

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;357:1487-95.

METHODS

Hypopharyngeal specimen for bacterial culture.

Aspiration from the hypopharyngeal region was performed under aseptic conditions. Cough reflex was elicited by the catheter tip approaching larynx, during which aspiration was performed. Eliciting this reflex helped define the position of the catheter tip in the same anatomical region between children. (Figure 1_On-line) Aspiration was done intermittently assuring not to apply suction while passing through the oro- and nasopharynx. Similar procedures were attempted during sedation in the neonates 1 month of age, and in the awake child at 12 months of age. The procedures caused no adverse events.

Symptom monitoring

Diary cards were integrated into a book on asthma in young children written for parents (see: www.copsac.com). The parents filled in days with symptoms and number of doses of inhaled β_2 -agonist (Figure 2_on-line).

Asthma Diagnosis by age 5:

The criteria used for the asthma diagnosis by age 5 is listed and compared with the relevant quotations from the updated GINA guidelines (1) in Table 1_On-line.

RESULTS

Baseline characteristics of infants with and without culture of *S.p.*, *M.c.* and/or *H.i.* were comparable with respect to gender, gestational age, mothers smoking and antibiotic use during 3rd trimester, infant solely breastfed for a minimum of 4 weeks and the neonatal baseline lung function (FEV0.5) and bronchial responsiveness (PD(TcO₂)(2) at one month. Neonates with older siblings at home were more often colonized (Table 2_On-line)

Table 1_On-line: Diagnostic criteria of asthma from age 5 in the longitudinal COPSAC birth cohort study.

Quotations from GINA guidelines for comparison.

Asthma diagnosis	GINA 2005	COPSAC
	Asthma diagnosed from age 5	Asthma diagnosed from age 5
SYMPTOMS		
Recurrent typical asthma symptoms	<p><i>“History of any of the following:</i></p> <p><i>Cough, worse particularly at night.</i></p> <p><i>“Some patients with asthma have chronic cough... as their principal if not only symptom”</i></p> <p><i>Recurrent wheeze</i></p> <p><i>Recurrent difficulty breathing</i></p> <p><i>Recurrent chest tightness”</i></p>	<p>Documentation of five wheezy episodes of at least three consecutive days over 6 months or one episode lasting 4 weeks.</p> <p>Wheeze translated as wheeze or whistling sounds, breathlessness or persistent troublesome cough severely affecting the well being of the infant.</p>
Night symptoms	<p><i>“Symptoms occur or worsen at night, awakening the patient”</i></p>	<p>History of typical asthma symptoms such as exercise induced symptoms,</p>
Precipitating and/or aggravating factors	<p><i>“Symptoms occur or worsen in the presence of: Animals with fur, exercise.....”</i></p>	<p>prolonged nocturnal cough,</p> <p>cough outside common cold,</p> <p>symptoms causing awakening</p>
Exclusion of alternative diagnoses	<p><i>“Alternative but very rare causes of recurrent wheezing, include cystic fibrosis...Chest radiography is an important diagnostic test to exclude</i></p>	<p>Chest X-ray</p> <p>Sweat chloride test</p>

	<i>such alternative causes of wheezing”</i>	
RISK FACTORS		
Risk factors	<i>“Eczema, hay fever or family history of asthma or atopic diseases are often associated with asthma.”</i>	Mothers with doctor verified asthma and a current or previous history of regularly anti-asthmatic treatment.
DOCUMENTATION		
Diary cards	<i>“important tool”</i>	Day-by-day recording
Wheezing at examination	<i>“A normal chest examination does not exclude asthma.”</i>	Recorded but not required for diagnosis
Reversibility/variability in airflow limitation	<i>“Asthma can often be diagnosed on the basis of symptoms. However, measurements of lung function abnormalities greatly enhance diagnostic confidence in children 5 years and older.”</i>	FEV ₁ + sRaw Baseline + reversibility Performed but not required for diagnosis
MEDICATION		
Trial of ICS	<i>“A trial of asthma medication is probably the most confident way to make a diagnosis of asthma in children.”</i>	Improvement on ICS + Relapse after stopping ICS
Trial of β_2 -agonist	<i>“Symptoms respond to anti-asthma therapy”</i>	In need of intermittent β_2 -agonist

Table 2_On-line. Perinatal and neonatal characteristics of neonates with and without colonization with *S. pneumoniae*, *H. influenzae* and/or *M. catarrhalis* in the airways at one month of age. Frequencies are calculated for the 279 neonates with non-missing characteristics and non-missing information on colonization. P-values correspond to Fishers exact test for independence. Baseline lung function and bronchial responsiveness was measured by the Raised Volume Rapid Thoracic Compression technique at one month.(2)

		Colonized		P-value
		Yes	No	
Male		53%	49%	0.66
Mother smoked during 3 rd trimester		18%	14%	0.53
Mother used oral antibiotics in 3 rd trimester		18%	11%	0.26
Infant fully breastfed >1 month		98%	94%	0.32
Gestational age	< 38 weeks	16%	19%	0.14
	38-41 weeks	77%	64%	
	> 41 weeks	7%	16%	
Older children at home	0	30%	72%	<0.0001
	1	54%	22%	
	2+	16%	7%	

Baseline FEV _{0.5} at 1 month	≤25% quintile	35%	23%	0.09
	25%-50% quintile	25%	25%	
	50%-75% quintile	14%	28%	
	≥75% quintile	26%	24%	
Bronchial responsiveness at 1 month	≤25% quintile	32%	23%	0.54
	25%-50% quintile	23%	26%	
	50%-75% quintile	26%	25%	
	≥75% quintile	19%	26%	

Figure 1_On-line: Hypopharyngeal specimen for bacterial culture.

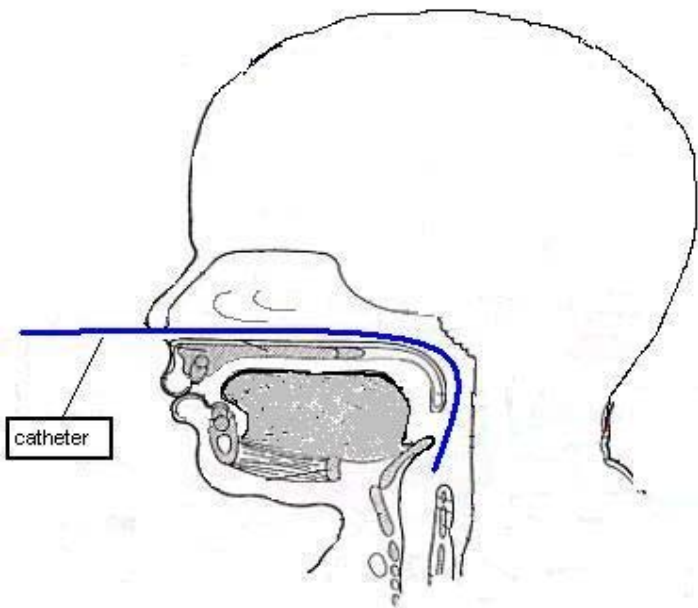


Figure 2_on-line. Diary cards from book “Asthma in Young Children” (http://ipaper.dk/copsac/Asthma_in_young/) given to parents to fill in dichotomized score for troublesome lung symptoms affecting the child wellbeing and use of inhaled β_2 -agonist.

Date

Month

Year

Lung symptoms
yes/no

Reliever
medication

		Januar 2007																																
Dato		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
		ma	ti	on	to	fr	lø	sø	ma	ti	on	to	fr	lø	sø	ma	ti	on	to	fr	lø	sø	ma	ti	on	to	fr	lø	sø	ma	ti	on		
Lunge Symptomer	Ja																																	
	Nej																																	
Antal Bricanyl behandling																																		

Reference List

- (1) Global Strategy for Asthma Management and Prevention. No 02-3659. 2005. NIH publication.
- (2) Loland L, Buchvald FF, Brydensholt HL, Anhoj J, Hall GL, Persson T et al. Sensitivity of Bronchial Responsiveness Measurements in Young Infants. *Chest* 2006; 129(3):669-675.