trial previously reported.<sup>10</sup>

#### COLONIZATION IN RELATION TO LUNG FUNCTION, BLOOD EOSINOPHIL COUNT, AND ALLERGY

The reversibility of airway resistance after  $\beta_2$ -agonist treatment was 23% in children colonized as neonates and 18% in those not colonized, for a difference of 5 percentage points (95% CI, 0 to 10); postbronchodilator airway resistance did not differ significantly between these groups of children (Table 2). The percentage change in blood eosinophil count increased significantly with age in children who were colonized ( $P=0.02$ ) (Table 2). Total IgE was significantly increased by 47% (95% CI, 1 to 115%) at 4 years of age in children who were colonized ( $P=0.05$ ), but specific IgE at 4 years was not significantly affected by colonization (odds ratio, 1.28; 95% CI, 0.65 to 2.54;  $P=0.48$ ) (Table 2).

#### COLONIZATION IN RELATION TO ASTHMA

The overall prevalence of asthma at 5 years of age was 14%; the prevalence was 33% in colonized children and 10% in those not colonized (odds ratio, 4.57; 95% CI, 2.18 to 9.57) (Table 2). The population attributable risk of asthma associated with colonization was 4.6% (95% CI, 1.9 to 7.3).

## DISCUSSION

Colonization of the airways with *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, or more than one of these organisms in asymptomatic neonates at 1 month of age was associated with increases by a factor of two to four in the risk of a first wheezy episode, persistent wheeze, acute severe exacerbation of wheeze, and hospitalization for wheeze, as well as increased blood eosinophil counts and total IgE and, eventually, increased reversibility of airway resistance and development of asthma by the age of 5 years.

These associations are compatible with the observation of a predominantly neutrophilic inflammation in young children with severe recurrent wheeze.<sup>3-6</sup> The association was specific for the typical pathogenic airway bacteria *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, whereas there was no such association for the typical skin bacterium *S. aureus*. The association was time specific: the symptoms were associated with colonization at 1 month but not at 12 months of age. The risk rates of the colonized and the noncolonized groups separated during the second year of life and remained separated through all 5 study years (Fig. 2).

Hypopharyngeal aspirates were obtained from

**Table 2.** Colonization in Relation to Asthma Diagnosis, Lung Function, and Allergy.

Outcome	Colonized	Not Colonized	Odds Ratio (95% CI)	Percent Estimated Difference (95% CI)*
Asthma at 5 yr (no.)				
Yes	17	20	4.57 (2.18 to 9.57)	
No	35	188		
Specific IgE at 4 yr (no.)				
>0.35 kU/liter	15	49	1.28 (0.65 to 2.54)	
≤0.35 kU/liter	36	151		
Mean postbronchodilator specific airway resistance at 5 yr (kPa·sec·liter <sup>-1</sup> )†	0.94	1.00		-7 (-13 to 1)
Reversibility of specific airway resistance after $\beta_2$ -agonist inhalation at 5 yr (%)‡	-23	-18		5 (0 to 10)
Blood eosinophil count at 4 yr ( $\times 10^{-9}$ /liter)§	0.42	0.29		31 (6 to 62)
Total IgE at 4 yr (kU/liter)¶	90	60		47 (1 to 115)

\* Relative differences are given, except for reversibility of specific airway resistance after  $\beta_2$ -agonist inhalation, for which absolute differences are given. Estimated relative differences were calculated as the ratio of geometric means for the colonized and not colonized groups.

† Of 213 infants, 39 were colonized and 174 were not colonized.

‡ Of 213 infants, 39 were colonized and 174 were not colonized.

§ Of 239 infants, 47 were colonized and 192 were not colonized.

¶ Of 248 infants, 49 were colonized and 199 were not colonized.

1-month-old infants while they were sedated for lung-function testing. Aspiration was guided by the cough reflex elicited when the laryngeal region was approached. Restricting cultures to those with representation of ciliated columnar epithelium (i.e., those probably originating from the lungs) confirmed the observed associations and suggest that the finding is independent of sampling site. However, the exact anatomical origin of the bacterial colonization in the airways cannot be determined. Bacterial colonization may have a larger role than that seen in our study, since we screened for only common pathogenic bacteria, and other bacteria may have a similar effect.

At 1 month, 61% of the infants were colonized with *S. aureus*, and 21% were colonized with *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or a combination of these organisms. At 12 months, this pattern had changed to a colonization pattern dominated by *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* (71%), and colonization with *S. aureus* had become rarer (13%). A very similar colonization pattern has been reported in other cohorts not selected for risk of asthma.<sup>17-19</sup> Colonization was independent of sex, maternal smoking during the third trimester, maternal use of antibiotics during the third trimester, breast-feeding, gestational age at birth, and baseline lung function measured at 1 month of age and was associated with the presence of older children at home<sup>17</sup>; however, adjustment for these confounders had no substantial effect on the association of colonization with wheeze and asthma.

Neonatal airway colonization with *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or more than one of these organisms was associated with several independent intermediate asthma phenotypes, including symptoms of persistent wheeze and acute severe exacerbation of wheeze, hospitalization for wheeze, and increased blood eosinophil counts<sup>20</sup> and total IgE.<sup>21</sup> This association between neonatal airway colonization and the progression of intermediate asthma phenotypes was confirmed by the increased reversibility of airway resistance and increased asthma prevalence at the age of 5 years. The diagnosis of asthma was made at the 5-year visit on the basis of the closely monitored history of the preceding year, together with a history of typical asthma symptoms and response to treatment in accordance

with the Global Initiative for Asthma guidelines (see the Supplementary Appendix).<sup>2</sup>

The population attributable risk in this high-risk population was estimated as 4.6 percentage points, a result suggesting that elimination of the risk associated with bacterial airway colonization should lead to a drop in overall asthma prevalence by the age of 5 years from 14.2 to 9.4% in similar high-risk populations. The generalizability of the findings is restricted by the high-risk nature of the cohort,<sup>7</sup> and validation in unselected populations is needed.

The intermediate asthma phenotypes vary with age, as seen in young children with persistent wheeze, who often outgrow their symptoms,<sup>22,23</sup> and in the contribution of allergy, which is more strongly associated with asthma risk when it occurs early in life. Likewise, it is possible that the observed association between bacterial airway colonization and the intermediate phenotypes of asthma and atopy would be different later in life.

The hypopharyngeal colonization status in neonates provides a predictive marker for the development of persistent wheeze, asthma, and atopy that may be useful for future targeted research in the prevention of early asthma and allergy. In conclusion, the association between early bacterial colonization of the airways of neonates and intermediate asthma phenotypes in the first years of life, as well as the development of asthma by the age of 5 years, opens new perspectives for the understanding and prediction of recurrent wheeze and asthma in young children.

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