



Original Article

Long-term effects of pneumococcal colonization during early childhood wheezing

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Abstract **Background:** Bacterial colonization during wheezing in early childhood has been associated with short-term relapses of wheezing, but no study has addressed the effects of *Streptococcus pneumoniae* colonization on long-term outcome of wheezing. The aim of the present study was therefore to evaluate whether pneumococcal (PNC) colonization during the first wheezing episode in early childhood is a determinant of asthma, atopy or lung function in the long term.

Methods: In 1981–82 83 infants were hospitalized for first wheezing episode at <24 months of age. PNC colonization was defined as positive nasopharyngeal aspirate for *S. pneumoniae* either in culture or antigen detection on hospital admission. Atopy and repeated wheezing or asthma were diagnosed on all follow-up visits from infancy until the age of 28–31 years. Spirometry was conducted at the ages of 8–10, 18–20 and 28–31 years.

Results: PNC colonization was found in 25/83 infants (30%) during hospitalization for wheezing in infancy. PNC colonization was not associated with later atopy, repeated wheezing, asthma or lung function at any time during the 30 year follow up.

Conclusion: PNC colonization during the first wheezing episode in early childhood is not a determinant of subsequent wheezing or later asthma, atopy or lung function in childhood, adolescence or adulthood.

Key words asthma, atopy, lung function, *Streptococcus pneumoniae*, wheezing.

It has been well documented that wheezing during viral respiratory infection in early childhood increases the risk of asthma and lung function deficits up to adulthood.^{1,2} Bacterial infection and colonization, however, are also independently associated with wheezing episodes in children,^{3,4} but the role of bacteria in wheezing illness is, compared with viruses, much less studied.^{3–5} Colonization of the nasopharynx by potential respiratory pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* is common in childhood, and *S. pneumoniae* is clinically the most important respiratory pathogen at all ages, with high colonization rates in young children.⁶ In addition, wheezing children with respiratory bacteria colonization at the time of the wheezing episode have a prolonged hospitalization time,^{7,8} increased prevalence of recurrent wheezing within 2 months after discharge,⁷ and the greatest burden of illness symptoms.^{5,8} Pneumococcal (PNC) colonization, however, was not associated with increased risk of wheezing 1 year after the initial wheezing episode in a previous Finnish study.⁷

There are currently no studies available on how PNC colonization during the first wheezing episode is associated with long-term

outcomes such as atopy, asthma or lung function in these children, and a call for studies on the long-term effects of colonization with respiratory bacteria during early childhood wheezing has been made.⁹

We have prospectively followed up a group of 83 children who were hospitalized for their first wheezing illness at <24 months of age in 1981–1982 from early childhood until the age of 28–31 years.^{1,2,10–13} On hospital admission *S. pneumoniae* was studied via culture and antigen detection in nasopharyngeal aspirates (NPA).¹⁴

Previous studies on PNC colonization during wheezing in children have suggested that *S. pneumoniae* may not be an innocent bystander during the respiratory infection.^{5,7,8} The aim of the present study was therefore to evaluate whether PNC colonization during the first wheezing episode in early childhood is a determinant of subsequent wheezing or later asthma, atopy and lung function in childhood, adolescence or adulthood. Although the influences of PNC colonization in the previous Finnish study were limited to the first 2 months after bronchiolitis,⁷ we hypothesized that longer influences might also be present.

Methods

This prospective study, conducted during the 12 months surveillance period 1981–1982, included all 83 infants who were hospitalized for bronchiolitis at <24 months of age at the Department of Pediatrics at Kuopio University Hospital, Finland.¹⁰ Patients who

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presented with acute lower respiratory tract infection associated with audible wheezing, prolonged expiration and signs of respiratory distress at the time of the hospital admission were diagnosed with bronchiolitis and were enrolled in the study. In addition to medical examination, chest radiography was done in all cases, and 48 patients had interstitial infiltration on chest radiograph.¹⁰

Viral etiology of infection was documented on antigen assay in NPA and/or antibody assay in paired sera for seven respiratory viruses, including respiratory syncytial virus (RSV), parainfluenza 1, 2 or 3 viruses, adenovirus, and influenza A and B viruses, as previously described.¹⁰ PNC colonization was studied on PNC culture and/or PNC antigen detection with serotype/group-specific counterimmunoelectrophoresis (CIEP), and types 7 and 14 with latex particle agglutination (LPA) in the same NPA as viruses.¹⁴ Bacterial culture was investigated in all 83 subjects and antigen detection test in 64 subjects. Patients with positive PNC culture or positive PNC antigen test were considered to have pneumococcal colonization. RSV was identified in 39%, other virus in 12%, and *S. pneumoniae* in 30% of the 83 subjects. *S. pneumoniae* strains isolated on culture were typed with LPA (types 7 and 14) and CIEP (other types) using serotype/group-specific antisera. Serum C-reactive protein (CRP) was measured via the routine method on admission.

The subjects have been followed up prospectively with repeated study visits until young adulthood. After the index bronchiolitis episode, four prospective follow-up studies were conducted to collect the early childhood data: one on convalescence 4–6 weeks after hospitalization; two between the ages 1 and 2 years; and one between the ages of 2 and 3 years. Since then four clinical follow-up visits have been conducted at the ages 4–6 years, 8–10 years, 18–20 years and 28–30 years.^{1,2,10–13}

Data on doctor-diagnosed wheezing or asthma were collected at follow-up visits. Doctor-diagnosed, repeated wheezing, defined as more than two wheezing episodes during the year preceding the control visit, was present in 44/76 children (58%) at 2–3 years, in 25/68 (37%) at 3–4 years and in 15/68 (25%) at 4–6 years of age.^{11,12} Thereafter, asthma status was evaluated at three follow-up visits. Doctor-diagnosed asthma was present at the age of 8–10 years in 9/62 (14.5%), at the age of 18–20 years in 16/54 (30%) and at the age of 28–31 years in 25/48 (31%) former bronchiolitis patients.^{1,2,13}

Flow-volume spirometry was measured at the ages of 8–10 years, 18–20 years and 28–31 years in 58, 53 and 47 subjects, respectively.^{1,13,15} The measured indices were forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1) and FEV1/FVC ratio. FVC, FEV1 and FEV1/FVC ratio are presented as percentage of the mean of age- and sex-specific, height-related reference values (FVC%, FEV1% and FEV1/FVC%).¹⁶

Serum allergen-specific immunoglobulin E (IgE; RAST, Pharmacia Diagnostica, Uppsala, Sweden) was measured in 67 children at 1.5–2 years of age and in 76 children at 2.5–3 years of age.¹⁷ The studied allergens were birch, timothy grass and mugwort pollens, house dust mite *Dermatophagoides pteronyssinus*, spores of *Cladosporium herbarum*, and dander of cat and dog. Atopic sensitization (AS) was defined as specific IgE to any allergen at the detection level > 0.35 kU/L.

Skin prick tests were performed at the ages of 8–10 years, 18–20 years and 28–30 years, including birch, timothy grass, mugwort, dog dander, cat dander, dust mites (*D. pteronyssinus*, *D. farinae*) and one mold (*C. herbarum*).^{1,2,13} Horse, cow, common alder and dust mite *Acarus siro* were also included in the panel at the age of 28–30 years. Weal with diameter greater than 3 mm and greater than half of the positive control (histamine dihydrochloride) was considered as positive. AS was defined as at least one positive reaction for tested allergens.

Statistical analysis

Data were analyzed using SPSS 21.0 (SPSS, Chicago, IL, USA). Chi-squared and Fisher's exact test were used in the univariate and logistic regression in the multivariate analysis of the categorized data. Linear mixed model was used in the analyses of continuous lung function data.

Ethics

The Ethics Committee of Kuopio University Hospital approved the original study plan and the 4–6, 8–10 and 18–20 year follow-ups. The Ethics Committee of Pohjois-Savo Health-Care District approved the 30 year follow-up study. At the beginning the parents were informed of the voluntary nature of the study, and consent for the participation of the child was obtained from the parents. Informed written consent was obtained from all subjects who participated in the adulthood follow-up visits.

Results

On admission for infant bronchiolitis, 25 (30%) of 83 patients were colonized with *S. pneumoniae*; 16/64 were PNC antigen positive and 18/83 were PNC culture positive. Twenty patients (24%) were aged <6 months, 29 (35%) were 6–12 months, and 34 (41%) were 12–24 months old; *S. pneumoniae* was found in 20%, 28% and 38% of these patients, respectively.

There were no significant differences in age or sex distributions in relation to positive PNC findings on admission to hospital (Table 1). Median serum CRP was higher in PNC-positive than in PNC-negative patients (Table 1). There was no significant association between serum CRP and presence of infiltration on chest radiography (data not shown) or between PNC colonization and presence of infiltration on chest radiography (Table 1).

Respiratory syncytial virus was identified in 32 subjects (39%; Table 2). PNC colonization was almost equally common in RSV-positive (28%) and RSV-negative subjects (16/51, 31%; Table 2). In all, 41 patients (49%) were negative for the studied viruses, and 34% of these were positive for *S. pneumoniae* at the time of the hospitalization (Table 2).

There were no significant associations between PNC colonization during hospitalization for wheezing in early childhood and AS at any age during the 28–30 year follow-up (Table 3). At the age of 2–2.5 years, however, 25% of those with PNC colonization were atopic, compared with 8% of those without such colonization ($P = 0.064$).

The PNC involvement was not associated with repeated wheezing at any age, including the age periods <12 months and 13–24 months, or with doctor-diagnosed asthma at any age (Table 3).

Table 1 Patient characteristics vs PNC colonization during hospitalization for wheezing in early childhood

	<i>S. pneumoniae</i> positive (n = 25)	<i>S. pneumoniae</i> negative (n = 58)	P-value
Sex (Male), n (%)	17 (68)	39 (67)	0.946
Age at hospital admission (months), mean (95%CI)	13.3 (10.7–15.9)	10.8 (9.2–12.5)	0.109
Infiltration present [†] n = 48 (58%)	15 (60)	33 (57)	0.793
CRP (mg/L), median (range)	26 (9–102)	9 (9–83)	0.020

[†]Interstitial infiltration on chest radiograph. CRP, C-reactive protein; PNC, pneumococcal.

Table 2 *Streptococcus pneumoniae* colonization vs viral findings[†]

Virus [†]	n (%)	<i>S. pneumoniae</i> positive n (%)
RSV	32 (39)	9 (28)
Adenovirus	5 (6)	1 (20)
Parainfluenza 1,2 or 3 virus	5 (6)	1 (20)
Negative	41 (49)	14 (34)
Total	83	25

[†]During hospitalization for the first wheezing episode in early childhood. Viral etiology of infection was documented on either antigen assay in nasopharyngeal aspirates and/or antibody assay in paired sera. RSV, respiratory syncytial virus.

There were no statistically significant differences between originally PNC-positive and PNC-negative patients in lung function at the age of 8–10 years, 18–20 years or 28–30 years (Table 4).

Subgroup analysis was done for those 49 subjects hospitalized at <12 months of age (12 were positive for *S. pneumoniae*), and the results in terms of AS, repeated wheezing, asthma or lung function remained negative (data not shown), as was found for the whole study group. In addition we studied the possible synergy between PNC colonization and viral infections. In these subgroup analyses PNC colonization did not increase the risk of AS, repeated wheezing, asthma or lung function abnormalities among those who had or did not have RSV infection or among those who had or did

not have confirmed viral infection during the first wheezing episode (data not shown).

Discussion

The present results are clearly negative. We were not able to identify any connection between PNC colonization during hospitalization for wheezing in early childhood and subsequent repeated wheezing, later asthma, AS or later lung function at any time during the 28–30 years of prospective follow up.

Pneumococcal colonization of upper airways in children is common, being at its highest at the age of 2 years.^{6,18} In the present study, 30% of children hospitalized for their first wheezing episode at the age of <24 months were colonized with *S. pneumoniae*, in line with another Finnish study in which the PNC colonization rate was 22% at 12 months, 37% at 18 months and 43% at 24 months of age in non-symptomatic children.¹⁹ Overall, children with viral respiratory infection have higher colonization rates than non-infected children,^{5,19} due to the greater mucosal adherence of bacteria, facilitated by viral infection.²⁰

In the present post-bronchiolitis follow up, PNC colonization at the time of hospitalization for the first wheezing episode was not associated with subsequent repeated wheezing in early childhood nor with asthma in later life. In addition, we were not able to demonstrate any synergy between viral infections and PNC colonization, or, more specifically, between RSV infection and PNC

Table 3 PNC colonization during hospitalization for wheezing in early childhood vs AS or respiratory symptoms at any age

	n (%)	<i>S. pneumoniae</i> positive (n = 25)	<i>S. pneumoniae</i> negative (n = 58)	P-value
AS				
1–1.5 years [†]	12/67 (18)	6/22 (27)	6/45 (17)	0.296
2–2.5 years [†]	10/76 (13)	6/24 (25)	4/52 (8)	0.064
8–10 years [‡]	8/23 (35)	2/7 (29)	6/16 (38)	1.000
18–20 years [‡]	32/51 (63)	11/16 (69)	21/35 (60)	0.549
28–30 years [‡]	30/48 (63)	9/14 (64)	21/34 (62)	0.870
Repeated wheezing [§]				
<12 months	18/69 (26)	6/22 (27)	12/47 (26)	0.878
1–2 years	36/69 (52)	13/22 (59)	23/47 (49)	0.431
2–3 years	44/76 (58)	15/24 (63)	29/52 (56)	0.581
3–4 years	25/68 (37)	10/22 (46)	15/46 (33)	0.304
4–6 years	17/68 (25)	6/22 (27)	11/46 (24)	0.765
Asthma [¶]				
8–10 years	9/62 (15)	5/20 (25)	4/42 (10)	0.106
18–20 years	16/54 (30)	3/16 (19)	13/38 (34)	0.256
28–30 years	15/48 (31)	5/14 (36)	10/34 (29)	0.669

[†]Specific IgE to any allergen tested > 0.35 kU/L. [‡]At least one positive skin prick test. [§]More than two wheezing episodes during the year preceding the control visit. [¶]Doctor-diagnosed asthma at the control visit. AS, atopic sensitization; PNC, pneumococcal.

Table 4 Lung function vs PNC colonization during hospitalization for wheezing in early childhood

Lung function	<i>S. pneumoniae</i> colonization during the first wheezing episode								
	Age 8–10 years			Age 18–20 years			Age 28–30 years		
	positive <i>n</i> = 20	negative <i>n</i> = 38	<i>P</i> -value	positive <i>n</i> = 16	negative <i>n</i> = 37	<i>P</i> -value	positive <i>n</i> = 14	negative <i>n</i> = 33	<i>P</i> -value
FVC%	100 (94–105)	96 (91–101)	0.202	112 (104–120)	106 (102–110)	0.089	96 (89–102)	99 (94–103)	0.469
FEV1%	93 (88–99)	95 (91–99)	0.815	102 (96–108)	96 (92–100)	0.097	86 (78–94)	86 (83–90)	0.902
FEV1/FVC%	85 (82–88)	88 (86–90)	0.148	84 (80–88)	83 (81–86)	0.710	77 (72–81)	75 (72–78)	0.497

FEV1%, forced expiratory volume in 1 s, presented as % of predicted value; FVC%, forced expiratory volume in 1 s, presented as % of predicted value; PNC, pneumococcal.

colonization, in relation to later outcome. In a previous Finnish study, nasopharyngeal bacterial colonization, documented on positive cultures for *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, during the first wheezing episode in children younger than 3 years of age was associated with a longer duration of hospitalization and higher risk of relapses of wheezing within 2 months after hospitalization.⁷ In addition, a recent study demonstrated the capability of *S. pneumoniae* to contribute to the severity of respiratory tract illnesses during rhinovirus infection, including asthma exacerbations.⁵ In the Finnish study, however, bacterial colonization was not associated with more long-term outcomes such as recurrent wheezing 12 months after the initial wheezing episode, in line with the present results.⁷ Thus, despite the short-term synergistic effect with respiratory viruses on respiratory symptoms, bacterial colonization during early-life wheezing plays either a minor or no role in the risk of subsequent wheezing, and PNC involvement does not increase the risk of repeated wheezing or childhood asthma.

Neonatal bacterial colonization of airways and post-natal antibiotic use in neonates seem to increase the risk of early-life wheezing and childhood asthma.^{21,22} Non-symptomatic neonates, who were colonized with *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* or with some combination of these organisms, were at increased risk for recurrent wheezing and asthma at the age of 5 years, but no association was seen between bacterial colonization at the age of 12 months and later asthma.²¹ In the present study, when we repeated the analyses in the subgroup of <12-month-old children, the results remained negative. Thus, after the neonatal period, PNC colonization seems not to be important for the risk of subsequent wheezing or later asthma.

In the present study, children who were colonized with *S. pneumoniae* on admission to hospital had a tendency towards increased risk for AS at 1–1.5 years and 2–2.5 years of age, but this tendency was lost with increasing age. Other studies have noted controversial results concerning the connection between PNC carriage and allergies: some studies noted higher colonization rates in atopic children,^{18,23} whereas others have not.^{4,7,24} In a previous Danish study, neonatal colonization with respiratory bacteria was associated with increased blood eosinophils and total IgE at 4 years of age,²¹ but these results are not completely comparable to the present ones because age at the time of colonization is important when assessing later outcomes. Asthma and other allergic illnesses (e.g. atopic eczema and allergic rhinitis) have been recognized as risk factors for severe PNC infection.^{25,26} This might account for

the impaired innate and adaptive immunity related to atopy^{25,26} or chronic airway inflammation, as seen in asthma.

The association between early-life viral infections and later lung function deficits that continue up to adulthood has been well established.^{1,15} No studies, however, have yet addressed the possible relationship between *S. pneumoniae* infection or colonization in early childhood and later lung function. In the present post-bronchiolitis cohort, PNC colonization at an early age caused no additional deterioration of lung function in former early-life wheezers in childhood, adolescence or adulthood.

In the present study, PNC colonization was associated, on average, with higher serum CRP concentration, suggesting some role for *S. pneumoniae* in the inflammatory process associated with wheezing. In another Finnish study, such an association was not found.²⁷ In our earlier study, the presence of infiltration on chest radiography during wheezing in early childhood was not associated with poorer outcome.²⁸ In the present study, PNC colonization was not associated with the presence of infiltration on chest radiography during wheezing episode. Only fragmentary data are available on the role of PNC colonization in innate or adaptive immunity in airway inflammation associated with wheezing. Interleukin-4 in sputum was higher if wheezing children were colonized with *S. pneumoniae*,²⁹ but no significant association was found between exhaled inflammatory markers and bacterial colonization of airways.³⁰

The main shortcoming of the present study was the small number of subjects. The attendance rate was good for such a long study, but one-third of the subjects dropped out during the 30 year follow up. There is a risk for type 2 error because only 14–22 children with PNC colonization attended the later follow-up visits. Subgroup analyses should also be interpreted with caution. There were not even suggestive differences, however, in the prevalence of asthma or in the results of lung function test at any age between the subjects with or without PNC colonization during first wheezing episode. Therefore, higher power would be unlikely to have changed the conclusions. Carriage and carriage acquisition have different immunological consequences,³¹ but the design of the study did not allow separation of long-term carriage from short-term colonization during viral infection. The main strengths of this post-bronchiolitis study were the long-term prospective follow up from early childhood to adulthood, the repeated and carefully done clinical studies, and the viral and bacterial methods, which were advanced at the beginning of the follow up 30 years ago, including PNC antigen studies.

In conclusion, PNC colonization is common in the respiratory tract of young children with wheezing during viral infection. The present data suggest that PNC colonization during the first wheezing episode in early childhood is not a determinant of subsequent wheezing or later asthma, atopy or lung function in childhood, adolescence or adulthood.

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Disclosure

The authors declare no conflicts of interest.

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