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Wheezing in Early Childhood

Predictive Factors for Asthma in the 11-year Follow-Up

Doctoral dissertation

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ABSTRACT

Wheezing in infants when they have a lower respiratory tract infection is not uncommon. However, some of these wheezing infants, specifically those requiring hospitalization, are at high risk of later asthma. Identifying at-risk infants would be helpful for monitoring and focusing treatment on the right subjects.

In 1992-1993, 100 infants aged 1-23 months were hospitalized for wheezing associated with respiratory tract infection. The baseline data, blood specimens and nasopharyngeal aspirates (NPAs) were collected on entry to the study. As an acute intervention, the clinical responses to racemic adrenaline and salbutamol were evaluated. The asthma and allergy status of the children have been clinically evaluated at the median ages of 4.0 and 7.2 years. In 2004, on average 11 years after the hospitalization for wheezing, 81/100 children attended the clinical follow-up in teenage years, i.e median age of 12.3 years (range 10.9-13.7). Symptoms suggestive of asthma and allergy were recorded and studies on allergy, baseline lung function and bronchial hyper-reactivity (BHR) were performed.

Teenage asthma was present in 40 % (32/81) of the children, and 25 % (20/81) of the children had asthma at all three visits and were considered to have persistent childhood asthma (PCA). Most of the children with teenage asthma (90%) or PCA (95%) were sensitized to at least one allergen.

Early factors predicting both teenage asthma and PCA were atopic dermatitis (adjusted odds ratios, aORs 3.5 and 4.5, respectively) and the presence of serum specific immunoglobulin E (sIgE) to inhalant allergens (aORs 11.3 and 13.1, respectively) and to wheat (aORs 20.2 and 9.7). Respiratory syncytial virus (RSV) aetiology of early wheezing was associated with a restrictive pattern of lung function, and early atopy and maternal smoking during pregnancy were associated with BHR. Though minor evidence was found that the children with later PCA may respond better to bronchodilators than those children with no PCA, the overall clinical responses to bronchodilators did not differ between these groups. Early exposures to cats and dogs were not associated with the prevalence of teenage asthma or PCA or atopic sensitization in infancy or at teenage. Neutrophil derived serum myeloperoxidase was not associated with later asthma.

Children with teenage asthma and children with PCA had higher blood eosinophil (B-EOS) count at the time of wheezing in infancy than their counterparts who did not develop teenage asthma (median $0.41x10^9$ cells/L vs. $0.17x10^9$, p=0.027) or PCA ($0.53x10^9$ vs. $0.20x10^9$, p= 0.006). The level of eosinophil cationic protein (ECP) in NPA (NPA-ECP), but not in serum (S-ECP), was higher in children with PCA than in children with no PCA (926 ng/g vs. 347 ng/g, p=0.013). At least one eosinophilic marker measured in infancy, was elevated (B-EOS $\geq$0.45 x 10^9 cells/L or NPA-ECP $\geq$815.0 ng/g or S-ECP $\geq$20.0µg/L) in 61% of the children with teenage asthma and in 75% of the children with PCA. In addition, elevated NPA-ECP predicted PCA independently of the presence of early atopic dermatitis (aOR 4.09).

In conclusion, a quarter of children hospitalized for wheezing in infancy will suffer from persistent asthma during childhood, and as many as 40 % will have asthma in teenage years. The features known to be related to chronic bronchial asthma, such as eosinophilic inflammation, atopic dermatitis and sensitization to inhalant allergens, are present already in some wheezing infants and in these children, their presence is predictive of later asthma.

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TIIVISTELMÄ

Varhaislapsuudessa uloshengitysvaikeus on yleistä alahengitystieinfektion aikana. Pikkulapsena sairastettu uloshengitysvaikeus ennustaa myöhempää astmaa ja riski sairastua astmaan on erityisen suuri jos uloshengitysvaikeus on johtanut sairaalahoitoon. Suuressa astmariskissä olevien pikkulasten tunnistaminen varhain olisi tärkeää, jotta lääkehoito ja tarkka seuranta osattaisiin kohdistaa oikein.

Tähän tutkimukseen otettiin 100 alle 2-vuotiasta lasta, joita hoidettiin sairaalassa alahengitystieinfektioon liittyvän uloshengitysvaikeuden vuoksi vuosina 1992-1993. Sairaalaan ottovaiheessa kerättiin tarkat tiedot lasten varhaisvaiheista ja lähisukulaisten atooppisista sairauksista sekä otettiin verinäytteitä ja nenänielun imulimanäytteitä (NPA). Lisäksi alkuvaiheeseen liitettiin lääkeinterventio, jossa selvitettiin kliinisin vastein raseemisen adrenaliinin ja albuterolin tehoa. Varhaisvaiheiden jälkeen lapset on tutkittu kliinisesti noin 4 ja 7 vuoden ikäisinä astman ja allergioiden esiintyvyyden selvittämiseksi. Vuonna 2004, noin 11 vuotta varhaisen sairaalahoitoa vaatineen uloshengitysvaikeuden jälkeen, 81/100 lasta osallistui seurantakäynnille teini-iässä, 11-14-vuotiaina. Tällä seurantakäynnillä kartoittettiin allergia- ja astmaoireet sekä tutkittiin herkistymistä allergeeneille ja keuhkojen toimintaa sekä keuhkoputkien supistumisherkkyyttä.

Lapsista 40 %:lla (32/81) oli astma ja 25 %:lla (20/81) lapsista oli astma kaikilla kolmella seurantakäynnillä (krooninen astma). Suurin osa astmaatikoista (90 %) ja kroonisista astmaatikoista (95 %) oli herkistynyt vähintään yhdelle hengitystieallergeenille.

Varhaiset tekijät, jotka ennustivat astmaa ja kroonista astmaa, olivat atooppinen dermatiitti (vakioidut oddsien suhteet, vORs, 3.5 ja 4.5, tässä järjestyksessä), seerumin spesifinen immunoglobuliini E (sIgE) inhalaatioallergeeneille (vORs 11.3 ja 13.1) ja vehnälle (vORs 20.2 ja 9.7). Respiratory syncytial virus (RSV) varhaisen uloshengitysvaikeuden aiheuttajana liittyi restriktiiviseen vt-spirometrialöydökseen ja varhainen atopia sekä äidin raskauden aikainen tupakointi keuhkoputkien lisääntyneeseen supistumisherkkyyteen. Vaikka viitteitä sille, että tulevat astmaatikot reagoisivat paremmin keuhkoputkia laajentaville lääkkeille kuin ei-astmaatikot löytyi, kokonaisuudessaan näiden ryhmien vasteet varhaiselle interventiolle eivät eronneet toisistaan. Varhainen altistuminen kissan ja koiran allergeeneille ei lisännyt astman, kroonisen astman tai allergeeneille herkistymisen riskiä. Varhaisen alahengitystieinfektion aikana mitattu seerumin myeloperoxidaasi ei ollut yhteydessä myöhempään astmaan.

Astmaatikoilla ja kroonisilla astmaatikoilla oli enemmän veren eosinofiilisiä soluja sairaalaan ottovaiheessa kuin ei-astmaatikoilla (mediaani 0.41 x 10^9 solua/l vs. 0.17 x 10^9, p=0.027) ja lapsilla, joilla ei ollut kroonista astmaa (0.53 10^9 vs. 0.20 10^9, p=0.006). Nenänielun imuliman, mutta ei seerumin, eosinofiilisen kationisen proteiinin (NPA-ECP) konsentraatio oli korkeampi kroonisilla astmaatikoilla verrattuna muihin lapsiin (926 ng/g vs. 347, p=0.013). Astmaatikoista 61 %:lla ja kroonisita astmaatikoista 75 %:lla oli vähintään yksi eosinofiilisten solujen aktivaatiota osoitava merkkiaine (B-EOS≥0.45x10^9 cells/l tai NPA-ECP≥815.0 ng/g tai S-ECP≥20 µg/L) koholla. Lisäksi koholla oleva NPA-ECP oli kroonisen astman itsenäinen riskitekijä (vOR 4.1).

Yhteenvetona voidaan todeta, että neljännekselle lapsista, jotka joutuvat varhaislapsuudessa sairaalahoitoon uloshengitysvaikeuden vuoksi, kehittyy krooninen astma ja jopa 40 %:lla on astma teini-iän kynnyksellä. Astmaan tunnetusti liittyvät atooppinen ihottuma, herkistyminen hengitystieallergeeneille ja eosinofiilinen tulehdus ovat todettavissa jo osalla uloshengitysvaikeudesta kärsivistä pikkulapsista ja merkitsee riskiä sairastua myöhemmin astmaan.

To Henri, Ilari and Elina

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Kuopio, October 2009

Mari Hyvärinen

ABBREVIATIONS

ANOVA	Analysis of variance
AOR	Adjusted Odds ratio
AP	Albuterol followed by placebo
BHR	Bronchial hyper-reactivity
CF	Complement fixation
CI	Confidence interval
ECP	Eosinophil cationic protein
ECT	Exercise challenge test
EIA	Enzyme immunoassay
ETS	Environmental tobacco smoke
FEIA	Fluoroenzyme-immunometric assay
FEV_1	Forced expiratory flow in one second
FVC	Forced vital capacity
FVS	Flow volume spirometry
HA	Hemagglutinin
ICAM	Intercellular adhesion molecule
IF	Immunofluorescence
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ISAAC	International study of asthma and allergies in childhood
HMPV	Human metapneumovirus
LRTI	Lower respiratory tract infection
MEF_{25}	Maximal expiratory flow when 25 % of the FVC remains to be expired
MEF_{50}	Maximal expiratory flow when 50 % of the FVC remains to be expired
MIC	Methacholine inhalation challenge test
MPO	Myeloperoxidase
NA	Neuraminidase
NPA	Nasopharyngeal aspirate
NPA-ECP	Eosinophil cationic protein in nasopharyngeal aspirate
OR	Odds ratio
PA	Placebo followed by albuterol
PCA	Persistent childhood asthma
PCR	Polymerase chain reaction
PRE	Placebo followed by racemic epinephrine
RAST	Radioallergosorbent test
RDAI	Respiratory distress assessment instrument
REP	Racemic epinephrine followed by placebo
ROC	Receiver operating characteristic
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription-polymerase chain reaction
SIgE	Specific immunoglobulin E
SPT	Skin prick test
Th-cell	T-helper cell
TR-FIA	Time-resolved fluoroimmunoassay
VmaxFRC	Maximal flow at the functional residual capacity

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred in the text by their Roman numerals. In addition, some unpublished data are presented.

I. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen T, Korhonen K, Korppi M Teenage asthma after severe early childhood wheezing: An 11-year prospective follow-up. Pediatr Pulmonol 2005; 40: 316-323.

II. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen T, Korhonen K, Kiviniemi V, Korppi M. Responses to inhaled bronchodilators in infancy are not linked with asthma in later childhood. Pediatr Pulmonol 2006; 41:420-7.

III. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen T, Korhonen K, Korppi M. Lung function and bronchial hyper-responsiveness 11 years after hospitalization for bronchiolitis. Acta Paediatr 2007; 96: 1464-9.

IV. Korppi M, Hyvärinen M, Kotaniemi-Syrjänen A, Piippo-Savolainen E, Reijonen T. Early exposure and sensitization to cat and dog – different effects on asthma risk after wheezing in infancy. Pediatr Allergy Immunol 2008; 19: 696-701.

V. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen T, Piippo-Savolainen E, KorppiM. Eosinophil activity in infants hospitalized for wheezing and risk of persistent childhood asthma. Pediatr Allergy Immunol 2009. Epub ahead of print.

VI. Hyvärinen MK, Kiviniemi V, Kotaniemi-Syrjänen A, Reijonen T, Piippo-Savolainen E, Korppi M. Serum myeloperoxidase in wheezing infants: no association with later asthma. Scientific letter to editor. Pediatr Pulmonol. In press.

CONTENTS

1. INTRODUCTION

Wheezing, referring to expiratory breathing difficulty, is common during lower respiratory tract infection (LRTI) in early childhood: more than a third of all children suffer from wheezing by the age of three years (Taussig et al. 2003, Illi et al. 2004) and about two percent of all infants suffer from wheezing severe enough to warrant hospitalization (Korppi et al. 1986, Boyce et al. 2000, Koehoorn et al. 2008). Viruses can be detected in the majority of wheezing infants, with respiratory syncytial virus (RSV) and rhinovirus being the most dominant agents (Kotaniemi-Syrjänen et al. 2003b, Heymann et al. 2004, Jartti et al. 2004).

Recurrence of wheezing and the subsequent development of asthma are common after early childhood wheezing, especially after a wheezing episode requiring hospitalization (Martinez et al. 1995, Wennergren et al. 1997, Kotaniemi-Syrjänen et al. 2002, Piippo-Savolainen et al. 2004, Sigurs et al. 2000 and 2005, Matricardi et al. 2008, Devulapalli et al. 2008). Small airway size and/or dysfunction in the regulation of airway tone seem to increase the risk of wheezing during LRTI in infancy and in childhood in otherwise healthy children (Turner et al. 2002, Taussig et al. 2003). For example, RSV-induced LRTI (RSV-LRTI) is a risk factor for subsequent wheezing up to the age of 11 years, but not any longer at age 13 (Stein et al. 1999b). Thus, the presence of wheezing in early puberty seems to be critical for the persistence of asthma.

Host, viral and other environmental factors are important determinants of the final outcome after early childhood wheezing (Castro-Rodriquez and Garcia-Marcos 2008, Singh et al. 2008, Martinez 2009). An atopic predisposition in infancy is a known risk factor for the development of asthma after wheezing in early childhood (Taussig et al. 2003, Matricardi et al. 2008, Kotaniemi-Syrjänen and Korppi 2007, Piippo-Savolainen and Korppi 2009). In most studies on early-life risk factors for asthma, clinical or non-specific markers of atopy, such as atopic dermatitis or elevated total serum immunoglobulin E (IgE), have been examined, and most studies have lacked either the determination of allergen-specific sensitization in infancy or the long-term follow-up revealing atopy becoming evident after infancy. There is recent epidemiologic evidence swhich uggests that early-life exposure to animals decreases subsequent allergy and

asthma (von Mutius 2007). In line with this, exposure to pets at the time of wheezing in infancy has not increased the risk of later asthma in the few long-term follow-up studies available (Goksör et al. 2006, Piippo-Savolainen et al. 2007a).

Rhinoviruses can provoke asthma exacerbations in adult asthmatics (Dougherty and Fahy 2009), and recent studies have stressed the role of rhinoviruses also during wheezing episodes in infants (Jartti et al. 2004, Kotaniemi-Syrjänen et al. 2003b, Jackson et al. 2008). Infants with rhinovirus induced wheezing seem to have an increased risk of suffering recurrent wheezing symptoms compared to wheezing induced by other viruses, like RSV (Lemanske et al. 2005). In many studies, this effect has been confirmed to continue until school age (Kotaniemi-Syrjänen et al. 2003b, Jackson et al. 2008), but there are no long-term follow-up studies with advanced viral diagnostic methods having been applied in infancy.

Eosinophilic inflammation is a characteristic feature of asthma, and it is often related to, and may even precede, the permanent structural changes in the airways (Barbato et al. 2006, Bush 2008). The age from one to three years has been suggested to be critical for the development of eosinophilic inflammation and the subsequent structural changes in the airways of young wheezing children at high risk of later asthma (Saglani et al. 2005 and 2007). Blood eosinophilia is a risk factor for later asthma in wheezy infants (Castro-Rodriquez et al. 2000, Ehlenfield et al. 2000, Just et al. 2008). However, there are no long-term follow-ups on the association between eosinophil activity in the blood or in the airways in wheezing infants and the risk of subsequent asthma. Neutrophils and their products seem to predominate in airway inflammation during acute viral wheeze in infancy (Kim et al. 2003, Marquet et al. 2008). In addition, neutrophils have an increased propensity to release myeloperoxidase (MPO), which is known to have the capability to damage epithelial cells (Haegens et al. 2008) and it also possesses pro-inflammatory properties (Lau et al. 2005). The role of neutrophil mediators such as serum MPO, measured in wheezing infants, is obscure – little is known about whether they elevate the later risk of asthma.

A positive response to bronchodilating agents, specifically to β-adrenergic agents, is considered as a sign of asthma in older children and in adults. These agents have not been as effective in infants with virus-induced wheezing (Chavasse et al. 2002, King et al. 2004). However, clinical experience suggests that a subset of wheezing infants

benefits from treatment with β-adrenergic agents. It is unclear whether the positive responses, as measured by clinical parameters, may be useful in predicting later childhood asthma.

The main purpose of this thesis was to examine the risk factors, which are present during hospitalization for virus-induced wheezing in infancy, for teenage asthma and for asthma lasting through early childhood until at least teenage.

2. REVIEW OF THE LITERATURE

2.1. Wheezing illnesses in early childhood

2.1.1. Incidence of wheezing in early childhood

Wheezing symptoms are common in infancy: in the developed countries more than a third of all children will suffer from wheezing by the age of three years (Taussig et al. 2003, Illi et al. 2004, Gillespie et al. 2006), and about 10-20 % of all children experience at least two wheezing episodes during the first years of life (Halken et al. 1991, Castro-Rodriguez et al. 2000, Devulapalli et al. 2004, Kummeling et al. 2007). In infants with with atopic predisposition, i.e. asthma or atopy in at least one first-degree relative, the figure for wheezing in early life is even greater, more than 50 % (Kuiper et al. 2007).

About 2 % of all infants suffer from wheezing severe enough to warrant hospitalization (Korppi et al. 1986, Boyce et al. 2000, Koehoorn et al. 2008). The figures for recurrent wheezing are high after hospitalization for wheezing in infancy: during the following year as many as 70-75 % of these infants will experience at least one physician-diagnosed wheezing episode (Korppi et al. 1993, Reijonen et al. 1998), and approximately 40-50 % of the infants wheeze at least twice (Reijonen et al. 1998, Lehtinen et al. 2007).

Bronchiolitis is often defined as wheezing at age less than 24 months (American Academy of Pediatrics 2006, Scottish Intercollegiate Guidelines Network 2006). However, this group of wheezy infants is heterogeneous with respect to host, viral and environmental risk factors evoking the wheezing symptoms in infancy, as well as in how this will impact on later childhood outcome (Jartti et al. 2009). Thus, identifying children at high risk of later asthma, and on the other hand, those who really are not at risk is clinically very important but challenging.

2.1.2. Viral recovery rates of wheezing illnesses and viral detection by molecular and non-molecular methods

Until the 1990s, viral diagnosis of wheezing illnesses was based on non-molecular methods, and viral recovery rates for wheezing in infancy were often below 50 % (Table 1) and for asthma exacerbations only about 10-30 % (Clarke et al. 1979, Pattemore et al. 1992). Non-molecular methods include conventional cell cultures (Ginocchio et al. 2007) and faster shell vial cultures (Ginocchio et al. 2007). Viral protein antigens can be detected directly in clinical specimens by immune fluorescence (IF)-based methods or by enzyme immunoassays (EIA) (Ginocchio et al. 2007, Mahony et al. 2008, Loens et al. 2009). A variety of serological tests including the hemagglutination inhibition test, complement fixation (CF) and EIA can be used for testing for antibodies in serum samples (Mahony 2008). The sensitivities of non-molecular methods vary greatly, e.g. depending on targeted viruses and age of specimens, and a combination of methods is often necessary to improve the diagnostics (Ginocchio et al. 2007, Mahony et al. 2008).

With the development of more sensitive molecular nucleic acid amplification techniques, multiple studies have shown that 80-95 % of wheezing in infancy (Table1) as well as most asthma exacerbations in children are induced by respiratory viruses (Johnston et al. 1995, Freymuth et al. 1999, Jartti et al. 2004, Grissel et al. 2005). These novel techniques include the polymerase chain reaction (PCR), which has led to the discovery of new viruses and a re-evaluation of the role of old viruses in LRTIs in children (Korppi 2008). Since most respiratory viruses are RNA-viruses, reverse transcriptase PCR (RT-PCR), in which nucleic acid is reverse transcribed into cDNA, is usually applied to detect respiratory viruses.

Different kinds of specimens can be used in respiratory virus diagnostics e.g. nasopharyngeal aspirate (NPA), nasopharyngeal wash, nasopharyngeal swab, oropharyngeal swab, sputum, tracheal aspirates and bronchoalveolar lavage (Ginocchio et al. 2007, Loens et al. 2009). It has been proposed that NPA is the optimal specimen to detect respiratory viruses by both non-molecular and molecular methods (Loens et al. 2009).

Table 1. Main studies with sampling period at least 12 months on viral aetiology of early wheezing

Study population	Study period	Wheezing episodes	Age , months	Methods	Viral detection rates (% of wheezing episodes)										
					RSV	Rhino-virus	Entero-virus	PIV 1-4	Influen-za virus A/B	Adeno-virus	Corona-virus	hMPV	Boca-virus	Other viruses	Total
Outpatients (Henderson et al. 1979)	1964-1975	909	0-24	culture	10	1	nt	6	nt	3	nt	nt	nt	1[a]	22
Outpatiets, birth cohort (Wright et al. 1989)	1980-1984	183	0-12	culture and antigen detection	58	see other	6	13	4	2	nt	nt	nt	7[b]	90
Inpatients (Wennergren et al. 1992)	1984-1985	94	0-24	serology, antigen detection	30	nt	7	2	0	10	nt	nt	nt	5[c]	43
Inpatients (Rylander et al. 1996)	1986-1988	103	4-18	culture	20	2	1	8	nt	3	nt	nt	nt	nt	33
Emergency department study (Duff et al. 1993)	1988-1992	20	0-24	culture, antigen detection	55	15	5	nt	5	nt	nt	nt	nt	nt	70
Inpatients, (Reijonen et al. 1997a and 1997b, Kotaniemi-Syrjänen et al. 2003b and 2005)[e]	1992-1993	100	0-24	antigen detection, serology, RT-PCR	29	27	10	12	nt	5	0	nt	nt	nt	69
Emergency department study (Rakes et al. 1999)	1993-1994	22	0-24	culture, antigen detection, RT-PCR	68	41	0.5	nt	nt	0	0	nt	nt	nt	82

Inpatients (Papadopoulos et al. 2002 and Xepapadaki et al. 2004)	1999-2000	118	0.5-12.5	RT-PCR	53	21	nt	3	3	8	3	16 (9/56)[d]	nt	nt	74
Outpatients, high-risk birth cohort study (Jackson et al. 2008)	1998-2000	442	0-36	RT-PCR	21	48	2	12	4	4	5	7	nt	nt	90
Inpatients (Jartti et al. 2009)	2000-2002	259	0-36	culture, antigen detection, serology, RT-PCR	38	38	20	8	3	10	3	5	20	nt	93
Outpatients (Regamey et al. 2008)	1999-2004	39	0-12	RT-PCR	13	18	nt	13	see other	see other	8	10	see other	8[f]	77

RSV=Respiratory syncytial virus, PIV 1-4=Parainfluenza virus 1-4, human Metapneumovirus=hMPV, nt=not tested

[a] *Mycoplasma pneumoniae*

[b] Cytomegalovirus, rhinovirus, *Chlamydia trachomatis* and *Mycoplasma pneumoniae*, each at less than 4 % frequency

[c] Rotavirus

[d] 56/118 samples were later evaluated for hMPV, in which hMPV was the unique pathogen in 5/9 samples

[e] In 2000-2002 supplementary studies on viral aetiology (RT-PCR) were performed for picornaviruses and coronaviruses (n=81/100) and RSV (n=61/100)

[f] Influenza A, adeno- or bocavirus

2.1.3. Infectious triggers of wheezing in infancy

Respiratory syncytial virus (RSV)

RSV (family *Paramyxoviridae*) is a single-stranded enveloped RNA-virus and the most common aetiological agent causing expiratory wheezing in infancy (Table 1). There are two subtypes of RSV, RSV A and B, wich possess antigenic differences and genetic diversity in the proteins (Ogra 2004). Important structural proteins include the fusion (F) protein and the attachment (G) protein (Ogra 2004). RSV accounts for about 40-70 % of the wheezing episodes in children aged less than three years (Table1), with detection rates being usually more than 50 % in children aged less than one year (Table1). During the winter months in temperate climates, RSV causes more than 60 % of the wheezing episodes in infants (Marguet et al. 2009, Mansbach et al. 2008). Nearly all children have been infected by the age of three years, and repeated, though clinically mild, infections are possible (Glezen et al. 1986, Ogra 2004). A seasonal variability is typical for RSV: in temperate climates the peak prevalence is in winter, with most infections occuring between late fall and early spring (Yusuf et al. 2007). In tropical climates, the virus can be detected all year round, and peaks are related to low temperatures (Stensballe et al. 2003). In northern Europe, RSV epidemics follow a regular long-term biannial pattern. For example in Finland, a minor RSV epidemic in every other spring is followed by a major epidemic in the following autumn and winter (Terlatskaia-Ladwig et al. 2005, Jartti et al. 2004). Children at the highest risk for suffering more severe symptoms are infants aged less than six months, particularly premature infants, and infants with bronchopulmonary dysplasia, congenital heart disease or immunodeficiency disorders (Boyce et al. 2000).

Picornaviruses

Rhinovirus (family *Picornaviridae*) is known as the common cold virus, but many studies using molecular techniques, specifically RT-PCR, have shown that rhinoviruses cause up to 40 % of wheezing episodes in infants and are the second most important aetiological agent after RSV in early wheezing (Table1). Rhinovirus is a dominant pathogen in wheezing illnesses in older children and adults and accounts for about 65 % of the respiratory viruses identified in these cases (Dougherty and Fahy 2009,

Friedlander 2005). Rhinoviruses are single-stranded non-enveloped RNA viruses, and more than 100 rhinovirus serotypes have been identified (Kelly and Busse 2008). The serotypes are classified into three phylogenetic groups, A, B and C (Kelly and Busse 2008), which all are able to cause wheezing respiratory diseases (Kelly and Busse 2008, Miller 2009). However, serotypes A and C seem to be more common than serotype B, at least in hospitalized children (Miller et al. 2009). The intra-cellular adhesion molecule (ICAM) -1 is a cell-surface receptor, which binds most rhinovirus serotypes (Kelly and Busse 2008). Rhinoviruses circulate all year round, with peaks in late spring and early autumn in temperate climates (Carroll et al. 2008). Most children have been infected by rhinovirus by the age of two years (Carroll et al. 2008).

Enteroviruses (family *Picornaviridae*) are capable of causing wheezing LRTIs in infants (Jacques et al. 2008), and trigger up to 20 % of the wheezing episodes in infancy (Table1). Enteroviruses have also been found in the airways of older children with symptomatic asthma, especially during the summer periods (Rawlingson et al. 2003, Jartti et al. 2004). Enteroviruses are single-stranded non-enveloped RNA viruses (Mahony 2008) and the non-polio-enteroviruses are divided into four species, A-D (Mahony 2008). There are almost 100 enterovirus serotypes (Jacques et al. 2008), and many different strains have been isolated from specimens collected from the respiratory tract (Jacues et al. 2008). In temperate climates, most enterovirus infections occur in spring, summer and autumn, with infections lasting into the winter (Mahony 2008, Jacques et al. 2008).

After the age of 12 months, the role of picornaviruses becomes more dominant in childhood wheezing, and rhinoviruses and enteroviruses together account for about 65 % of all cases (Jartti et al. 2004).

Human metapneumovirus (hMPV)

Human metapneumovirus (family *Paramyxoviridae*) is a single-stranded enveloped RNA-virus, which has probably been circulating for decades, but was not discovered until 2001 (Kahn 2006, Mahony 2008). At least two distinct genotypes cause respiratory tract diseases in humans (Kahn 2006). hMPV is related to RSV, and the clinical manifestations of these viruses are indistinguishable (Kahn 2006, Mahony 2008). The age-related incidence of hMPV infection is highest in children aged less than

two years (Heikkinen et al. 2008), and most children have been infected by the age of five years (Kahn 2006). hMPV causes up to 16 % of all wheezing episodes in infancy (Table 1), and is second or third to RSV and rhinovirus as a cause of bronchiolitis (Table1, Wolf et al 2006). Co-infections with RSV and other viruses are rather common (Kahn 2006, Xepapadaki et al. 2004). hMPV has a worldwide distribution, and in temperate climates, it is detected throughout the year, but it circulates mainly in the late winter and spring, and often coincides with or follows the peaks of RSV epidemics (Kahn 2006).

Adenovirus

Adenoviruses (family *Adenoviridae*) are double-stranded non-enveloped DNA viruses with more than 50 serotypes, which are classified into six subtypes (A-F) (Mahony 2008). Type C seems to be the most common subtype found in the respiratory tract (Echavarria 2006). Adenoviruses circulate all year round, but infections are most frequent in late winter and early spring (Mahony 2008). Most adenovirus infections occur in early life (Mahony 2008) and can be considered as being responsible for up to 20 % of wheezing episodes in infancy (Table1).

Coronavirus

Coronaviruses (family *Coronaviridae*) are single-stranded, large and enveloped RNA viruses, which are found in up to 8 % of the specimens obtained from wheezing infants (Table 1). There are two main serogroups, group I includes coronaviruses 229E and NL63 and group II coronaviruses OC43, SARS and HKU1 (Mahony 2008). Coronaviruses 229E and OC43, identified in the mid 1960s, are capable of causing not only mild (i.e. common cold) but also severe (i.e. bronchiolitis and pneumonia) respiratory tract infections (Mahony 2008). A novel coronavirus, SARS, associated with severe acute respiratory syndrome, was discovered in 2003 (Williams 2005). The most recently discovered coronaviruses, NL63 and HKU1, have been found to cause a variety of respiratory tract diseases worldwide including wheezing-associated illnesses (Mahony 2008, Ebihara 2005). In the temperate climates, coronavirus infections occur mainly in the winter months, often jointly with other viruses (Mahony 2008).

Parainfluenza and influenza viruses

Parainfluenza viruses (family *Paramyxoviridae*) are single-stranded enveloped RNA-viruses, and four parainfluenzavirus serotypes (1-4) cause symptomatic respiratory infections (Mahony 2008), being responsible for up to 13 % of wheezing episodes in infants (Table1). Parainfluenza viruses circulate all year round, and type 3 is able to cause most of the severe lower respiratory tract infections in infants, and is most prevalent in the spring and winter months (Mahony 2008).

Influenza viruses (family *Orthomyxoviruses*) are single-stranded enveloped RNA-viruses, which cause annual epidemics in temperate climates (Mahony 2008, Chen and Deng 2009). The surface glycoproteins of influenza virus, hemagglutinin (HA) and neuraminidase (NA), are highly variable which complicates the development of vaccines (Chen and Deng 2009). Pandemics may follow the emergence of a new virus with a novel HA and NA combination (Chen and Deng 2009). The rates of wheezing episodes associated with influenza virus vary greatly, depending on the attack rates in the general population (Rojo et al. 2006). In temperate climates, epidemics occur during the winter months and last from three to eight weeks (Mahony 2008, American Academy of Pediatrics, Committee on infectious diseases 2008). There are three genera within the influenza family (A, B and C) and only A and B viruses are capable of causing significant diseases (Carrat and Flahaut 2007). Influenza A virus infections tend to occur at a younger age than influenza B virus infections (Rojo et al. 2006, Peltola et al. 2002), and children younger than two years are at the highest risk of LRTI (Rojo et al. 2006). Influenza viruses cause up to 5 % of wheezing episodes in infants aged less than three years (Table 1).

Bocavirus

Human bocavirus (family *Parvoviridae*) is the most recently discovered wheezing-associated respiratory tract virus (Allander et al. 2001). Bocavirus is a single-stranded non-enveloped DNA-virus with two distinct genotypes circulating worldwide (Mahony 2008). Infections have been diagnosed by molecular methods and by serology, but bocavirus has not been isolated in cell cultures (Mahony 2008, Kantola et al. 2008). Bocavirus has been associated with varying degrees of respiratory illnesses, ranging from common cold to bronchiolitis and pneumonia (Arnold et al. 2006, Bastien et al.

2007, Mahony et al. 2008). The symptoms most commonly associated with bocavirus infections are cough, fever and rhinitis (Bastien et al. 2007). In several studies, bocaviruses have been most prevalent in children aged less than three years (Mahony 2008). It has been reported that bocavirus is present in 20 % of the wheezing children at that age (Jartti et al. 2004), but most of the bocavirus findings have been co-infections with other viruses (Allender et al. 2007), and the role of bocavirus as a true pathogen has been questioned (Allender et al. 2007). However, viral loads have been high if bocavirus has been the only detected virus and antibody responses have been associated with high numbers of viral copies in NPA detected by PCR, evidence for a causative role in wheezing infections (Allender et al. 2007). In addition, bocavirus is rarely detected in asymptomatic individuals (Mahony 2008). In temperate climates, bocavirus infections occur mainly in winter (Mahony 2008), but seasonality has not been found in all studies (Bastien 2007.

2.1.4. Mechanisms of virus-induced wheezing in infancy

Wheezing during respiratory virus infection means that viruses have infected the lower respiratory tract. Airway obstruction is caused by epithelial swelling and plugging of the airway lumen by mucus and cellular debris (Tuffaha and Busse 2001). In addition, viruses can enhance bronchial hyper-reactivity (BHR) by altering neural control mechanisms, mainly by inducing cholinergic activity and by inducing the release of neuropeptides (Tuffaha and Busse 2001, Gern and Lemanske 2003, Gern 2008). The direct effects of the virus and infection-related immune responses both cause these changes, with the airway epithelial cells, granulocytes and lymphocytes being the main cells involved in the process (Tuffaha and Busse 2001, Gern 2008). The subsequent symptoms and signs include expiratory wheezing, tachypnea and hypoxia.

Epithelial cells

The airway epithelial cells form the primary target of the viruses which infect the respiratory tract (Gern and Lemanske 2003, Gern 2008). Respiratory viruses, especially RSV, can cause direct lung injury by replication in the epithelium (Gern and Lemanske 2003, DeVincenzo 2007), leading to epithelial oedema and shedding which together with mucus production can provoke wheezing symptoms (Gern 2008). Epithelial damage may cause enhanced allergen exposure since it can increase permeability of the mucosal layer (Gern 2008). Rhinoviruses usually are responsible for minor cellular damage, i.e. the mechanisms leading to the symptoms are mainly immunological (Kelly and Busse 2008). On the other hand, persistence of rihonoviruses in the lower airway epithelial cells of recurrently wheezing infants has been reported (Malmström et al. 2006). Viruses bind to receptors, RSV mainly to Toll-like receptors (TLRs) (Murawski et al. 2009) and rhinovirus mainly to ICAM-1 receptors (Kelly and Busse 2008). Host cell recognition of viral antigens leading to viral replication in epithelial cells leads to activation of transcription factors of several cytokines and chemokines, which attract leucocytes to the site of inflammation (Schuh et al. 2003, Gern and Lemanske 2003, Kelly and Busse 2008).

Granulocytes, monocytes and macrophages

Activation of cytokines and chemokines is followed by immune cell influx and activation (Tuffaha and Busse 2001, Gern 2008). Neutrophils are the most common cell type in virus-induced respiratory tract infection in wheezing infants and are present also in the respiratory secretions of subjects with acute asthma (Kim et al. 2003, Marquet et al. 2008, Barbato et al. 2001, Norzila et al. 2000). Sputum neutrophils as well as their degranulation products, such as elastase, interleukin (IL)-8 and MPO, are likely to contribute to airway obstruction by mucus secretion and by further secretion of chemokines and cytokines (Gern 2008, Jaovisidha et al. 1999).

Mononuclear cells, such as macrophages and dendritic cells, are important cells in the early responses to respiratory viruses and these cells secrete adhesion molecules such as ICAM-1 and pro-inflammatory cytokines such as IL-1, IL-8, tumor necrosis factor alpha, interferon (IFN)-γ, IFN- α, and monocyte inflammatory protein -1α (Gern 2008, Kelly and Busse 2008, Tuffaha and Busse 2001). These products are important mediators of inflammation and they act to attract and recruit further inflammatory cells, but type 1 IFNs, such as IFN-α, may also limit viral spread and take part in the recovery process (Gern 2008, Kelly and Busse 2008).

There is evidence that eosinophils can also be activated during respiratory tract infection (Tuffaha and Busse 2001). Eosinophil activation during rhinovirus infection is common (Gern 2008, Kelly and Busse 2008), and an eosinophil-positive subgroup has been identified in infants suffering from RSV-induced bronchiolitis (Kim et al. 2003). The infected epithelial cells secrete a mediator called regulated upon activation normal T-cell expressed and secreted (RANTES), which is an important chemotactic factor in the activation of eosinophils (Kelly and Busse 2008). Eosinophil cationic protein (ECP), secreted by eosinophils, may even have antiviral properties (Kelly and Busse 2008). On the other hand, elevated sputum eosinophils, seen often in asthmatic subjects when there are no infections, may also enhance an individual′s susceptibility to suffer a rhinovirus infection (Kelly and Busse 2008).

Lymphocytes

Cell-mediated immune responses are involved in virus-induced wheezing in infancy (Tuffaha and Busse 2001, Gern 2008, Kelly and Busse 2008). Natural killer T-cells,

CD8+ cytotoxic T-cells (T-killer) and CD4+ T-cells (T-helper) are involved in virus-induced wheezing in infancy during the early stages of the illness (Tuffaha and Busse 2001). During viral infection, CD8+ T-cells produce IFN-γ which has an ability to lyse infected cells (Tuffaha and Busse 2001). CD4+ T-cells differentiate into T-helper (Th) 1 cells or Th2 cells depending on cytokines produced by monocytes or antigen presenting cells (Tuffaha and Busse 2001, Pinto et al. 2006). Viral infections typically induce Th1-type cells, which produce cytokines, including IFN-γ and IL-2, two cytokines which are essential in cell-mediated immunity (Tuffaha and Busse 2001, Pinto et al. 2006, Gern 2008, Kelly and Busse 2008). The Th2 cells tend to produce other cytokines including IL-4 and IL-5, which are typical of asthma and allergy and important in humoral immunity and in the immediate-type hypersensitivity (Pinto et al. 2006, Smyth and Openshaw 2006).

The first B-cell response to a respiratory tract virus infection is the generation of mucosal IgA within a few days this being followed by the production of specific IgM and IgG antibodies (Kelly and Busse 2008). Thus, lymphocytes serve to clear infected epithelial cells and to limit the extent of infection (Gern 2008).

2.1.5. Factors contributing to susceptibility to virus-induced wheezing in infancy

Age, sex and co-morbidity

Population-based birth cohort studies have shown that wheezing during lower respiratory tract infection is most common during first year of life (Matricardi et al. 2008, Polk et al. 2004, Sandin et al. 2004, Taussig et al. 2003). In community-based studies, the prevalence of wheezing has been 18-32%, 9-17% and 4-12% in the first, second and third year of life, respectively (Taussig et al. 2003, Matricardi et al. 2008). Bronchiolitis cases occur most frequently in the first months of life (Koehoorn et al. 2008, Scottish Intercollegiate Guidelines Network 2006). In addition, younger infants are at higher risk than older infants for requiring hospitalization for virus-induced wheezing (Scottish Intercollegiate Guidelines Network 2006). In infancy, acute respiratory tract infections are equally common in males and females (Wright et al. 1989, von Linstow et al. 2008). Nonetheless, wheezing is more common in boys, as observed both in epidemiological (up to 1.4-fold) and in hospital-based studies (1.4-2.3-

fold) studies (Linneberg et al. 2006, Matricardi et al. 2008, Korppi et al. 1986, Reijonen et al. 1995, Koehoorn et al 2008, Marquet et al. 2009). Infants born prematurely or those who have congenital heart disease or chronic lung disease are at the highest risk of requiring hospitalization for bronchiolits (Scottish Intercollegiate Guidelines Network 2006).

Premorbid lung function

If the airway calibre is diminished at birth or in early life, LRTIs further reduce the calibre and thus facilitate wheezing symptoms. The measurement of maximal flow at the functional residual capacity ($V_{max}FRC$) by the tidal rapid non-invasive thoracoabdominal compression technique has yielded most of the information about small airway function in young infants (Hoo et al. 2002, Taussig et al 2003). In these studies, diminished airway function, as reflected by a reduced length-adjusted $V_{max}FRC$, has often preceded the first wheezing episode in infancy, and the length-corrected flows have been higher in female infants (Murray et al. 2002, Taussig et al. 2003). The sex difference in length-adjusted $V_{max}FRC$ is significant during the first year of life (Hoo et al. 2002), and this may account for the early sex-related differences in airway structure and tone as well as by slower airway growth in boys (Hoo et al. 2002). In addition, there is evidence that small infants have BHR to non-specific stimuli with diminishing reactivity with age (Taussig et al. 2003). Diminished lung function and BHR in infants before any LRTIs have been identified both in community-based studies and in a population with an atopic predisposition (Murray et al. 2002, Taussig et al. 2003).

In some studies, flow-limitation in infancy has been an independent risk-factor for wheezing illnesses, not related to known risk factors for asthma such as atopy or atopic predisposition (Turner et al. 2002, Turner et al. 2004). On the other hand, in a study which applied a plethysmographic method, infants with a family history of asthma displayed significantly reduced values of specific airway conductance compared to those with no family history of asthma (Dezateux et al. 1999). In addition, other factors, such as exposure to environmental tobacco smoke (ETS) and excessive weight gain, have been associated with reduced lung growth in infancy (Dezateux et al. 1999, Turner et al. 2008 and 2009).

Immunologic factors

Both innate immune responses, such as production of inflammatory mediators in response to host cell recognition of virus, and acquired immune responses, such as T-cell and B-cell mediated responses, are important in protecting the host against virus infections (Heymann et al. 2005, Singh 2007). Thus, variability in these immunologic responses may play an important role in host susceptibility to experience wheezing illnesses (Heymann et al. 2005, Oh 2006, Singh 2007, Juntti et al. 2009). Recent evidence has suggested that classical Th1-lympocyte cytokines, IL-2, IL-4, IFN-γ and IL-17, were virtually undetectable in secretions obtained from infants suffering from a severe infection caused by RSV (Welliver et al. 2007). In addition, high viral loads and activation of apoptotic mechanisms were associated with severe disease caused by RSV or influenza virus (Welliver et al. 2007). Thus, the pathogenesis of severe viral LRTI may be related to a failure to develop the appropriate cytotoxic T-cell response (Welliver et al. 2008), and this defect might be age-related, affecting mostly infants aged less than six months (Chung et al. 2007). A deficiency in the appropriate Th1-type responses is supported by the finding that infants with atopic predisposition exhibited an inverse correlation between cord blood IFN-γ responses of mononuclear cells and moderate to severe viral infections during the first year of life (Friedlander et al. 2005). In addition, excess Th2 type responses in atopic individuals may dimish viral clearance through suboptimal Th1 responses, and predispose patients to recurrent wheezing symptoms or asthma exacerbations (Papadopulos et al. 2002, Malmström et al. 2006).

Genetic factors

Genetic loci that contribute to the variability in innate and acquired immune responses have been studied in order to evaluate the contribution of genetic predisposition to wheezing in infancy (Singh 2007). Studies performed thus far are limited and concern mainly RSV-associated disease in hospitalized infants and are based on the candidate gene approach (Singh 2007, Miyairi 2008). It has been estimated that the genetic contribution accounts for approximately 20 % of the variance in RSV disease severity (Miyairi 2008). Genes associated with viral entry to the host cell, genes encoding surfactant proteins functioning as recognition molecules, Th1 versus Th2 cytokine response genes, neutrophil response genes and genes associated with antibody responses

and with HLA type I antigens have been studied (Miyairi 2008). The evidence from these studies suggests that the genes related to the limitation of the extent of the infection, specifically genes associated with IFN system, are important (Miyairi 2008). Deficient Th1-type responses and excessive Th2-type responses have been reported in RSV disease, and IL-13 has been speculated to play an important role in the Th2 skew (Miyairi 2008, Forton et al. 2009). Polymorphisms in a region which is associated with increased IL-13 production, have been linked to asthma and atopy, and in a recent study also to increased risk of severe RSV bronchiolitis in infants aged less than six months, pointing to a common genetic component in these diseases (Forton et al. 2009).

There are only a few genetic studies on the susceptibility of early wheezing illnesses caused by respiratory tract viruses other than RSV. However, there is evidence that immune responses as well as candidate genes in non-RSV bronchiolitis may be somewhat different than in RSV bronchiolitis. Expression of IFN-inducible genes has been higher in RSV bronchiolitis than in bronchiolitis caused by rhinovirus (Scagnolari et al. 2007). IL-10 gene polymorphism, connected to asthma and atopy, was associated with severe rhinovirus bronchiolitis, but not to RSV-bronchiolitis at the age of less than six months (Helminen et al. 2008).

Social and environmental factors

The population-based cohort studies have shown that exposure to infections as reflected in attendance to day-care or by the number of older siblings (Taussig et al. 2003, Polk et al 2004, Sandin et al. 2004, Linneberg et al. 2005, Koehoorn et al. 2008), increases the risk of wheezing in early childhood. In some studies, young maternal age, low maternal occupation level and low household income level have been related to an increased risk of wheezing episodes in early childhood (Koehoorn et al. 2008), but these associations have not been confirmed in all population-based cohorts (Linneberg et al. 2006). In most studies, breastfeeding has been protective for wheezing illnesses in infancy (Wright et al. 2001, Oddy et al. 2003, Polk et al. 2004, Linneberg et al. 2006, Koehoorn et al. 2008). The role of exposure to ETS as a risk factor for early wheezing in infancy is evident, (Wright et al. 1991, Polk et al. 2004, Carrol et al. 2007, Håberg et al. 2007) and some studies have highlighted the association between maternal smoking during pregnancy and increased risk of wheezing in infancy (Linneberg et al. 2006,

Håberg et al. 2007). There are also other factors, such as exposure to air pollution (Ryan et al. 2005), dampness in the home environment (Emenius et al. 2004) and antibiotic exposure in early life (Kummeling et al. 2007), which have been linked to an increased risk of wheezing in infancy.

The social and environmental risk-factors for hospitalization due to virus-induced wheezing are mostly the same as those for wheezing not requiring hospitalization (Nielsen et al. 2003, Koehoorn et al. 2008). Exposure to ETS has been related to prolonged hospitalization (Chatzimichael et al. 2007, Marquet et al. 2009) and low oxygen saturation (Bradley et al. 2005), and maternal smoking during pregnancy has been associated with hospitalization for bronchiolitis (Nielsen et al. 2003, Koehoorn et al. 2008). The role of air pollutants in the risk of hospitalization for wheezing in infancy is not clear; exposure to air pollutants has been claimed to increase the risk, specifically in infants born prematurely (Karr et al. 2006) or in infants born just before or during the peak of an RSV seasonal epidemic (Karr et al. 2009).

Atopy

Allergic individuals may have impaired antiviral responses, leading to more severe infections and further to wheezing (Papadopoulos et al. 2002, Gern 2008). It has been proposed that there exists a subgroup of infants with early respiratory allergy and eosinophilic inflammation in the airways, and this subgroup is particularly prone to wheeze during respiratory viral infections (Singh 2007, Gern 2008). Several studies have evaluated whether personal atopy or atopic predisposition increase the risk of wheezing illnesses in infancy. Population based-studies have found only weak (Illi et al. 2004, Linneberg et al. 2006, Håberg et al. 2007) or even no associations (Martinez et al. 1995, Sandin et al. 2004) between atopic manifestations or atopic predisposition and wheezing illnesses in infancy. In addition, the risk factors for atopic dermatitis seem to differ from those for wheezing in infancy suggesting that these two conditions have a different aetiology (Linneberg et al. 2006). However, some birth cohort studies have suggested that wheezing episodes are more frequent in infants with an atopic predisposition compared to infants with no atopic predisposition (Kuiper et al. 2007), and it is believed that other environmental factors, such as exposure to ETS, are particularly harmful in these predisposed infants (Kuiper et al. 2007).

A few studies have found an association between early atopy or atopic predisposition and bronchiolitis requiring hospitalization (Carrol et al. 2007, Stensballe et al. 2006), but many studies have failed to detect any such association (Singh 2007). In a recent study, atopic manifestations in infants hospitalized for bronchiolitis increased wih age and were more common in infants with recurrent wheezing (Jartti et al. 2009). Thus, the question of whether there is a subgroup of wheezing infants with allergies and possible asthma-like inflammation in the airways, is unclear, and might depend on the age of the studied infants.

Viral factors

In a birth cohort study conducted in infants with atopic predisposition, RSV caused more severe infections in infants than influenza A virus, parainfluenza virus or rhinovirus (Gern et al. 2002), which has also reported in small infants requiring hospital care (Marguet et al. 2009). There is some evidence that the presence of rhinovirus together with RSV increases the severity of bronchiolitis compared to RSV aetiology alone (Papadopoulos et al. 2002), but again some studies have failed to detect this relationship (Marguet et al. 2009). In other studies, there have been no differences in the clinical severity between rhinovirus induced bronchiolitis compared to RSV bronchiolitis in hospitalized infants (Kellner et al. 1989, Korppi et al. 2004a). However, infants hospitalized for rhinovirus induced wheezing were 6-24 months old, whereas infants with RSV aetiology of wheezing were less than 12 months old (Korppi et al. 2004a). In addition, rhinovirus infection was more often associated with atopy, eosinophil activation (Korppi et al. 2004a, Jartti et al. 2007) and a more favourable response to oral prednisolone treatment than was the cases for wheezing related to RSV infection (Jartti et al. 2007).

2.2. The prevalence of asthma symptoms in the child population, and the figures in short- and long-term follow-ups after early childhood wheezing

Approximately one-third of the children wheezing in the first three years of life continue to wheeze after the age of three years (Martinez et al. 1995, Kotaniemi-Syrjänen et al. 2002, Matricardi et al. 2008). There are studies on population-based birth

cohorts, high-risk birth-cohorts and hospital-based cohorts revieling that even mild wheezing in infancy is a significant risk factor for wheezing and asthma later in life.

2.2.1. Worldwide prevalence of asthma symptoms

According to the International Study of Asthma and Allergies in Childhood (ISAAC), the global prevalence of wheezing is 11.5 %, and frequent or severe asthma symptoms occur in 4.9 % of children at the age of six to seven years (Lai et al. 2009). The respective figures are 14.1 % and 6.9 % for 13-14 years old children (Lai et al. 2009). As seen in Table 2, the reported prevalence rates of asthma symptoms vary considerably; the rates of frequent asthma symptoms in the developed countries are highest in Australia and New Zealand (8.4-12.1%), and lowest in Europe (3.2-6.2%) including Finland (5.1%) and Sweden (3,4%). The lifetime prevalence of asthma was estimated to be 4.0 % in a non-selected population-based study in children aged 7-12 years living in Kuopio, Finland, and an additional 3.0 % had displayed some evidence of asthma during the preceding year (Remes et al. 1996).

Table2. Prevalence of wheezing and frequent wheezing or severe asthma symptoms at the ages of 6-7 and 13-14 years according to the ISAAC study (Lai et al. 2009)

	At the age of 6-7 years		At the age of 13-14 years	
Region	**Current wheeze (%)**	**Symptoms of frequent or severe asthma (%)**	**Current wheeze (%)**	**Symptoms of frequent or severe asthma (%)**
Global total	11,5	4,9	14,1	6,9
Finland[a] (Kuopio)	-	-	19,0	5,1
Sweden	10,2	4,2	9,7	3,4
Northern and Eastern Europe	8,7	3,2	9,7	3,8
Western Europe	9,6	3,6	14,3	6,2
North America	19,1	7,1	21,6	11,3
Australia	20,0	8,4	30,6	12,1
New Zealand	22,2	9,8	26,7	10,9

[a] Data not available for the age of 6-7 years

2.2.2. Short-term outcome after early childhood wheezing

Wheezing symptoms and asthma

The prospective birth-cohort-studies, based usually on self-reported symptom-history or current symptoms, have shown that about 30-40 % of infants will have wheezing symptoms until pre-school age if they have wheezed in their first three years of life (Martinez et al. 1995, Sherriff et al. 2001, Sandin et al. 2004, Matricardi et al. 2008). A retrospective population-based study, which applied a health-care specialist-confirmed diagnosis of bronchiolitis, revealed that 16 % of infants with an outpatient visit for bronchiolitis at less than one year of age had frequent wheezing symptoms or asthma at age 4-5.5 years (Carroll et al. 2009). In a high-risk birth cohort study, which included only children with an atopic predisposition, the risk of frequent symptoms or asthma at age six was 30-60 %, depending on the viral aetiology of early wheeze (Jackson et al. 2008).

After hospital admission for wheezing in infancy 18-53 % of pre-school children experience frequent symptoms or asthma (Kotaniemi-Syrjänen and Korppi 2007,

Valkonen et al. 2009), and the prevalence of school-age asthma, at 7.6-9.5 years of age, has been 15-40 % (Korppi et al. 1994, Sigurs et al. 2000, Henderson et al. 2005, Kotaniemi-Syrjänen et al. 2005).

Lung function

In the population-based lung function studies, it was found that pre-school children who continued to wheeze from infancy until six years of age had lower lung function in infancy, as indicated by their values for $V_{max}FRC$, compared to those who had never wheezed (Martinez et al. 1995). In another population-based study, a third of the early wheezers had BHR to histamine at the age of seven years, and this figure rose to more than 50 % in those children with both early and current wheeze, but early wheezing was not a significant predictor of BHR at early school age (Illi et al. 2006).

There is evidence that children hospitalized for RSV bronchiolitis at less than one year of age have reduced lung function, as measured by flow volume spirometry (FVS), at age seven years compared to age-matched controls (Fjaerli et al. 2005). In addition, a reduction in lung function and increased BHR have been observed at school age in children hospitalized for virus-induced bronchiolitis when they were less than two years of age compared to controls (Korppi et al. 1994). Further, seen in the present cohort followed up until early school age, 23 % of the children who had been hospitalized for virus-induced bronchiolitis in infancy exhibited reduced indices in FVS and 13 % had BHR to exercise, and BHR was significantly associated with rhinovirus aetiology of early wheezing (Kotaniemi-Syrjänen et al. 2008).

2.2.3. Long-term outcome after early childhood wheezing

Wheezing symptoms and asthma

In the birth cohort studies, about 30-40 % of the children who suffered wheezing at less than three years of age have continued to display symptoms until the age of 11-13 years (Morgan et al. 2005, Matricardi et al. 2008), but some birth cohort studies have reported lower figures, 13-24 %, for wheezing symptoms (Sporik et al. 1991, Lodrup Carlsen et al. 2006). There are only few birth cohorts which have been followed up until adulthood. In the birth cohort study from Tucson, Arizona, one third of the early

wheezers were regarded as asthmatic at the age of 22 years, and an additional third of early wheezers had inactive asthma, i.e. an asthma diagnosis but no current symptoms (Stern et al. 2008). In another birth cohort study in subjects with an atopic predisposition, 38 % of infants with wheezing before the age of two years still were displaying symptoms at the ages of either 11 or 22 years (Rhodes et al. 2001).

There are only few long term follow-up studies after hospitalization for wheezing in infancy. Wheezing or asthma has been demonstrated in 17-39 % of the children followed up to 10-13 years of age after hospitalization for wheezing in infancy (Table 3), and these figures are higher than in controls or in the general population in all of the studies. There are only two prospective follow-up studies after hospitalization for early childhood wheezing which have continued until adulthood (Piippo-Savolainen et al. 2004, Goksör et al. 2006). These studies were conducted in Finland and Sweden, and the prevalence of asthma at the age of 17-20 years was similar, 41-43 %, in both of these studies (Piippo-Savolainen et al. 2004, Goksör et al 2006).

Lung function

In the population-based lung function studies, LRTI in infancy has been associated with lowered lung function, as measured by forced expiratory flows, until the age 10-11 years (Stein et al. 1999b, Håland et al. 2009), and adolescents who had a history of wheezing before the age of three years, have had significantly lower forced expiratory flows than those with no history of early wheezing (Morgan et al. 2005). However, in some studies it was lung function at birth rather than LRTIs in the first years of life which has explained the later deficits in lung function (Håland et al. 2009).

In the lung function studies after hospitalization for early wheezing, forced expiratory flows have been reduced at the age of 10-13 years (Noble et al. 1997, Sigurs et al. 2005). Increased BHR has been demonstrated to dry-air (Sigurs et al. 2005) or to methacholine (Gurwitz et al. 1981, Wennergren et al. 1997). Abnormal lung function parameters, measured by FVS, were seen in 31-36 % of the subjects, compared with 11-16 % of the controls, in the bronchiolitis cohorts followed-up until adulthood (Piippo-Savolainen et al. 2004, Goksör et al. 2008).

Table 3. Hospitalization for wheezing in infancy: Asthma prevalence at the age of 10-13 years.

Reference	Study population/ design	Number of children with early wheezing/ number of controls	Age (months)on admission	Age (years)at the follow-up in cases/controls	Prevalence (%) of asthma in cases/ in controls	Definition of symptoms
Gurwitz et al. 1981	Bronchiolitis/ retrospective	48/-	7.6 (mean, range 2-23 months)	10.4/-	17	Physician-diagnosed asthma
Noble et al. 1997	Bronchiolitis/ prospective	61/47	3.6 (mean)	10.1/10.0	39/13	Self-reported current respiratory symptoms or maintenance asthma medication
Wennergren et al. 1997	Wheezing bronchitis/ prospective	92/-	< 24 (median 10)	≥ 10/-	30/-	From mild to severe asthma based on the current need of the bronchodilators and/or maintenance asthma medication
Sigurs et al. 2005	RSV-bronchiolitis/ prospective	47/93	< 12 (mean 3.7)	13.4/13.4	28/3.3	≥ 3 episodes of physician-diagnosed wheezing in the past year

2.3. Early-life predictive factors for long-term outcome after early childhood wheezing

The question of whether acute virus-induced wheezing in infancy can cause asthma, or is only a marker of a predisposition towards asthma has been addressed in many studies. Viruses cause direct cytopathic effects to the epithelium, which may evoke physiologic changes in the airways. In addition, viruses can interact with both innate and adaptive immune responses, and these effects may be different depending on the environmental factors and genetic predisposition of the host. The march from early life wheezing to persistent asthma is complex and evidently multi-factorial with many host- and environmental factors involved in the process.

2.3.1. Age

In Tucson's study, 20 % of the children with wheezing only during the first year of life, wheezed after the age of three years, with the figures for second and third year wheezing being 40 % and 60-70 %, respectively (Taussig et al. 2003). However, the follow-up of the Tucson's cohort has shown that persistent wheezing (from infancy until age six) is a risk factor for asthma in adulthood, suggesting that most forms of asthma have their origins in early childhood (Stern et al. 2008). An increasing number of asthma risk factors become involved already in early life, probably between the ages one to two years, which might explain the increasing risk of recurrent wheezing at this age (Jartti et al. 2009). However, there are also opposite findings: in infants hospitalized for RSV-induced wheezing at age less than 12 months, the occurrence of wheezing symptoms was high and persisted up to the teenage years (Sigurs et al. 2005), suggesting that severe disease in a very young infant may have a different impact on the development of asthma than a milder disease.

2.3.2. Lung function in infancy

In the Tucson birth cohort, $V_{max}FRC$ levels, measured shortly after birth, was lowest in children who wheezed only during the three first years of life but not thereafter (Morgan et al. 2005). In addition, wheezing persisting from early childhood up to the age of 11 years has been associated, independent of atopy, with reduced $V_{max}FRC$

measured in infancy (Turner et al. 2004). Increased BHR to histamine shortly after birth has been related to transient wheezing (Wilson et al. 2004), but when BHR is present at the age of 12 months, it is often persistent and associated with the development of asthma by the age of 11 years (Turner et al. 2009). Furthermore, there is also evidence, that at least in atopic infants, BHR, measured shortly after wheezing in infancy, is related to the development of asthma in a ten-year follow-up (Saga et al. 2001).

In clinical practice, a response to bronchodilators is often considered to be a sign of asthma. The early bronchodilator response, as evaluated by tidal lung function measurements in asymptomatic children with a history of wheezing at less than two years of age, was significantly larger than in non-wheezing controls, and the value increased with an increasing number of asthma risk factors (Lodrup Carlsen et al. 2004). However, unfortunately the early bronchodilator responses with regard to later asthma status of the children were not reported in that study.

2.3.3. Viruses

RSV

In the Tucson birth cohort, the children with a history of RSV-LRTI, including pneumonia cases, during the first three years of life were more likely to still be having episodes of wheezing up to the age of 11 years, but no longer at the age of 13 (Stein et al. 1999b). Furthermore, at the age of 11 those children with a history of RSV-LRTI had a lower forced expiratory volume in one second (FEV_1) and they were more likely to respond bronchodilators than children with no history of LRTI in infancy (Stein et al. 1999). A history of RSV-LRTI in infancy was unrelated to skin prick-test (SPT) positivity at age 11 (Stein et al. 1999b). Similarly, in another birth cohort study, RSV-bronchiolitis requiring hospitalization at the age less than 12 months was associated with asthma, but not with the development of atopy compared to the other population in the cohort by the age of seven years (Henderson et al. 2005).

There are a limited number of hospital-based long-term follow-up studies examining the association between RSV-induced wheezing and asthma. RSV-LRTI requiring hospitalization during the first year of life was not associated with asthma in a

retrospective study conducted in Northern Finland, and even a reduction in SPT positivity was seen at the age of 6-10 years in children with a history of hospitalization for RSV-LRTI in infancy when compared to matched controls (Juntti et al. 2003). In a prospective Swedish study, hospitalization for RSV bronchiolitis at the age of less than 12 months was found to be a risk factor for asthma compared to the matched controls at the follow-up visits at the ages of three, seven and 13 years. However, a reduction in risk ratios was seen with increasing age (Sigurs et al. 1995, 2000 and 2005, Perez-yarza et al. 2007), pointing to a disappearance of the effect of early RSV infection. However, decreased lung function and increased BHR to dry-air hyperventilation compared to matched controls could still be demonstrated at age 13 (Sigurs et al. 2005). Sensitization to common allergens was more common in RSV subjects than in matched controls at the age of 13 (Sigurs et al. 2005). In a 20-year prospective follow-up, asthma and SPT positivity were not more common after RSV bronchiolitis in infancy than than corresponding value in controls (Korppi et al. 2004b). However, indices in the FVS, FEV_1/forced vital capacity (FEV_1/FVC) and maximal expiratory flow when 25 % of the FVC remains to be expired (MEF_{25}), were lower in subjects with a history of RSV bronchiolitis compared to controls (Korppi et al. 2004b). In studies comparing RSV bronchiolitis to non-RSV bronchiolitis, no long-term differences in asthma prevalence have been observed between the groups (Wennergren et al. 1997) or asthma prevalence has been even lower, as seen in the present cohort at an early school age (Kotaniemi-Syrjänen et al. 2002), and in another bronchiolitis cohort in adolescence (Hyvärinen et al. 2005) and in early adulthood (Piippo-Savolainen et al. 2007b). However, there are no long-term follow-ups which have used the novel molecular methods available for viral diagnoses.

Rhinovirus

Many studies have demonstrated that rhinoviruses are the major cause of asthma exacerbations in both children and adults (Jartti et al. 2004, Friedlander et al. 2005). Impaired innate antiviral responses in asthmatics have been observed (Kelly and Busse 2008), and persistence of rhinovirus in asthmatic bronchial epithelium, also in infants, has been demonstrated (Kelly and Busse, Malmström et al. 2006). It has been postulated that rhinovirus plays a role in the pathogenesis of remodelling by generating the

mediators involved in the process (Leigh et al. 2008). However, population-based long-term follow-up-studies on the association between early rhinovirus-induced wheezing and subsequent asthma are lacking, since no molecular viral diagnostics were available before the 1990s. The Childhood Origins of ASThma cohort, a birth cohort consisting of infants with at least one atopic parent, demonstrated that rhinovirus-induced viral wheezing, though not requiring hospitalization, was a significant predictor of asthma at the age of six years (Jackson et al. 2008). In another high-risk cohort, wheezing induced by rhinovirus either alone or in combination with RSV at less than one year of age was associated with wheezing at age five years, but only in children sensitized at less than two years of age (Kusel et al. 2007), and these authors concluded that viral infections may interact with the onset of atopy in infancy to promote later asthma.

Seen in the present cohort at early school age, asthma was more common after rhinovirus aetiology of early wheezing compared the situation when the wheezing was attributable to RSV or other viral causes (52% vs. 15%) (Kotaniemi-Syrjänen et al. 2003b), and rhinovirus aetiology associated with early wheezing was linked with BHR to exercise (Kotaniemi-Syrjänen et al. 2008). However, there are no long-term follow-up studies with which to conduct a comparison of the outcomes after rhinovirus and RSV-bronchiolitis.

Other viruses

Other respiratory viruses causing early wheezing, including probably also the newly discovered hMPV and bocavirus, have been included into the non-RSV-group in studies which have evaluated the role of viral aetiology of early wheeze on the later respiratory morbidity. Hospitalization for hMPV bronchiolitis has been associated with asthma at pre-school-age (García-García et al. 2007), but the long-term outcome is unknown. In addition, the outcome after bronchiolits caused by bocavirus is unknown.

2.3.4. Atopy

As presented in Figure 1, the Tucson birth cohort study classified wheezing during the first three years of life into transient wheezing, non-atopic persistent wheezing and atopic persistent wheezing or asthma based on the development of atopy and the persistence of wheezing symptoms by the age of six years (Taussig et al. 2003). Transient wheezers are individuals who wheeze only during the first three years of life and are not likely to be atopic or to have an atopic predisposition, and in most of these children, the wheezing symptoms will have disappeared before school age. Children with non-atopic persistent wheezing may wheeze both during the first three years of life and at the age of six years, but the risk of wheezing decreases with age and is no longer significant at the age of 11-13 years. Atopic persistent wheezers are often sensitized to inhalant allergens, and the risk of wheezing persists from infancy at least up to early adolescence, and this group also shows the lowest level of lung function at the ages six and 11 years (Taussig et al. 2003, Morgan et al. 2005). Thus, an early onset of the symptoms and atopic sensitization are believed to be important risk factors for severe and persistent disease (Taussig et al. 2003, Morgan et al. 2005). However, sensitization to allergens was not studied before six years of age in the Tucson birth cohort. The German Multicenter Allergy Study cohort is the only birth cohort study which has evaluated the role of early atopic sensitization, as defined by serum specific IgE (sIgE), on wheezing at 11-13 years of age in children with a history of wheezing before three years of life. In that study, a positive family history of atopy and early sensitization to perennial allergens were the strongest risk factors for persistent wheezing (Matricardi et al. 2008). Early sensitization to food allergens predicted the persistence of wheezing in the univariate analyses, but no longer in the multivariate analyses (Matricardi et al. 2008).

In the present cohort, early atopic dermatitis and sensitization (defined by serum sIgE) to wheat, egg white or inhalant allergens were predictive for asthma at early school age (Kotaniemi-Syrjänen et al. 2003a). In the Finnish cohort which was followed up until adulthood, parental asthma predicted asthma and parental atopy was associated with pathological lung function (Piippo-Savolainen et al. 2004). In addition, early sensitization to common inhalant allergens, as measured by non-automated radioallergosorbent tests (RAST), predicted asthma until the age of 13.5-16 years

(Piippo-Savolainen et al. 2007a). However, there are no other long-term follow-up studies after hospitalization for virus-induced wheezing with measurements of serum sIgE to common food and inhalant allergens determined in infancy.

2.3.5. Early exposure and sensitization to animal danders

In the 2000′s, many studies have documented that early-life exposure to animals can decrease the subsequent development of allergy and asthma (Ownby et al. 2002, von Mutius et al. 2007). The theory known as the hygiene hypothesis proposed that exposure early in life to house dust, animals and livestock increases an infant′s exposure to endotoxins or other protective factors originating from the animals or their microbial flora (Strachan 1989, Ownby and Johnson 2003, von Mutius et al. 2007). Increase in the Th1 immunity leading to increase in IFN- γ production, but not in Th2 interleukins, such as IL-4 and IL-13, may be the mechanism to explain why exposure to infective agents could reduce the production of sIgE and the prevalence of allergic diseases and asthma (von Mutius 2007). In addition to Th1/Th2 balance, T regulatory cells may have a central role in controlling immune responses such as the rate of secretion of sIgE from the B cells (Akdis et al. 2005, von Mutius 2007).

Birth cohort studies on early exposure to furry pets and the later development of asthma have given inconsistent results (Apelberg et al. 2001 and Ownby and Johnson 2003), from an increased wheezing risk in children with asthma in parents (Sandin et al. 2004) to a decreased asthma risk, at least in low-risk children, continuing until adolescence and even longer (Remes et al. 2001).

In the German birth cohort, intense exposure to cat or mite allergens in wheezing infants at age less than three years was not associated with wheezing at the age 13 years (Matricardi et al. 2008), but sensitization to cat or mite allergens was a strong predictor of wheezing (Matricardi et al. 2008). In the Finnish post-bronchiolitis study, neither exposure nor sensitization to cat or dog dander predicted the appearance of asthma in early adulthood (Piippo-Savolainen et al. 2007a).

2.3.6. Environmental tobacco smoke exposure

Exposure to ETS in utero and in infancy reduces lung function and affects lung function development (Moshammer et al. 2006, Carlsen et al. 2008, Landau et al. 2008). In particular, there is an association between maternal smoking and development of asthma in childhood (Cook et al. 1999), and the effect of maternal smoking during pregnancy on lung function at school age may be greater than the effect of exposure to ETS after birth (Moshammer et al. 2006). There are no long-term follow-up studies which have evaluated the role of ETS on the development of asthma after early childhood wheezing. In the Tucson birth cohort, maternal smoking during pregnancy was linked to low levels of lung function before any LRTI had developed and to the transient wheezing phenotype (Stein et al. 1999). In the German birth cohort, in early wheezers, parental smoking was not a risk factor for the persistence of wheezing at puberty (Matricardi et al. 2008).

In the Swedish long-term follow-up, exposure to ETS at the time of hospitalization for virus-induced wheezing in infancy was predictive of asthma at the age of 10 years, and both pre- and postnatal exposure to tobacco smoke were independent risk factors for asthma in early adulthood, and maternal smoking during pregnancy was an independent risk-factor for BHR (Wennergren et al. 1997, Goksör et al. 2007). The authors concluded that small airway function in early adulthood may be attributable to by prenatal exposure to tobacco smoke, but post-natal exposure to ETS increases the asthma risk by increasing the risk of active smoking in early adulthood (Goksör et al. 2007). In the Finnish post-bronchiolitis study, exposure to maternal smoking at less than two years of age was not associated with asthma in early adulthood, but it was a significant risk factor for lung function abnormalities at that age (Piippo-Savolainen et al. 2006).

2.3.7. Eosinophilic inflammation

The role of eosinophilic inflammation is obscure in early childhood wheezing. It is unclear which age is critical for the development of eosinophilic inflammation and the appearance of permanent structural changes typical for asthma in the airways of wheezing children. In recent bronchial biopsy studies, evidence was found that the age from one to three years is probably the most critical period, since changes were not seen

even in children with a history of severe wheezing younger than 24 months but the changes could be demonstrated in wheezing children by three years of age (Saglani et al. 2005, Saglani et al. 2007). In addition, there seems to be a subgroup of RSV-positive wheezing infants presenting with airway eosinophilia and increased IL-5 levels and IL-5/ IFN-γ –ratios, which points to a heterogeneity of airway inflammation in RSV-induced wheezing and Th2-type-response in a subgroup of wheezing infants with airway eosinophilia (Kim et al. 2003). Furthermore, in a recent study, blood eosinophilia was postulated to be an early sign of allergic inflammation, even before eosinophilia could be detected in alveolar or bronchial tissue (Just et al. 2008).

In the birth cohort studies, blood eosinophilia has been considered as a significant risk factor when assessing asthma risk in children with repeated wheezing at less than three years of age (Castro-Rodriquez et al. 2000, Guilbert et al. 2004). In addition, lack of eosinophilia in infants with a history of recurrent wheezing has been predictive of future remission (Just et al. 2008). Blood eosinophilia during hospitalization for RSV bronchiolitis at less than 18 months of age has been predictive for wheezing until the age of seven (Ehlenfield et al. 2005). Activated eosinophils release a wide spectrum of proteins, such as eosinophil cationic protein (ECP). Accordingly, elevated levels of ECP in serum and/or the presence of ECP in respiratory secretions have been associated with subsequent wheezing and even asthma in infants with RSV bronchiolitis (Pifferi et al. 2001), and as seen in the present cohort, in young children wheezing with other viruses at less than two years of age (Reijonen et al. 1997a and b). However, the role of blood eosinophils and ECP in the prediction of persistent asthma has not been established in long-term follow-up studies. Neither blood eosinophilia nor elevated serum levels of ECP evaluated at less than two years of age were associated with asthma at the age of 10 years in a Swedish study (Wennergren et al. 1997). Likewise, no association was seen between blood eosinophilia during an acute viral wheeze in infancy and later asthma in a Finnish bronchiolitis cohort followed-up until adulthood (Piippo-Savolainen et al. 2007c). However, the lack of eosinopenic response, i.e. a physiologic decrease in blood eosinophil count, to infection and blood eosinophilia when there was no infection in infancy were significant risk factors for the development of asthma by the teenage years and in adulthood (Hyvarinen et al. 2005 and Piippo-Savolainen et al. 2007c).

2.3.8. Neutrophilic inflammation

Neutrophil accumulation is more predominant than eosinophil accumulation in airway inflammation associated with acute viral wheeze in infancy (Kim et al. 2003, Marguet et al. 2008). MPO is the most abundant protein in neutrophils (Klebanoff et al. 2005), and the main function of MPO is to kill bacteria and other pathogens phagocytised by neutrophils (Klebanoff et al. 2005). MPO is released from neutrophils also during RSV infection (Jaovisidha et al. 1999), and it possesses the capacity of damaging lung epithelial cells (Haegens et al. 2008) and in addition, it may have pro-inflammatory properties (Lau et al. 2005). However, the role of neutrophils and their products, such as MPO, in asthma and other respiratory disorders has been poorly studied. In adults, there is preliminary evidence to suggest that neutrophils from patients with asthma release more MPO than do cells from subjects without asthma (Carlson et al. 1991). An inverse relationship has been reported between lung function and serum MPO levels in patients with allergic asthma (Monteseirin et al. 2001). The results from the studies conducted in children suggest that serum MPO levels are higher in children with symptomatic or unstable asthma, when compared to children with episodic or non-symptomatic asthma (Kristjansson et al. 1994, Scher et al. 1996, Carlsen et al. 1997, Kalayci et al. 2000), and bacterial infections increase serum MPO levels in both asthmatic and non-asthmatic children (Tauber et al. 1999). No association has been reported between serum MPO levels and atopy (Boner et al. 1993, Carlsen et al. 1997).

No significant differences in serum MPO levels have been found between infants with recurrent wheezing and healthy controls, and furthermore, serum MPO showed no association with BHR assessed by tidal flow volume responses to inhaled salbutamol (Lodrup-Carlsen et al. 1995). There is only one earlier study which has examined the role of serum MPO, measured in early-life wheezers, in the prediction of subsequent wheezing and later asthma. In that study, serum MPO was increased in infants less than 12 months old who had been hospitalized for RSV-induced bronchiolitis but the level decreased within a few weeks, but serum MPO at any level was not predictive for subsequent wheezing and asthma in the two-year follow-up (Sigurs et al. 1994). However, there are no long-term follow-up studies on serum MPO levels in wheezing infants and the subsequent risk of developing asthma.

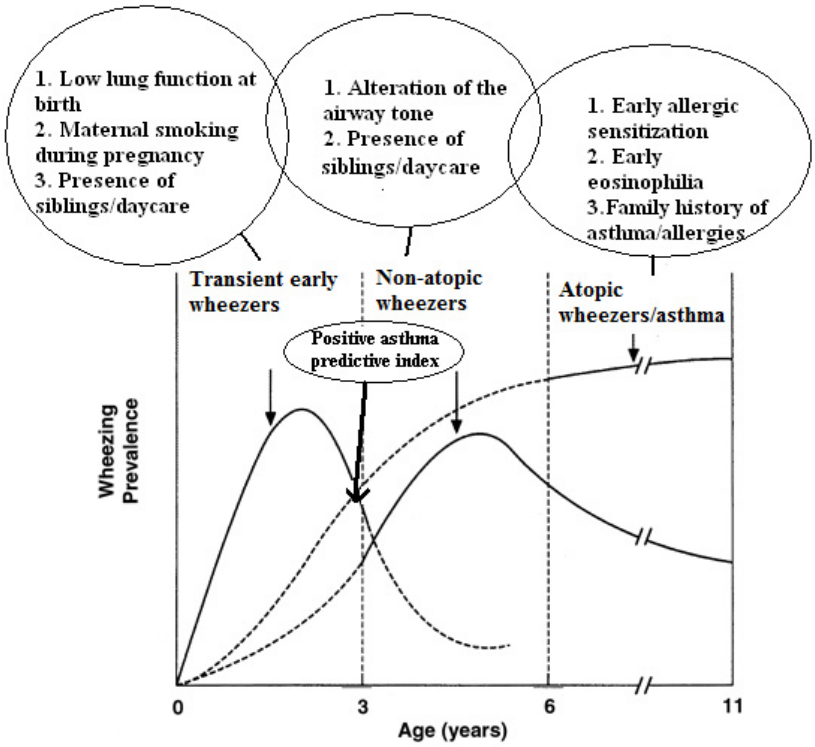

Figure 1. Hypothetical wheezing phenotypes and related risk-factors. Modified from Stein et al. 1997, Taussig et al. 2003 and Gastro-Rodriguez and Garcia-Marcos 2008. Dashed lines suggest overlap between the groups.

2.3.9. Clinical tools for identifying the child at high risk of developing asthma after early childhood wheezing

Wheezing in infancy is a heterogenic condition. Most of the children with wheezing episodes in infancy have normalized by school age, but in some infants, early wheezing is the first sign of childhood asthma (Figure 1). At present, there is no laboratory marker, biological or genetic, available which can recognize those wheezing infants who are at high risk of developing asthma. A combination of clinical characteristics and

simple biological markers has been created to form algorithms, which may identify, at an early stage of the disease, those wheezing children at a high risk of later asthma (Figure 1). The algorithms developed to facilitate the prediction of later asthma in wheezing children aged less than three years are presented in Table 4. Castro-Rodriquez et al. introduced the first algorithm “asthma predictive index” (API) (Castro-Rodriguez et al. 2000), to be applied to predict which subjects who suffer wheezing at less than three years of age will have asthma between the ages six and 13 years. The algorithm exhibited good specificity of 85-97%, but a low sensitivity of 16-42 % only. The API was later modified (mAPI) for the use in the Prevention of Early Asthma in Kids trial to select recurrently wheezing children for secondary asthma prevention with inhaled corticosteroids (Guilbert et al. 2004). In addition to API and mAPI, the algorithms have been revised, based on the results of the Finnish and Swedish post-bronchiolitis studies, to be more suitable for evaluating infants and toddlers being treated for wheezing in hospital (hAPI, Piippo-Savolainen and Korppi 2008).

Table 4. Algorithms to identify wheezing children at high risk of asthma: Asthma predictive index (API), modified API (mAPI) and API to define children hospitalized for wheezing at high risk for subsequent asthma (hAPI)

	API (Castro-Rodriguez et al. 2000)	mAPI (Guilbert et al. 2004)	hAPI (Piippo-Savolainen and Korppi 2008)
Prediction of asthma	1. Frequent[a] wheezing and ≥ 1 major criteria or		1. Hospitalization for wheezing and ≥1 major criteria [c] or
Either 1 or 2	2. Frequent[a] wheezing and ≥ 2 minor criteria		2. Hospitalization for wheezing and ≥2 minor criteria
Major criteria	1. Parental history of asthma[b]	1. Parental history of asthma[b]	1. Parental history of asthma [b]
	2. Atopic dermatitis[b]	2. Atopic dermatitis[b]	2. Atopic dermatitis[b] and/or sensitization to food allergens
		3. Allergic sensitization to ≥ 1 aeroallergen	
Minor criteria	1. Allergic rhinitis[b]	1. Allergic sensitization to milk, egg or peanuts	1. Sensitization to inhaled allergens
	2. Wheezing unrelated to colds	2. Wheezing unrelated to colds	2. Wheezing induced by other viruses than RSV
	3. Blood eosinophils ≥ 4 %	3. Blood eosinophils ≥ 4 %	3. Blood eosinphilia or lack of eosinopenic response during viral infection

[a] API: ≥ 3 episodes, mAPI: ≥ 4 episodes, hAPI:hospitalization for early wheezing

[b] Diagnosed by phycisian

[c] Early exposure to environmetnal tobacco smoke as well as current smoking has further increased the asthma risk

3. AIMS OF THE STUDY

The main aims of this thesis were to evaluate the outcome 11 years after wheezing requiring hospitalization in infancy, and in this way to identify at-risk infants, to find risk factors present already during wheezing in infancy for teenage asthma and for asthma lasting throughout childhood until teenage.

The specific aims of the study were:

1. To evaluate the prevalence of teenage asthma and the prevalence of persistent childhood asthma after hospitalization for wheezing in infancy.
2. To study which host, viral, and environmental risk factors, present during hospitalization for wheezing in infancy, would be associated with teenage asthma and/or persistent childhood asthma.
3. To study whether early exposure to dog and cat allergens would be related to early teenage asthma and/or persistent childhood asthma and to sensitization to these allergens.
4. To study whether clinical responses to bronchodilating agents in infancy can be used to identify those wheezing children at a high risk of developing later asthma in childhood.
5. To elucidate whether markers of eosinophilic activation, i.e. elevated blood eosinophil count or elevated levels of eosinophil cationic protein in serum or in nasopharyngeal aspirate, predict later childhood asthma after hospitalization for wheezing in infancy.
6. To evaluate the role of neutrophil-derived serum myeloperoxidase, when measured durig infancy, in the prediction of subsequent asthma.
7. To find risk factors, already present during hospitalization for wheezing in infancy, for abnormal flow volume spirometry and bronchial hyper-reactivity in the teenage years.

4. SUBJECTS AND METHODS

4.1. Enrolment and acute-phase study

4.1.2 Study subjects

Between January 1st 1992 and November 2nd 1993, 100 consecutive children, aged 1-23 months, were recruited into this study using the inclusion criteria of respiratory infection-related wheezing (the index episode of wheezing) and respiratory distress requiring inpatient treatment in the Department of Paediatrics, Kuopio University Hospital, Finland (Reijonen et al. 1995). Patients were excluded if they had a history of chronic cardiorespiratory disease, including asthma, or if they received regular medication for any pulmonary disease, or if they had taken bronchodilators in the preceding six hours, or if acute respiratory failure was threatening (Reijonen et al. 1995).

4.1.3. Outcome measures and acute-phase intervention study

In all infants, a baseline clinical evaluation was performed, consisting of measurements of respiratory rate, heart rate (Component Central Monitor, Hewlett Packard GMBH, Böblingen, Germany), oxygen saturation (Ohmeda Biox 3700e Pulse Oximeter, Ohmeda, Louisille, Columbia), body temperature (°C, rectally) and clinical assessment of Respiratory Distress Assessment Instrument (RDAI) score (Reijonen et al. 1995). The RDAI score, assessed by one of the four study doctors, was based on two respiratory variables, wheezing and retractions (Lowell et al. 1987).

In order to investigate whether racemic epinephrine (adrenaline) and/or albuterol (salbutamol) would improve the clinical condition of infants, patients were randomly assigned to one of four treatment groups. To prevent confounding by viral aetiology and wheezing history, the randomisation was performed in clusters of 12 cases, and the 13 children with and the 87 children without any previous history of wheezing were randomised separately (Kernan et al. 1999).

The crossover study design is presented in Table 5. The REP (racemic epinephrine followed by placebo) group first received nebulized racemic epinephrine, and the AP

(albuterol followed by placebo) group first received nebulized albuterol; both groups received nebulized physiological saline (0.9% sodium chloride) solution 30 minutes later. The PRE (placebo followed by racemic epinephrine) and the PA (placebo followed by albuterol) groups first received physiological saline, and 30 minutes later either racemic epinephrine (PRE) or albuterol (AP).

Racemic epinephrine was given at a dose of 0.9 mg/kg (Micronefrin, Bird Products Corp, Palm Springs, California), and albuterol (Salbuvent, Orion Pharma, Espoo, Finland) was given at a dose of 0.15 mg/kg. Solutions were added to 2 ml of physiological saline solution. All inhalations were administered by a trained nurse using a Spira Module 2 Nebulizer (Respiratory care Center, Hämeenlinna, Finland) with a continuous 5-L/min flow of 100% of oxygen. The design of the administration of the drugs was double-blind; neither the patients or their parents, nor the study doctors knew which drug (or placebo) was being administered.

The clinical assessment of oxygen saturation, respiratory rate, heart rate and RDAI score was repeated, by the same study doctor as at the baseline assessment, 15 and 30 minutes after each inhalation (Reijonen et al. 1995). Sixty minutes after the first inhalation, intramuscular administration of epinephrine (0.01mg/kg) was given to all patients (Reijonen et al. 1995).

Table 5. The crossover study design of the acute-phase intervention study

Periods	Period 1				Period 2
Clinical evaluations	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**
REP [a] n=24	RE		P		i.m. epinephrine
AP [b] n=27	A		P		i.m. epinephrine
PRE [c] n=24	P		RE		i.m. epinephrine
PA[d] n=25	P		A		i.m. epinephrine

[a] Racemic epinephrine (RE) followed by placebo (P)

[b] Albuterol (A) followed by P

[c] Placebo (P) followed by racemic epinephrine (RE)

[d] Placebo (P) followed by albuterol (A)

4.1.4. Baseline data

The baseline and family data were collected by interviewing the parents during the hospitalization by using a structured questionnaire including questions on the history of wheezing and atopic dermatitis, as well as on the family history of asthma and atopic diseases. Only diagnoses made by a physician were accepted. In addition, information on maternal smoking during pregnancy and early exposure to ETS and pets at home or at day care in infancy was recorded (Reijonen et al. 1995 and 1996).

4.1.5. Laboratory studies

Determination of blood eosinophils, serum eosinophil cationic protein and serum total immunoglobulin E

A venous blood sample was drawn from each patient within the 24 hours after admission for the determination of blood eosinophils (B-EOS), serum eosinohil cationic protein (S-ECP) and serum total IgE. B-EOS were counted by using an automated cell counter (Coulter Counter STKS, Coulter Electronics, Hialeah, FL) and expressed as cells $x10^9$ /L (Reijonen et al. 1997a). S-ECP was determined according to the instructions of the manufacturer (Pharmacia ECP RIA, Pharmacia, Uppsala, Sweden), as described earlier in detail (Reijonen et al. 1997a). The cut-off limits for the elevated values applied in the later studies were 0.450 cells x 10^9/L for B-EOS (Eisen 1980, Reijonen et al. 1997a, Kotaniemi-Syrjänen et al. 2002, Simon et al. 2007), 16.0 µg/L or 20.0 µg/L for S-ECP (Peterson et al. 1991, Koh et al. 2007) and 60 kU/l (144 µg/l) for serum total IgE (Saarinen et al. 1982).

Determination of nasopharyngeal eosinophil cationic protein

NPA samples, obtained within 24 hours after hospital admission, were taken by placing a catheter into the nasopharynx, followed by aspiration into a mucus trap (Reijonen et al. 1997b). Each sample was stored frozen in a tightly capped tube at -70 °C until processed within four months. After thawing and weighing, the sample was suspended in 2-5 ml of ice-cold 0.9% NaCl solution to produce a homogenous suspension and then centrifuged for 10 min (1.400g), and the precipitate was discarded

(Reijonen et al. 1997b). The ECP concentration in the supernatant was determined according to the instructions of the manufacturer (Pharmacia ECP RIA, Pharmacia, Uppsala, Sweden). ECP was expressed as nanograms (ng)/g of NPA (ECP-NPA) after correction for dilution with 0.9 % NaCl (Reijonen et al. 1997b). The receiver operating characteristic (ROC) curve analysis was applied to find the cut-off concentration for NPA-ECP which would maximize true positive cases (sensitivity) and minimize false positive cases (1-specificity). The ROC analyses were performed in relation to both asthma at the median age of 12.3 years (teenage asthma) and persistent childhood asthma (PCA), and the best cut-off limit for NPA-ECP was 815.0 ng/g with relation to both teenage asthma (sensitivity 0.42, specificity 0.83, positive likelihood ratio (LR+) 2.5) and with relation to PCA (sensitivity 0.58, specificity 0.83, LR+ 3.4).

Determinations of serum myeloperoxidase

The level of serum myeloperoxidase (MPO) was determined in frozen serum samples obtained on admission (Measurement 1; S-MPO1) by radioimmunoassay (MPO RIA, Pharmacia AB, Uppsala, Sweden), and the concentrations were expressed as micrograms (µg)/L. The total variation of the method was 5,8 - 12,3 % in the concentration range of 32 - 730 ug/L. S-MPO measurements were repeated by the same method 6 weeks (measurement 2; S-MPO2), 4 months (measurement 3; S-MPO3) and 12 months (measurement 4; S-MPO4) later at the median ages of 0.98 years (range 0.20-2.10), 1.15 years (0.39-2.26) and 1.86 years (1.14-3.12), respectively.

Determination of serum allergen -specific IgE antibodies

In 2000, serum specific IgE (sIgE) antibodies (Phadiatop Combi®) were determined in frozen serum samples, which had been obtained during the index episode of wheezing in 1992-1993, by fluoroenzyme-immunometric assay (FEIA), UniCAP™ (Pharmacia, Uppsala, Sweden) (Paganelli et al. 1998). The presence of serum sIgE antibodies in response to the mixtures of inhalant and food allergens was screened first by the detection limit (≥0.35 kU/l), and if positive, serum sIgE antibodies were measured, with the same limit. Phadiatop combi® panel for food allergens included egg white, cow's milk, fish, wheat, peanut, and soy bean, and for inhalant allergens timothy grass pollen, birch pollen and mugwort pollens, cat dander, dog dander, horse

dander, house dust mite *D. pteronyssinus*, and spores of the mould *C. herbarum*.

Viral studies

Viral infections were studied by antigen detection in the NPAs taken at entry and by antibody measurements in paired sera. Direct detection of viral antigens by time-resolved fluoroimmunoassay (TR-FIA) was available for RSV, parainfluenzaviruses 1-3, influenza A and B viruses, and adenoviruses (Reijonen et al. 1998). Serology by CF was studied for the same respiratory viruses in paired sera taken six weeks apart: four-fold or greater increase in titres was defined as a positive result (Reijonen et al. 1997b).

In 2000, 81 frozen good-quality NPA samples taken on admission in 1992-1993, were evaluated available for RT-PCR for detection of rhinoviruses, enteroviruses and coronaviruses (strains 229E and OC-43) (Kotaniemi-Syrjänen et al. 2003b). In 2002, an adequate NPA sample was available for the detection of the RSV genome by RT-PCR in 61 cases (Kotaniemi-Syrjänen et al. 2005).

4.2. Intervention with anti-inflammatory medication and short-term follow-up

Nebulized anti-inflammatory therapy was randomly given for 16 weeks from admission: 34 patients received cromolyn sodium, 34 received budesonide, and 32 control patients received no anti-inflammatory medication (Reijonen et al. 1996). For the first eight weeks, 20 mg of cromolyn sodium was given four times a day and 500 µg of budesonide twice a day. For the second eight weeks, the doses were reduced to 20 mg of cromolyn sodium three times a day and 250 µg of budesonide twice a day (Reijonen et al. 1996).

After the index episode of wheezing, several study visits were organized in order to follow-up the respiratory status and allergic manifestations of the children. SPTs were studied for the first time eight months after the hospitalization at the median age of 1.5 years. A trained nurse performed the tests by using the ALK SPT extracts including cat and dog epithelial dander allergens (ALK Laboratories, Copenhagen, Denmark).

Three years after the index episode of wheezing, at the median age of 4.0 years (range 3.2-5.1 years), 89/100 children attended the pre-school follow-up visit (Reijonen et al. 2000). Children who were either on continuous anti-inflammatory medication for

asthma or had suffered from at least two doctor-diagnosed wheezing episodes after the index episode of wheezing were considered to have asthma (Reijonen et al. 2000).

4.3. Follow-up visit at early school age

In 1999, at a median 6.3 years (range 5.3-7.2 years) after the index episode of wheezing 82/100 children attended the follow-up visit at a median age of 7.2 years (range 5.8-8.8 years). Symptoms suggestive of asthma and allergies were recorded and the baseline pulmonary function was examined by FVS (Medikro, Kuopio, Finland). The FVS was followed by the exercise challenge test (ECT) (Kotaniemi-Syrjänen et al. 2002), which consisted of 8-minute free running outdoors at the heart rate of ≥ 80% of maximum. The ECT was considered as positive if there was auscultatory wheezing present after the exercise and/or a 15 % or greater fall in FEV_1 compared to baseline value (Kotaniemi-Syrjänen et al. 2002). Asthma was considered to be present if 1) the child had suffered from at least two episodes of wheezing and/or prolonged cough apart from infection for at least four weeks during the preceding 12 months and the exercise challenge test was pathological or 2) the child was on continuous inhaled anti-inflammatory medication for asthma (Kotaniemi-Syrjänen et al. 2002).

4.4. Follow-up visit at early teenage

4.4.1. Study subjects

In 2004, from January to March, 81/100 children, 21 girls and 60 boys, attended the clinical follow-up visit in the Department of Paediatrics, Kuopio University Hospital, Finland. The median follow-up time from the index episode of wheezing was 11.4 years (range 10.3-12.2) and the median age at the follow-up visit was 12.3 years (range 10.9-13.7).

4.4.2. Follow-up data and clinical examination

Before the study visit in 2004, a structured questionnaire was sent to the study subjects. Symptoms suggestive of asthma associated with exercise, infections and allergens were recorded. The study doctor (Mari Hyvärinen) checked the answers by interviewing the children and their parents during the clinical study. In addition to

asthma symptoms, the symptoms suggestive of allergic rhinitis, conjunctivitis and atopic dermatitis occurring during the preceding 12 months were recorded, and if present classified as current. In addition, the ongoing asthma medication was registered.

Skin prick tests (SPT) were performed, and the allergens (ALK SPT extracts, ALK Laboratories, Copenhagen, Denmark) tested were the outdoor allergens birch, common alder and mugwort pollens, pollens of meadow fescue and timothy grass, and spores of *C. herbarum*, and the indoor allergens cat, dog, horse and cow epithelial danders and home dust mites *D. pteronyssinus* and *D. farinae* and spores of the mold *Alternaria alternata*. The concentrations of standardized allergen extracts were 10 histamine equivalent points except for cow (1/100 w/v), *C. herbarum* (1/20 w/v) and *A. alternata* (100 BU/ml) extracts. Histamine hydrochloride (10 mg/ml) was used as a positive control and 50 % glycerol as a negative control. Wheals with a mean (half of the sum of the largest diameter and its perpendicular measurement) diameter of at least 3 mm were regarded as positive. No reactions were allowed to negative controls.

4.4.3. Studies on lung function and BHR

Baseline lung function

Baseline flow-volume spirometry (FVS) was examined by a pneumotachographic spirometer (Medikro, Kuopio, Finland). Before the visit, the children were instructed not to use inhaled long-acting β2-agonists for 48-72 hours or short-acting β2-agonists for 12 hours before the lung function studies. In addition, no symptoms suggestive of respiratory tract infections were allowed. First, the children were carefully instructed on how to perform the test. Thereafter, the measurements were repeated three times, and accepted if the variation in FEV_1 was less than 5 % and the graphic curves were appropriate and equal in shape. The FVS was appropriately performed by 80/81 children. Lung function parameters were expressed as percentages of the gender-specific and height-related reference values (% of predicted) for Finnish children (Salorinne 1989). The parameters registered were FVC, FEV_1, FEV_1/FVC, MEF_{50} (maximal expiratory flow at 50 % of FVC) and MEF_{25}. From the acceptable FVS curves, the best FVC and FEV_1 were recorded for further analyses. The other flow-volume parameters were obtained from three technically satisfactory FVS curves by the

envelope method, in which the best superimposed curves from total lung capacity were applied to form a composite maximal curve (Quanjer et al. 1993). The cut-off limits for abnormal results were < 80 % for FEV_1, < 88 % for FEV_1/FVC, < 62 % for MEF_{50} and < 48 % for MEF_{25} (Viljanen 1982).

Tests of BHR

The baseline FVS was followed by ECT that consisted of 8-minute free running outdoors at the heart rate of ≥ 80 % of the predicted maximum. During running, the heart rate was monitored by telemetry (Polar Sport Tester, Polar Elektro Ltd, Kempele, Finland) at 1-minute intervals. FVS was measured 5, 10 and 15 minutes after the exercise. The FEV_1 obtained from the baseline FVS was used in later comparisons. FEV_1 changes were calculated as follows: [(pre-exercise FEV_1 – post-exercise FEV_1) / pre-exercise FEV_1] x 100 %. The ECT was regarded as positive if there was a 10 % or greater fall in FEV_1 values at 5, 10 or 15 minutes after the running (Godfrey 2000). One of the study doctors (Mari Hyvärinen) was responsible for both the performance FVS and ECT and the interpretation of the results. The ECT was appropriately performed by 78/81 children.

The methacholine inhalation challenge (MIC) test was performed on a separate day from the other lung function studies. The children were given instructions and the baseline FVS was performed as described above. Methacholine was inhaled from a Spira Electro-2 dosimeter (Spira Respiratory Care Center Ltd, Hämeenlinna, Finland) allowing the calculation of the total amount of methacholine inhaled by each subject. First, FVS was performed before and after an inhalation of physiological saline, to obtain the baseline FEV_1. Then, methacholine was inhaled, and the numbers of breaths required to achieve a cumulative dose of 20, 80, 300, 900, 2900 and 4900 µg of methacholine were 1, 3, 11, 3, 10 and 10, respectively. The concentration of methacholine increased from 2.5 mg/ml to 25 mg/ml after the cumulative dose of 300 µg. The baseline FEV_1 value was applied in the comparisons with the measurements done 1.5 minutes after each methacholine dose. The test was continued until a 20 % fall in FEV_1, or until a cumulative dose of 4900 µg of methacholine was reached. The exact cumulative dose causing 20 % fall in FEV_1 was determined by semilogarithmic interpolation and termed the provocative dose (PD_{20}). The methodology of the MIC was

the same as that routinely used in our clinic (Pöysä et al. 1992). The MIC test was appropriately performed by 69/81 children.

4.4.4. Asthma definition

Asthma at the median age of 12.3 years (teenage asthma) was considered to be present if 1) the child was on a continuous maintenance or intermittent anti-inflammatory medication for asthma or if 2) she/he had suffered from repeated (≥2) episodes of wheezing or prolonged (≥4 weeks) cough apart from infections during the preceding 12 months as reported by parents, and the ECT was regarded as positive.

Children, who were regarded to have asthma at all the three follow-up visits, at the median ages of 4.0 (Reijonen et al. 2000), 7.2 (Kotaniemi-Syrjänen et al. 2002) and 12.3 (present study) years, were considered to have persistent childhood asthma (PCA).

4.5. Ethics

The study was approved by the Research Ethics Committee of Kuopio University and Kuopio University Hospital. Informed written consent was obtained from the children and from the parents of the children.

4.6. Statistical analyses

The data were analyzed using SPSS 11.5-16.0 software (SPSS Inc.Chicago, IL, USA). In univariate analyses, Pearson's Chi square test and Fisher's exact test were used for dichotomous data. Fisher's exact test was used when the expected frequency for any cell was < 5. The risks are expressed as odds ratios (OR) and their 95% confidence intervals (95% CI). Continuous data were analyzed by the independent sample T-test or Mann-Whitney U-test.

The logistic regression was used to calculate the adjusted ORs (aOR) and related 95% CIs. Analysis of variance (ANOVA) was applied to assess the significance of the differences in the continuous baseline FVS parameters (percentages of the gender-specific height-related reference values) between children with and without a certain risk factor present. Each risk factor was analyzed separately. In this model, covariates acted as effect modifiers with a bilateral interaction with the risk factor. The association between S-MPO1 and oxygen saturation, heart rate, respiratory rate, body temperature

and RDAI score on admission as well as the length of hospital stay (in days), was studied by linear regression adjusted for age (in months) on admission. The skewed MPO data were logarithmically (log_{10}) transformed (LogMPO1). Each variable was entered into the age-adjusted linear model separately with LogMPO1 as a dependent variable.

Repeated measures analyses of variance (ANOVA) was applied to study whether the change in LogMPO concentration was significant at a various time points (within-subject effect), and whether there were any differences in the change between intervention groups or later asthma groups (between-subject effects). Separate analyses were performed for each group, and the analyses between asthma groups were adjusted for age on admission (<12 months/ ≥12 months), atopic dermatitis in infancy and RSV aetiology of early wheezing, which have associated with asthma in the earlier studies in this cohort (Reijonen et al. 2000, Kotaniemi-Syrjänen et al. 2002).

The initial response to acute treatment with regard to later asthma was analysed as a crossover study using mixed models (Brown and Prescott 2001). The pooled responses used as dependent variables were the mean values of respiratory rates, heart rates, oxygen saturations and RDAI scores after the first (mean of the 15 and 30 minute assessments of responses, period 1) and second (mean of the 45 and 60 minute assessments of the responses, period 2) administration of racemic epinephrine, albuterol or placebo. The standard elements of a cross-over trial, such as the period (1 or 2), sequence (whether the drug or placebo was given first), treatment (racemic epinephrine, albuterol, or placebo) and the random effect of the patient (describing the natural heterogeneity of individuals and accounting for the dependence of repeated measures from the same subject) were included into the model. In addition, PCA and the interaction of PCA and treatment were also included into the model in order to address the study hypothesis. Analyses were performed separately applying either15- and 45-minute (15 minutes after inhalations) or 30- and 60-minute (30 minutes after inhalations) responses as dependent variables.

5. RESULTS

5.1. Outcomes in the 11-year follow-up

5.1.1. Prevalence of teenage asthma and persistent childhood asthma (I)

Teenage asthma was considered to be present in 32 (40 %) children, and 29 of them were on inhaled anti-inflammatory asthma medication; 17 were using inhaled steroids and one cromones as continuous medication, and 11 used inhaled steroids intermittently during the pollen season and/or infections. The other three children with asthma had suffered from repeated wheezing, and as an objective asthma criterion, displayed a diagnostic fall in FEV_1 (range 12 -31 %) in the ECT. Even though they were taking anti-inflammatory asthma medication, 22/29 (76 %) children had suffered from repeated wheezing episodes or prolonged cough apart from infection during the preceding 12 months or had a pathological result in the ECT. The asthma criteria were not fulfilled in 6/31 (19%) children who reported some kind of wheezing and in 5/27 (19%) of those who reported repeated wheezing during the preceding 12 months.

PCA was present in 20/81 (25%) children, and 27 (33%) children had not had an asthma diagnosis between the ages of 4-12 years. As seen in Figure 1, pre-school asthma had remitted by school-age in 14/45 (31%) children but relapsed again in 7/49 (14%) children at teenage. Only 7/33 (21%) children with asthma at school age had experienced a remittance by teenage, and whereas, 22/33 (67%) continued to have asthma (Figure 2).

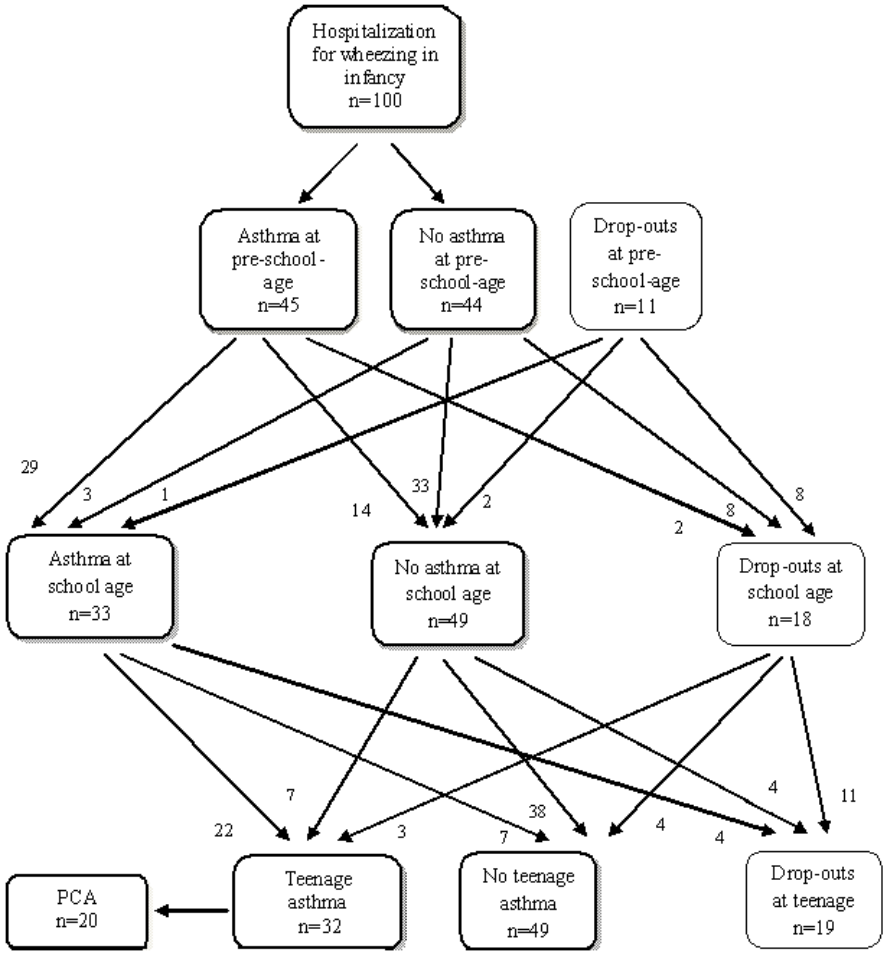

Figure 2. Asthma at the three clinical follow-up visits: at pre-school age, at school-age and in teen years (teenage asthma). PCA=Persistent Childhood Asthma, asthma present at all three follow-up visits.

5.1.2. Prevalence of atopy (I)

Allergic rhinitis and/or conjunctivitis was present in 30/81 (37%) and atopic dermatitis in 26/81 (32%) children. SPTs were performed in all 81 cases, but one child was excluded from the analyses because of a positive reaction to the negative control, and 50/80 (63%) children displayed at least one positive reaction. The figures for indoor and outdoor allergens were 40/80 (50%) and 42/80 (53%), respectively.

Allergic rhinitis, conjunctivitis and SPT positivity were more common in children with teenage asthma and in children with PCA compared to children with no teenage asthma or no PCA (Table 6). Atopic dermatitis was more common in children with PCA compared to children without PCA, but not in children with teenage asthma compared to children with no asthma at that age (Table 6).

Table 6. Atopic manifestations in children with teenage asthma and in children with PCA.

	Teenage asthma			**PCA**		
Atopic manifestations	**Yes (n=32)**	**No (n=49)**	**OR [a] (95% CI)**	**Yes n=(20)**	**No (n=61)**	**OR [a] (95% CI)**
Allergic rhinitis n(%)	19(59)	8(16)	7.49 (2.66-21.09)	14(70)	13(21)	8.62 (2.77-26.83)
Allergic conjunctivitis, n(%)	14(44)	5(10)	6.84 (2.15-21.81)	8(40)	11(18)	3.03 (1.00-9.17)
Atopic dermatitis, n(%)	14(44)	12(24)	2.40 (0.92-6.23)	11(55)	15(25)	3.75 (1.30-10.78)
SPTs[b]:						
At least one positive reaction, n(%)	28(90)	22(45)	11.46 (3.07-42.75)	18(95)	32(52)	16.31 (2.05-129.98)
to indoor allergens[c], n(%)	24(77)	16(33)	7.07 (2.52-19.85)	17(89)	23(38)	14.04 (2.97-66.43)
to outdoor allergens[d], n(%)	24(77)	18(37)	5.91 (2.12-16.42)	15(79)	27(44)	4.72 (1.40-15.89)

[a] Non-adjusted analyses.

[b] SPTs were available in 80 children (31 with current asthma and 19 with PCA).

[c] There were 31 positive reactions to cat, 29 to dog, 20 to horse and 11 to cow's epithelial dander, and 12 were positive to D.*pteronyssinus* and 6 to D. *farinae.*

[d] There were 24 positive reactions to birch, 25 to common alder, 34 to meadow fescue, 35 to timothy grass, to 3 to mugwort pollen and 4 were positive to *C. herbarum*.

5.1.3. Lung function and bronchial hyper-reactivity (III)

Baseline flow volume spirometry

The FVS was appropriately performed by 80/81 (99 %) children (Table 7). On average, all parameters were within normal limits when compared to gender-specific and height-related references. Children with asthma at early school age and PCA had higher FVC than children with no teenage asthma or no PCA, and FEV_1/FVC values were lower in children with teenage asthma compared to children with no asthma at that age (Table 7). In the supplememtary analyses, in which only children without asthma at any time formed the non-PCA group, FEV_1/FVC values were significantly lower in the PCA group than in the non-PCA-group.

Twenty-six (33 %) children exhibited an abnormal value in one or more of the four FVS parameters. The figure for teenage asthma was 11/32 (34 % vs. 31 % in those with no teenage asthma, OR 1.2, 95% CI 0.45-2.98) and for PCA 6/20 (30 % vs. 33 % in those with no PCA, OR 0.86, 95% CI 0.29-2.57). Among FVS parameters, children with teenage asthma had more often a pathological finding in MEF_{25} (8/32, 25%) compared to children with no asthma at that age (3/48, 6%, OR 5.0, 95%CI 1.21-20.61), but children with and without PCA did not differ in terms of any of the FVS parameters (data not shown). The three medication groups, i.e. continuous, intermittent and no anti-inflammatory medication groups differed significantly only in terms of the abnormal MEF_{25} value, the group with continuous medication having higher numbers for pathological values than those not taking any medication.

Table 7. Baseline lung function by FVS (% of predicted) in all teenage children and in children with asthma at that age (teenage asthma) and PCA.

Parameters in FVS	All	Teenage asthma	PCA
	n=80	n=32	n=20
FVC, mean	97.79	101.19 [a]	102.93 [c]
(95% CI)	(95.04-100.55)	(96.08-106.30)	(96.74-109.12)
FEV_1, mean	92.23	93.15	95.17
(95% CI)	(89.35-95.12)	(87.55-98.76)	(88.05-102.30)
FEV_1/FVC, mean	94.55	92.22 [b]	92.59
(95% CI)	(92.67-96.43)	(88.70-95.75)	(87.84-97.34)
MEF_{50}, mean	81.43	78.12	80.29
(95% CI)	(77.20-85.67)	(70.22-86.01)	(69.84-90-73)
MEF_{25}, mean	71.68	67.57	70.02
(95% CI)	(67.10-76.27)	(59.12-76.02)	(59.10-80.95)

[a] FVC= 95.53 in non-asthmatics (p=0.044 compared to children with asthma at early teenage)
[b] FEV1/FVC=96.09 in non-asthmatics (p=0.044 compared to children with asthma at early teenage)
[c] FVC=96.08 in children with no PCA (p=0.031 compared to children with PCA)
P-values determined by independent sample t-test.

Tests of bronchial hyper-reactivity

The ECT was appropriately performed by 78/81 (96%) children, and 21 (27%) children had a pathological (≥ 10 % fall in FEV_1) result. Children with continuous or intermittent anti-inflammatory medication had more often a pathological result in the ECT (range 11-34 %) compared to those not taking medication (13/27, 48%, vs. 8/51, 16%, OR 4.99, 95%CI 1.72-14.51).

In the MIC test, some kind of BHR (PD_{20}≤4900 µg) was demonstrated in 41 (59 %), intermediate to high BHR (PD_{20}≤1600 µg) in 32 (46%), and high BHR (PD_{20}≤400 µg) in 11 (16 %) children. As shown in Table 8, children with intermittent or continuous anti-inflammatory medication displayed more often a BHR to methacholine at all three levels compared to the children with no anti-inflammatory medication. Likewise, BHR to methacholine at all three levels was more common among children with teenage asthma or PCA compared to children with no asthma at that age or PCA. The only

exception was the BHR to methacholine when one compared children with PCA to those without PCA (Table 9).

Both the MIC test and the ECT were appropriately performed by 66 children, and 15 of them had a positive result in both tests (≥10 % fall in FEV_1 in the ECT and PD_{20}≤1600 µg in the MIC test). Sixteen children were positive in the MIC test alone and only three in the ECT alone.

Table 8. BHR to methacholine by consumption of anti-inflammatory asthma medication in teenage years.

	Intermittent or continuous anti-inflammatory medication		
BHR to methacholine n=69	**Yes [a] (n=24)**	**No (n=45)**	**OR[b] (95% CI)**
Any, n (%)			
PD_{20}≤4900µg	19 (79)	22 (49)	3.97 (1.26-12.49)
Intermediate to high, n (%)			
PD_{20}≤1600µg	16 (67)	16 (36)	3.63 (1.27-10.31)
High, n (%)			
PD_{20}≤400µg	7 (29)	4 (9)	4.22 (1.09-16.32)

[a] Intermittent anti-inflammatory medication in 10 and continuous medication in 14 children.

[b] Non-adjusted analyses

Table 9. BHR to methacholine according to the presence of teenage asthma and PCA.

BHR to methacholine n=69	Teenage asthma Yes (n=27)	Teenage asthma No (n=42)	Teenage asthma OR [a] (95% CI)	PCA Yes n=(17)	PCA No (n=52)	PCA OR [a] (95% CI)
Any, n (%) PD_{20}≤4900µg	22 (81)	19 (45)	5.33 (1.69-16.75)	14 (82)	27 (52)	4.32 (1.11-16.84)
Intermediate to high, n (%) PD_{20}≤1600µg	19 (70)	13 (31)	5.30 (1.85-15.20)	12 (71)	20 (38)	3.84 (1.18-12.54)
High, n (%) PD_{20}≤400µg	9 (33)	2 (5)	10.00 (1.20-51.04)	5 (29)	6 (12)	3.19 (0.83-12.28)

[a] Non-adjusted analyses

5.2. Early-life predictive factors for asthma in the 11-year follow-up (I, II, IV, V, VI)

5.2.1. Age, gender and history of earlier episodes of wheezing (I)

Children with teenage asthma had been significantly older on admission than children with no asthma at that age (median age 12.02 months vs. 8.77, p=0.033). The respective figures for children with and without PCA were 13.75 vs. 9.46 months (p=0.002). Teenage asthma was present in 16/52 (31%), and PCA in 7/52 (13%) of those hospitalized at less than 12 months of age with the respective figures being 55% (OR 2.76, 95% CI 1.08-7.09) and 45% (OR 5.22, 95% CI 1.77-15.40) for those who were hospitalized when they were older than 12 months. Gender was not significantly associated with teenage asthma or PCA; 8/21 (38%) of females had teenage and 5/21 (23 %) had PCA, the respective figures for males were 24/60 (40%) and 15/60 (25 %). A history of earlier physician-diagnosed wheezing was more common among children with teenage asthma or PCA compared to children with no teenage asthma (7/32, 22% vs. 3/49, 6%, aOR 3.73, 95% CI 0.87-16.04) or PCA (5/20, 25% vs. 5/61, 8%, aOR 3.16, 95% CI 0.76-13.10). However, in the age- and sex-adjusted logistic regression, these differences were not significant.

5.2.2. Family history of asthma and atopy and environmental factors (I, IV)

A parental and maternal history of asthma were at least twice as common in children with teenage asthma and in children with PCA in comparison with the children without these conditions, but the differences were not statistically significant in the adjusted analyses (Table 10). Likewise, no significant differences were seen between parental atopy, exposure to ETS or maternal smoking during pregnancy and teenage asthma or with PCA (Table 10), and there were no associations between paternal asthma, maternal atopy, paternal atopy, paternal smoking or maternal smoking and teenage asthma or PCA (data not shown).

Early exposure to cats or dogs seemed to be associated with some reductions in the risk of teenage asthma or PCA (Table 10). In addition, only 2/12 (17%) children exposed to cat and 3/18 (17%) children exposed to dog during infancy had a positive SPT reaction to the respective allergens in their teens compared to 29/68 (43%) and 26/62 (42%) for non-exposed children. These inverse relationships between early exposure and later sensitization to cat or dog were not statistically significant after adjustment for the presence of atopic dermatitis in children or atopy in parents between children with or without teenage asthma (aOR 0.30, 95% CI 0.06-1.57) or between children with or without PCA (aOR 0.35, 95% CI 0.09-1.38). In addition, only 3/22 (14%) of the families with atopic dermatitis in children vs. 19/59 (32%, p=0.095) with no atopic dermatitis in children, and 7/38 (18%) with asthma or allergy in parents vs. 15/43 (35%, p=0.096) with no asthma or allergy, had a dog or cat at home at the time of admission.

Table 10. Early family and environmental factors in relationship to teenage asthma and PCA.

	Teenage asthma			PCA		
Family and environmetnal factors	**Yes (n=32)**	**No (n=49)**	**aOR[d] (95% CI)**	**Yes n=(20)**	**No (n=61)**	**aOR[c] (95% CI)**
Parental history of asthma [a], n(%)	8 (25)	5 (10)	3.355 (0.92-12.24)	5 (25)	8 (13)	2.74 (0.67-11.24)
Maternal history of asthma [a], n(%)	5 (16)	2 (4)	5.08 (0.87-29.65)	3 (15)	4 (7)	3.22 (0.56-18.49)
Parental history of atopy [b], n(%)	15 (47)	21 (43)	1.17 (0.47-2.95)	9 (45)	27 (44)	1.02 (0.35-3.03)
Exposure to ETS during infancy, n(%)	15 (47)	22 (45)	1.35 (0.52-3.51)	11 (55)	26 (43)	3.19 (0.93-10.92)
Maternal smoking during pregnancy, n(%)	9 (28)	8 (16)	2.02 (0.68-6.05)	5 (25)	12 (20)	1.62 (0.45-5.75)
Exposure to cat during infancy, n(%)	2 (6)	10 (20)	0.30[d] (0.05-1.61)	1 (5)	11 (18)	0.34[d] (0.04-3.20)
Exposure to dog during infancy, n(%)	6 (19)	12 (24)	1.21[d] (0.35-4.16)	1 (5)	17 (28)	0.235[d] (0.03-2.09)

[a] Diagnosed by a physician

[b] Atopic dermatitis or allergic rhinitis diagnosed by a physician

[c] Logistic regression adjusted for sex and age (in months) on admission. After adjustment for RSV-aetiology of the index episode of wheezing, the results remained the same.

[d] Logistic regression adjusted for age (in months), atopic dermatitis in infants, parental history of asthma and RSV aetiology of the index episode of wheezing on admission.

5.2.3. Viruses related to the index episode of wheezing (I)

A third of the index episodes were related to RSV and another third to rhinovirus infection and in most of the RSV- and rhinovirus-positive cases, no other viruses were identified (Table 11).

Table 11. Viral aetiology of the index episode of wheezing in 81 children attending to the follow-up visit in teenage years.

Viral findings [a]	All n=81
RSV, n (%)	25 (31)
as a single viral finding	17
in mixed viral infections [b]	8
Picornaviruses :	
Rhinovirus, n (%)	24 (30)
as a single viral finding	19
in mixed viral infections [c]	5
Enterovirus, n (%)	10 (12)
as a single viral finding	5
in mixed viral infections	5
Other viruses alone or in combinations, n (%)	8 (10)
No viral findings, n (%)	22 (27)

[a] Antigen detection (RT-FIA) and serology (CF) for RSV, adenovirus, influenza viruses A and B and parainfluenza viruses 1-3 performed in all 81 children. RT-PCR for RSV performed in 48/81 and RT-PCR for picornaviruses in 66/81 children.

[b] 1 RSV-rhinovirus, 4 RSV-enterovirus, 2 RSV-parainfluenza virus-3 and 1 RSV-adenovirus-parainfluenza virus-3 co-infections

[c] 1 rhinovirus-RSV, 1 rhinovirus-enterovirus, 2 rhinovirus-adenovirus and 1 rhinovirus-parainfluenza virus-3-adenovirus co-infections were detected

Teenage asthma was present in 5/25 (20%) of the children if the index episode of wheezing in infancy was related to RSV, and in 11/19 (58%) if the index episode was related to rhinovirus. The respective figures for PCA were 2/25 (8%) and 9/19 (47%). Although aORs were 0.29-0.45 for RSV-related and 1.27-2.51 for rhinovirus-related early wheezing, the associations between viral findings during the index episode of wheezing and teenage asthma or PCA were not statistically significant (Table 12 and

Table 13). Fourteen of the former 25 (56%) RSV-positive children had at least one positive reaction in SPTs in their teenage years, compared to 37/56 (66%, OR 0.65, 95% CI 0.25-1.71) of the RSV-negative children. The respective figures were 14/19 (74%) and 23/42 (55%) for rhinovirus positive and negative cases, respectively (OR 2.31, 95%CI 0.71-7.59).

The index episode of wheezing was related to RSV in 14/27 (52%) children who never experienced asthma in childhood, and the respective figure for rhinovirus aetiology alone was 3/27 (11%). In the supplementary analyses, with adjustment for age, sex and early atopic dermatitis, where the no PCA group consisted of the "no asthma ever" group, early wheezing related to neither RSV (aOR 0.23, 95% CI 0.04-1.42) nor to rhinovirus (aOR 2.53, 95% CI 0.37-17.32) was significantly associated with PCA.

Table 12. RSV aetiology of the index episode of wheezing in infancy in relation to teenage asthma and PCA.

	Teenage asthma			**PCA**		
RSV	**Yes (n=32)**	**No (n=49)**	**aOR[b] (95% CI)**	**Yes n=(20)**	**No (n=61)**	**aOR[b] (95% CI)**
RSV alone or in combinations with other viruses, n (%)	5(16)	20(41)	0.40 (0.11-1.40)	2(10)	23(38)	0.40 (0.07-2.20)
RSV alone, n (%) [a]	4(13)	13(31)	0.45 (0.11-1.88)	1(5)	16(30)	0.29 (0.03-2.85)

[a] The 8 RSV co-infections were excluded from the analyses. The numbers for teenage asthma/no teenage asthma were 31/42, and for PCA/no PCA=19/54.

[b] Logistic regression adjusted for sex , age (in months) and atopic dermatitis on admission. After further adjustment for passive smoking in infancy, the results remained the same.

Table 13. Rhinovirus aetiology of the index episode of wheezing in infancy in relation to teenage asthma and PCA.

	Teenage asthma			PCA		
Rhinoviruses	Yes (n=27)	No (n=39)	aOR[c] (95% CI)	Yes n=(16)	No (n=50)	aOR[c] (95% CI)
Rhinovirus alone 1, n (%) [a]	11(41)	8(21)	1.41 (0.40-4.93)	9(56)	10(20)	2.51 (0.62-10.21)
Rhinovirus alone 2, n (%) [b]	11(44)	8(22)	1.39 (0.38-5.10)	9(56)	10(22)	1.93 (0.45-8.21)
Rhinovirus alone or in combinations with other viruses, n (%)	13(48)	11(28)	1.27 (0.39-4.15)	9(56)	15(30)	1.30 (0.33-5.20)

[a] Rhinovirus alone compared to other viruses, rhinoviruses in combinations or no viral identification.

[b] Rhinovirus alone compared to other or no viral identification. Rhinovirus in combinations with other viruses excluded from the analyses. The numbers for teenage asthma /no teenage asthma were 25/36, and for PCA/no PCA 16/45.

[c] Logistic regression adjusted for sex , age (in months) and atopic dermatitis on admission. After further adjustment for passive smoking in infancy, the results remained the same.

5.2.4. Atopy in children (I, IV, unpublished data

Atopic dermatitis and serum total IgE (I)

As presented in Table 14, atopic dermatitis at the time of the index episode of wheezing in infancy was predictive for both teenage asthma and PCA, but serum total IgE≥60 kU/L associated significantly only with PCA.

The presence of serum specific IgE to food allergens (I, unpublished data)

The presence of serum sIgE of ≥0.35 kU/L to the mixture of food allergens at the time of the index episode of wheezing was quite common, but it was not significantly associated with the development of teenage asthma or with PCA (Table 15). With respect to the individual allergens, cow's milk was predictive for teenage asthma and wheat was a strong predictor of both teenage asthma and PCA (Table 15).

The presence of serum specific IgE to inhalant allergens (I, unpublished data)

The presence of serum sIgE of ≥0.35 kU/L to the mixture of inhalant allergens was significantly associated with teenage asthma and PCA (Table 16). Sensitization to individual inhalant allergens was rare at the time of the index episode of wheezing in infancy, and only the presence of sIgE to dog and cat allergens, which were detectable in more than five subjects were included in the analyses. No significant associations were found between teenage asthma or PCA and the presence of serum sIgE to either cat or dog allergens in infancy (Table 16).

Table 14. Atopic dermatitis and serum total IgE≥60 kU/L at the index episode of wheezing in infancy in relation to teenage asthma and PCA.

	Teenage asthma			**PCA**		
Atopy	**Yes (n=32)**	**No (n=49)**	**aOR[b] (95% CI)**	**Yes n=(20)**	**No (n=61)**	**aOR[b] (95% CI)**
Atopic dermatitis, n(%)	14(44)	8(16)	3.48 (1.20-10.09)	11(55)	11(18)	4.53 (1.42-14.44)
Serum total IgE ≥60 kU/L [a], n(%)	11(37)	5(11)	3.51 (0.95-12.95)	10(53)	6(10)	5.82 (1.51-22.42)

[a] Data were available for 77/81 children. The numbers for teenage asthma/teenage asthma were 30/47 and for PCA /no PCA 19/58.

[b] Logistic regression adjusted for sex and age (in months) on admission

Table 15. The presence of allergen-specific IgE antibodies to food allergens measured from the 73/81 children at the index episode of wheezing in relation to teenage asthma at and PCA.

	Teenage asthma			PCA		
Specific IgE of ≥0.35 kU/l n=73	**Yes (n=29)**	**No (n=44)**	**aOR[a] (95% CI)**	**Yes (n=20)**	**No (n=53)**	**aOR[a] (95% CI)**
Food allergens, n(%)	18(62)	16(36)	2.28 (0.78-6.66)	14(70)	20(38)	2.66 (0.80-8.91)
Egg white, n(%)	15(52)	13(30)	2.01 (0.68-5.96)	12(60)	16(30)	2.38 (0.72-7.80)
Cow's milk, n(%)	13(45)	7(16)	3.43 [b] (1.08-10.86)	9(45)	11(21)	2.09 (0.64-6.79)
Wheat, n(%)	10(34)	1(2)	20.24 [c] (2.37-172.86)	8(40)	3(6)	9.66 [d] (2.11-44.14)
Peanut, n(%)	9(31)	5(11)	2.76 (0.74-10.29)	8(40)	6(11)	3.77 (0.98-14.53)

[a] Logistic regression adjusted for sex and age (in months) on admission. Allergens with > 5 positive detections are presented.
After further adjustment with atopic dermatitis on infancy; [b] aOR 3.89, 95% CI 1.12-13.50 ,[c] aOR 13.11, 95% CI 1.44-119.27, [d] aOR 6.09, 95% CI 1.21-30.57

Table 16. The presence of allergen specific IgE antibodies to inhalant allergens measured from 73/81 children at the index episode of wheezing in infancy in relation to teenage asthma and PCA.

	Teenage asthma			PCA		
Specific IgE of ≥0.35 kU/l n= 73	**Yes (n=29)**	**No (n=44)**	**aOR[a] (95% CI)**	**Yes n=(20)**	**No (n=53)**	**aOR[a] (95% CI)**
Inhalant allergens, n(%)	11(38)	2(5)	11.30 [b] (1.89-67.60)	10(50)	3(6)	13.07 [c] (2.39-71.42)
Cat, n(%)	4(14)	2(5)	1.93 (0.292-12.78)	4(20)	2(4)	3.19 (0.47-21.43)
Dog, n(%)	5(17)	2(5)	2.59 (0.41-16.48)	4(20)	3(15)	1.87 (0.32-10.88)

[a] Logistic regression adjusted for sex and age (in months) on admission. Allergens with > 5 positive detections are presented. In addition, specific IgE was detectable to birch pollen in 4, timothy grass pollen in 1, mugworth pollen in 1, horse dander in in 4 and dust mites in 1 children.

After further adjustment with atopic dermatitis in infancy;[b] aOR 7.26, 95% CI 1.11-47.61 , [c] aOR 8.64, 95%CI 1.44-51.97

Early sensitization to cat and dog assessed by serum allergen-specific IgE or positive skin prick test reactions (IV)

In order to evaluate whether early sensitization to dog or cat is predictive for asthma at early teenage or PCA, the data on the presence of serum sIgE to cat or dog at the time of the index episode of wheezing and the respective SPT reactions eight months later, at the median age of 1.5 years (range 0.8-2.7), were combined. Early sensitization to cat and dog was more common among children with asthma at early teenage and among children with PCA (28-40%) than in children with no asthma at early teenage or PCA (6-8%) (Table17).

Only 1/12 (8%) children exposed to cat and 1/18 (6%) children exposed to dog in infancy were sensitized to respective allergens by the median age of 1.5 years compared to the respective figures of 11/68 (16%, aOR 0.48, 95% CI 0.06-4.14) and 11/62 (18%, aOR 0.34, 95% CI 0.04-2.95) for non-exposed children.

Table 17. Early sensitization to cat and dog in relation to teenage asthma and PCA.

	Teenage asthma			**PCA**		
Sensitization [a] to cat or dog n=80	**Yes (n=32)**	**No (n=48)**	**aOR[b] (95% CI)**	**Yes n=(20)**	**No (n=60)**	**aOR[b] (95% CI)**
Sensitization to cat, n(%) sIgE≥0.35 kU/L / SPT+	9 (28)	3 (6)	4.76 [c] (1.10-20.58)	7 (35)	5 (8)	3.80 [d] (0.94-15.30)
Sensitization to dog, n(%) sIgE≥0.35 kU/L / SPT+	9 (28)	3 (6)	4.51 [e] (0.93-21.93)	8 (40)	4 (7)	5.01 [f] (1.07-23.52)

[a] Specific IgE to cat or dog at the time of the index epsode of wheezing was available in 73/81 and SPTs eight months later in 77/81 children: either sIgE or SPTs were available in 80/81 children.

[b] Logistic regression adjusted for sex and age (in months) on admission. Further adjustment with parenthal asthma, atopic dermatitis in infancy and RSV aetiology of the index episode of wheezing: [c] aOR 5.52, 95%CI 1.15-26.61, [d] aOR 4.21, 95% CI 0.93-19.07, [e]aOR=3.00, 95% CI=0.53-17.09, [f] aOR=2.87, 95% CI 0.51-16.08.

5.2.5. Responses to nebulized bronchodilating agents during the index episode of wheezing in infancy (II)

Before the crossover trial conducted on admission, there were no significant differences in the baseline clinical parameters of the bronchiolitis severity between the two acute treatment groups, i.e. infants who received racemic epinephrine (REP and PRE groups) and infants who received albuterol (AP and PA) (Table 18). The asthma cases at the median ages of 4.0, 7.2 and in the teenage years were evenly distributed between these acute treatment groups. Children with teenage asthma had lower respiratory rates before the start of the trial compared to children without asthma at that age (48.7±9.8 vs. 55.6±10.8, p=0.005), but children with PCA did not differ from children without PCA in terms of respiratory rate, oxygen saturation, heart rate or RDAI score (Table 19).

In the mixed model analyses, RDAI scores had been lower after racemic epinephrine and albuterol administrations in children with teenage asthma (5.27 and 5.39, respectively) compared to children with no asthma at that age (5.57 and 6.19, respectively), whereas the figures were the opposite (6.76 in children with teenage asthma vs. 5.59 in non-asthmatics) after placebo administration (p=0.04). The other clinical responses to treatment did not differ between children with and without teenage asthma (data not shown). Figure 2a shows that, oxygen saturations were higher in children with PCA than in children without this disorder after albuterol administration, but lower, after racemic epinephrine and placebo administrations (Figure 2a). No differences were observed in respiratory rates, RDAI scores, and heart rates between those developing later PCA and non-PCA cases (Figure 2b-d).

A supplementary analysis was performed by comparing findings between the 20 children with PCA and the 27 children with no signs of asthma in childhood. No differences in pooled responses, including oxygen saturation, to given inhalations between children with PCA and no asthma ever were seen. However, the analysis of the oxygen saturations assessed 15 minutes after inhalations revealed a statistically significant result (p=0.03, data not shown).

Table 18. Baseline clinical evaluation (mean±SD) between the two therapeutic groups [a]

Variables before treatment	REP/PRE [b] group n=48	AP/PA [c] group n=52
Oxygen saturation (%)	93.25±2.21	93.49±1.92
Respiratory rate (breaths/min)	51.75±9.60	53.22±11.98
Heart rate (beats/min)	150.70±20.75	150.06±14.04
RDAI score (points)	8.63±2.83	8.96±3.22

[a] There were no statistically significant differences between the groups for any variable (independent sample t-test)

[b] REP= racemic epinephrine (adrenaline) followed by placebo, PRE= placebo followed by racemic epinephrine

[c] AP= albuterol (salbutamol) followed by placebo, PA=placebo followed by albuterol

Table 19. Baseline values (mean±SD) in children with and without PCA[a]

Variables before treatment	PCA n=20	No PCA n=61
Oxygen saturation (%)	93.65±2.11	93.43±2.08
Respiratory rate (breaths/min)	50.15±8.59	53.71±11.50
Heart rate (beats/min)	156.00±15.56	149.90±17.62
RDAI score (points)	9.30±4.00	8.84±2.72

[a] There were no statistically significant differences between the groups in any of the variables (independent sample t-test)

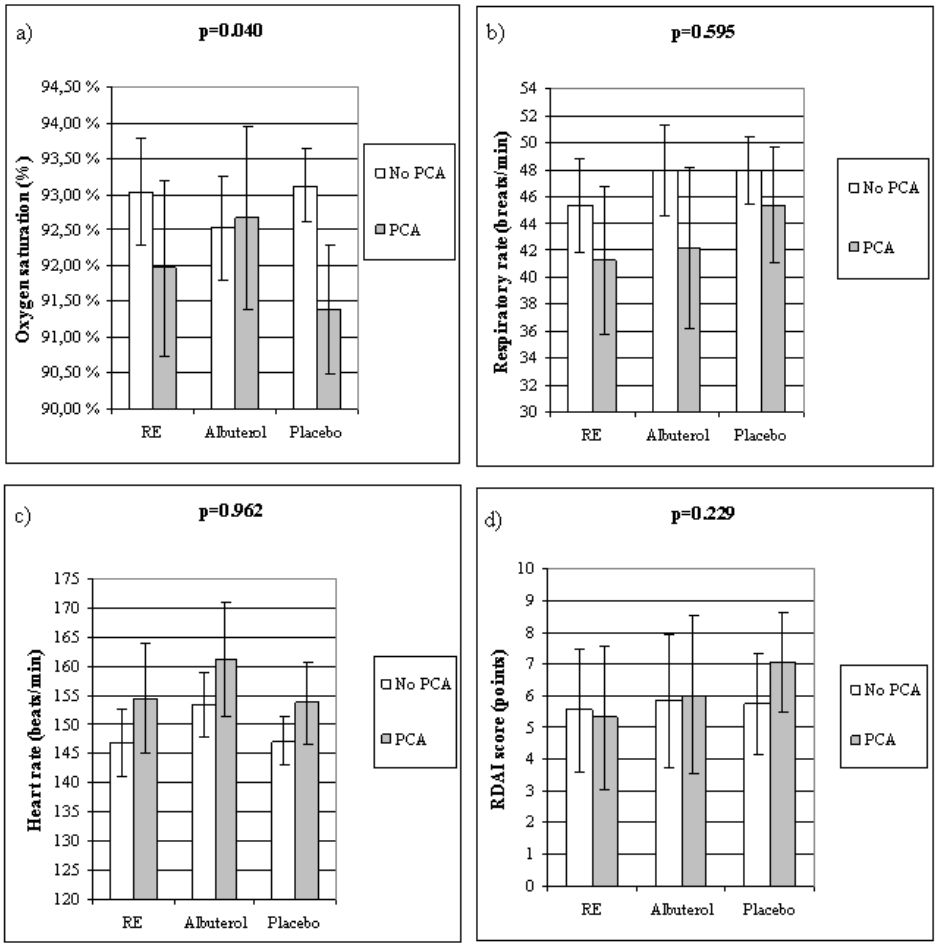

Figure 3. The pooled responses (combining the 15- and 30-minute results), oxygen saturation (a), respiratory rate (b), heart rate (c) and RDAI score (d) (means and 95% CIs) after inhalations of racemic epinephrine (RE, n=39), albuterol (n=42) or placebo (n=81) in children with and without PCA. The p-values (the mixed models analyses) indicate the interaction between the given inhalations and PCA.

5.2.6. Eosinophilic inflammation during the index episode of wheezing (IV)

Among the 81 children attending the clinical follow-up in their teenage years, there were 79 children (teenage asthma in 31, PCA in 20) for whom there was data on B-EOS, 81 children (teenage asthma in 32, PCA in 20) with data on S-ECP and 78 children (teenage asthma 31, PCA in 19) with data on NPA-ECP measured on admission available. Table 20 reveals that all the three markers were higher in children aged ≥12 months on admission, in children with atopic dermatitis or serum sIgE to food

or inhalant allergens in infancy, in children wheezing with viruses other than RSV and in children wheezing specifically with rhinoviruses. No statistically significant associations were found between the markers and gender, parental asthma, passive smoking during infancy or maternal smoking during pregnancy (data not shown).

Children with teenage asthma differed significantly from children with no signs of asthma for B-EOS obtained on admission (median 0.410 x 10^9 cells/L, range 0.050-2.451 vs. 0.168, 0.060-1.201, p=0.027). The values of S-ECP and NPA-ECP in children with teenage asthma did not differ significantly from the corresponding S-ECP (median 3.7 µg/L, range 1.9-116.6 vs. 3.2, 1.9-33.0, p=0.196) and NPA-ECP (398 ng/g, 33-3950 vs. 472, 2-2782, p=0.388) values in non-asthmatic children. The values of both B-EOS and NPA-ECP were significantly higher in children PCA when compared to children without PCA (median 0,531 x 10^9 cells/L, range 0.005-2.451 vs. 0.197, 0.006-1.201, p=0.006 and 926 ng/g, 97-3950 vs. 347, 2-3510, p=0.013). In the case of S-ECP, the difference between children with and without PCA did not reach statistical significance (5.6 µg/l, 1.9-116.6 vs. 3.20, 1.9-33.0, p=0.054).

As seen in Table 21, elevated levels NPA-ECP in infancy were a significant predictor of teenage asthma. However, neither elevated B-EOS nor elevated S-ECP was predictive for teenage asthma. However, elevated B-EOS, S-ECP and NPA-ECP were significant risk factors for PCA. On average, an elevated B-EOS increased the PCA risk by 2.9-fold, elevated S-ECP by 6.1-fold and elevated NPA-ECP by 6.7-fold. All three markers were elevated in 8 children, and 6 of them had PCA.

As presented in Table 22, none of the three eosinophil activity markers was a significant predictor of teenage asthma in the multivariate analyses. An elevated NPA-ECP level associated significantly with PCA. Instead, the associations between elevated B-EOS and PCA, and between S-ECP and PCA, which were significant in the univariate analyses, were not robust to adjustments for atopic dermatitis in infancy, RSV aetiology of the index episode of wheezing and age on admission.

Table 20. Association between baseline characteristics present in infancy and B-EOS, S-ECP and NPA-ECP obtained on admission in 81 children attending the clinical follow-up at in their teenage years.

Baseline characteristics in infancy	B-EOSx10^9cells/L Median (range)	p [a]	S-ECP µg/L Median (range)	p [a]	NPA-ECP ng/g Median (range)	p [a]
Age						
< 12 months	0.140 (0.005-2.451)	< 0.001	2.6 (1.9-49.4)	< 0.001	286.5 (2.0-2714.0)	0.005
> 12 months	0.504 (0.056-1.516)		8.4 (1.9-116.6)		590.5 (101.0-3950.0)	
Atopic dermatitis						
Yes	0.554 (0.056-2.451)	0.001	5.9 (1.9-116.6)	0.004	536.5 (51.0-3950.0)	0.055
No	0.212 (0.005-1.201)		2.9 (1.9-33.0)		357.0 (2.0-2782.0)	
Serum specific IgE [b] to food						
Yes	0.426 (0.006-2.451)	0.004	4.6 (1.9-116.6)	0.009	593.0 (2.0-3950.0)	0.004
No	0.150 (0.005-1.201)		3.2 (1.9-23.3)		299.0 (15.0-1290.0)	
Serum specific IgE [b] to inahalant allergens						
Yes	0.792 (0.080-1.516)	<0.001	19.5 (1.9-116.6)	<0.001	1005.5 (144.0-3950.0)	0.033
No	0.197 (0.005-2.451)		3.2 (1.9-49.49		384.5 (2.0-2782)	
RSV identification [c]						
Yes	0.097 (0.006-0.613)	0.001	2.0 (1.9-11.5)	< 0.001	214.0 (15.0-879.0)	<0.001
No	0.400 (0.005-2.451)		4.5 (1.9-116.6)		531.0 (2.0-3950.0)	
Rhinovirus alone [d]						
Yes	0.500 (0.028-1.432)	0.017	6.2 (1.9-116.6)	0.001	508.0 (142.0-3950.0)	0.012
No	0.174 (0.006-2.451)		2.8 (1.9-55.6)		265.5 (15.0-2714.0)	

[a] Mann-Whitney U-test, [b] ≥0.35 kU/L, [c] Mixed infections with other viruses included, [d] Rhinovirus as only identified virus

Table 21. Elevated B-EOS, S-ECP and NPA-ECP obtained on admission as predictive factors for teenage asthma and PCA

	Teenage asthma			PCA		
Elevated eosinophil marker on admission	**Yes (n=32)**	**No (n=49)**	**OR [a] (95% CI)**	**Yes n=(20)**	**No (n=61)**	**OR [a] (95% CI)**
B-EOS [b], n (%)	12(39)	13(27)	1.70 (0.65-4.46)	10(50)	15(25)	2.93 1.02-8.42
S-ECP [c], n (%)	6(19)	4(8)	2.60 0.67-10.06	6(30)	4(7)	6.11 1.52-24.61
NPA-ECP [d,] n (%)	13(42)	8(17)	3.52 1.24-9.99	11(58)	10(17)	6.74 2.16-21.00
At least one elevated marker, n (%)	19(61)	19(40)	2.33 0.92-5.90	15(75)	23(40)	4.57 1.46-14.28

[a] Non-adjusted analyses
[b] B-EOS was available in 79/81 (teenage asthma in 31, PCA in 20), [c] S-ECP in 81/81 (teenage asthma in 32, PCA in 19) and [d] NPA-ECP in 78/81 (teenage asthma in 31, PCA in 19)

Table 22. Logistic regression: Elevated B-EOS, S-ECP and NPA-ECP obtained on admission as predictive factors for teenage asthma and PCA

	Teenage asthma			PCA		
Elevated eosinophil marker on admission	**Yes (n=32)**	**No (n=49)**	**aOR [a] (95% CI)**	**Yes n=(20)**	**No (n=61)**	**aOR [a] (95% CI)**
B-EOS [c], n (%)	12(39)	13(27)	0.66 (0.20-2.21)	10(50)	15(25)	1.01 (0.27-3.76)
S-ECP [d], n (%)	6(19)	4(8)	0.92 (0.19-4.51)	6(30)	4(7)	1.77 (0.34-9.12)
NPA-ECP [e,] n (%)	13(42)	8(17)	2.17 (0.68-6.98)	11(58)	10(17)	4.45 [f] (1.18-16.75)
At least one elevated marker, n (%)	19(61)	19(40)	1.10 (0.36-3.33)	15(75)	23(40)	2.33 (0.61-8.84)

[a] Logistic regression adjusted for age on admission, sex, atopic dermatitis in infancy and RSV etiology of early wheezing. The markers were analyzed separately.

[c] B-EOS was available in 79/81 (teenage asthma in 31, PCA in 20), [d] S-ECP in 81/81 (teenage asthma in 32, PCA in 19) and [e] NPA-ECP in 78/81 (tenage asthma in 31, PCA in 19)

After further adjustment for sensitization to either food or inhalant allergens at the time of admission: aOR 4.97, 95%CI 1.18-20.95 [f]

5.2.7. Neutrophil activation measured by serum myeloperoxidase (VI)

Of the original 100 children enrolled into the present study, there were S-MPO results on admission available for 98 children, and 6 weeks, 4 months and 12 months later, for 94, 89 and 90 children, respectively. The level of S-MPO1 was higher in children aged ≥ 12 months, in children with serum sIgE to food or inhalant allergens and in children with wheezing induced by rhinoviruses, compared to children without these characteristics (data not shown). However, children with exposure to ETS before hospitalization for wheezing had lower S-MPO1 values compared to children with no such exposure (median 241.5 µg/L range 107.2-829.6 vs. 315.5, 136.6-1051.4, p=0.007). When each variable was included separately into the age-adjusted analyses, all other significant associations disappeared (data not shown), except for the association between exposure to ETS in infancy and LogMPO1 (p=0.007). No statistically significant associations were seen between MPO at any of the four measurements and maternal smoking during pregnancy, exposure to maternal smoking in infancy, gender, parental asthma or atopy, atopic dermatitis in infancy or RSV identification (data not shown).

The S-MPO concentration was highest on admission and significantly decreased thereafter. According to the repeated measures ANOVA, the decrease in LogMPO was significant between the measurements 1 and 2 as well as between measurements 3 and 4 (Figure 4).

The levels of S-MPO1, S-MPO2, S-MPO3 and S-MPO4 did not differ between children with and without teenage asthma or PCA (data not shown). The effect of the early four-month intervention on the change in LogMPO was analyzed by the repeated measures ANOVA, and there were no significant differences between the budesonide, cromolyn sodium and control groups (data not shown). Finally, the changes in LogMPO measured in infancy were analyzed in relation to the appearance of later asthma adjusted for age on admission (<12 months/ ≥12 months), atopic dermatitis in infancy and RSV aetiology of the index episode of wheezing in nfancy. In repeated measures ANOVA, the changes in LogMPO showed an identical pattern between children with and without teenage asthma (data not shown), and as presented in Figure 5, the changes in LogMPO were nearly identical in children whether or not they had PCA.

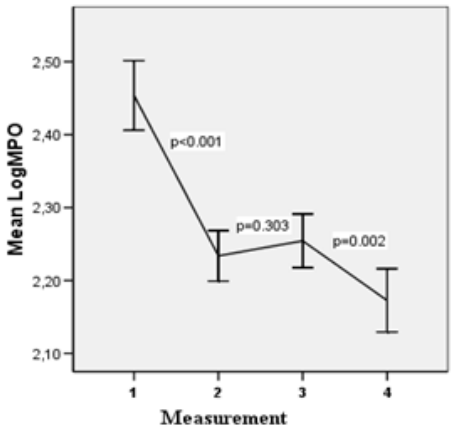

Figure 4. The changes in LogMPO (ug/L, means and 95% CIs) measured four times in infancy assessed by the repeated measures ANOVA.

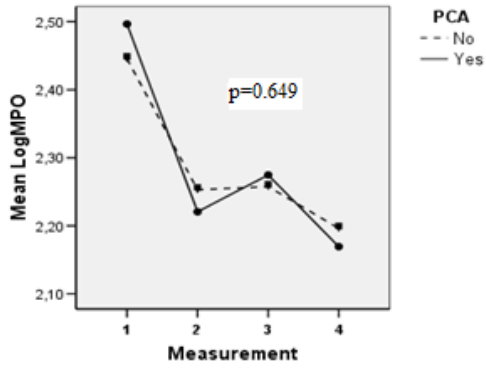

Figure 5. The changes in LogMPO (ug/L, means and 95% CIs) measured four times in infancy between children with and without PCA assessed by the repeated measures ANOVA adjusted for age on admission, atopic dermatitis in nfancy and RSV aetiology of the index episode of wheezing in infancy.

5.2.8. Early-life predictive factors for abnormal lung function and bronchial hyper-reactivity (III)

Parental history of asthma, maternal smoking during pregnancy, early exposure to ETS, elevated serum total IgE, the presence of serum sIgE to inhalant or food allergens, elevated NPA-ECP or elevated S-ECP on admission or the presence of atopic dermatitis on admission, were not significantly associated with any parameter in FVS (data not shown). There was a trend that elevated B-EOS, assessed on admission, was associated with lower FEV_1 (89.54 (mean, % of predicted) ± 12.11 (SD) vs. 93.64 ± 13.48, p=0.081), MEF_{50} (76.16 ± 18.32 vs. 83.81 ± 19.33, p=0.059) and MEF_{25} (64.98 ± 19.08 vs. 74.72 ± 21.11, p=0.065). RSV aetiology of the index episode of wheezing in infancy was associated with a higher FEV_1/FVC and a lower FVC when compared with non-RSV aetiology (Table 23). There were no statistically significant differences in any baseline FVS parameters between rhinovirus positive and negative cases (data not shown).

Table 23. Findings in the baseline FVS in teenage years in relation to RSV- and non-RSV aetiology of the index episode of wheezing in infancy.

Parameters in FVS % predicted, mean ±SD	**RSV+ [a] n=24**	**RSV- n=56**	**p [b]**
FVC	93.65±11.05	99.57±12.59	0.009
FEV_1	91.87±10.65	92.39±13.93	0.267
FEV_1/FVC	98.35±6.53	92.91±8.70	0.033
MEF_{50}	85.81±17.62	79.56±19.43	0.802
MEF_{25}	77.78±20.35	69.07±20.34	0.303

[a] Mixed infections included

[b] ANOVA adjusted for age on admission (<12/≥12 months), atopic dermatitis in infancy and their interactions with RSV aetiology of the index episode of wheezing in infancy.

None of the early-life risk-factors was a significant predictor of abnormal lung function, as defined as one or more abnormal value in the FVS parameters (data not shown).

The early-life factors were tested also as predictors of BHR to exercise and to methacholine. As shown in Table 24, the presence of serum sIgE to inhaled allergens on admission and maternal smoking during pregnancy were associated with BHR to exercise, and furthermore atopic dermatitis in infancy was associated with intermediate to high BHR to methacholine. No other early-life risk factor, present during the index hospitalisation, was predictive of BHR in teenage years (data not shown).

Table 24.Early-life risk factors for BHR in teenage years.

Early-life risk-factors, present/tested	**BHR[a]**	**aOR [b]**	**95% CI**
	Positive ECT (Fall in FEV_1≥10%)		
Serum sIgE (≥0.35 kU/L) to inhalant allergens, 13/73	10	12.57	2.30-68.77
Maternal smoking during pregnancy, 17/18	8	4.58	1.28-16.39
	Positive MIC test (PD_{20}<1600μg)		
Atopic dermatitis in infancy, 19/69	13	3.48	1.09-11.10

[a] ECT=Exercise challenge test, MIC= Methacholine inhalation challenge

[b] Logistic regression adjusted for sex and age (<12/≥12 months) on admission.

6. DISCUSSION

6.1. Methodological aspects

6.1.2. The study design

One hundred children requiring hospitalization for wheezing during an acute respiratory tract infection at age less than two years were recruited to the present prospective follow-up study. The study was originally planned to address the problems faced daily by paediatricians working in hospital: How to treat young wheezing children, and how to prevent recurrent bronchial obstructions? Because of this clinical approach, a positive viral identification was not an inclusion criteria and previous wheezing was not an exclusion criteria. However, with the availability of advanced methods, the viral identification rate was 73 % for the 81/100 children of the present study. In most of the cases, the index episode of wheezing in infancy was the first: 88% of the children of the present study had not wheezed before. The recruitment period lasted for almost two years, and children were recruited during and between two RSV outbreaks.

Despite the long (median 11.4 years) follow-up of the children, the attendance rate was as high as 81/100 (81%). The basic characteristics and laboratory findings of the 19 drop-outs at the time of the index episode of wheezing in infancy did not differ from those of participating children, except for maternal smoking, which was more common in the drop-outs (56% vs. 28%, p=0.027). The present study did not include healthy non-wheezing controls.

Asthma in teenage years was strictly doctor-diagnosed; the child needed to be on continuous or intermittent anti-inflammatory asthma medication, or needed to have repeated wheezing symptoms, and to have an objective evidence of asthma. Persistent childhood asthma, PCA, was regarded as being present if the child had doctor-diagnosed asthma, at both of the two previous follow-up visits at the median ages of 4.0 and 7.2 years and also in the present study in their teenage years. All other children, including 12 children with teenage asthma, were included in the non-PCA group.

Since the study group was highly selected, i.e. children hospitalized for wheezing in infancy, our results reflect only the outcome in children who suffered a clinically severe

form of bronchiolitis or early wheezing. Since the Kuopio University hospital is the only hospital which provides care for acute viral wheeze requiring hospitalization in the area of north-eastern Finland, the results of the prospective follow-up of the present hospital-based cohort do provide representative information at the population level on the outcome of early severe wheezing.

6.1.3. Strengths of the study

On admission, one of the four investigators decided whether the patient was suitable for inclusion in the study, and performed the pre-treatment and post-treatment evaluations. The evaluations consisted of measurements of respiratory rate, heart rate and oxygen saturation (Reijonen et al. 1995). In addition, a standardized RDAI scoring based on two respiratory variables, wheezing and retractions, was utilized (Lowell et al. 1987). The inter-observer variation was small: the two investigators did not differ in ratings by more than one unit in the eight-point retraction scale and the nine-point wheezing scale. The κ-values of 0.82-0.87 indicated excellent agreement. In addition, the administration of the medication was double-blind (Reijonen et al. 1995).

A variety of clinical and laboratory data related to allergy, eosinophil and neutrophil activation and viral aetiology of the index episode of wheezing were collected on admission (Reijonen et al.1997b, Kotaniemi-Syrjänen et al. 2002, 2003a, 2003b and 2005). Only diagnoses made by a physician were considered when the history of atopic dermatitis and earlier wheezing as well as the parental history of asthma and allergy were being recorded (Reijonen et al. 1996). Before the present follow-up visit, a structured questionnaire was filled in by the study subjects and their parents, and all the answers were checked thoroughly at the clinical visit. The criteria for the presence of teenage asthma were rather strict, and self-reported wheezing symptoms needed to be combined either with continuous or intermittent asthma medication or a pathological result in the exercise challenge test in the free running outdoors. This test has been specific but not very sensitive for the diagnosis of asthma, and the 10-15 % falls in FEV_1 have been used as cut-off points (Godfrey et al. 1999). In order to improve the sensitivity of the test, we chose a 10 % limit for the present study. FVS was performed twice, two weeks apart, with nearly identical results, and BHR was studied by two different methods, by ECT and MIC test.

There are only two previous studies with corresponding designs and long, over 10 years, follow-ups, as was the case in the present study. Both the Swedish and Finnish post-bronchiolitis studies with follow-up lasting until adulthood have provided important information on the early-life risk factors for asthma (Piippo-Savolainen et al. 2004, Goksör et al. 2006). However, in the Swedish study, early sensitization to allergens and eosinophilic markers were not studied, and in both the Swedish and Finnish studies, advanced viral diagnostic methods were not available at the time of recruitment. In addition, data on neutrophilic markers and early-life responses to medication were not available in either study.

6.1.4. Shortcomings of the study

The main shortcoming of the present study was the absence of a non-wheezing control group. However, the prevalence of asthma at the age of 7 to 12 years has been carefully studied in our area in two epidemiological population-based studies, and the prevalence of asthma at school age is known to be between 4.0-5.0 % (Timonen et al. 1995, Remes and Korppi 1996). Confirmation of these values came in a more recent study performed in our area; asthma prevalence was about 5 % at the age of 13-14 years (Lai and the ISAAC Phase Three Study Group 2009). Thus, the present study focused on predictive factors within the study group, but it was also possible to compare asthma in these children with carefully studied asthma prevalence in the same area.

Although the asthma criteria were strict, the inclusion of the children with maintenance anti-inflammatory asthma medication may have biased the results, at least with respect to lung function tests and tests of BHR. However, over 75 % of the children with either continuous or intermittent asthma medication had either experienced repeated episodes of wheezing, prolonged cough or a positive result in the ECT. In addition, measurable BHR was more common in children with continuous or intermittent asthma medication than in those children with no maintenance medication for asthma.

We consider PCA, such as defined in the present study, to be a very reliable measure of chronic atopic asthma in childhood. In the present study, 95 % of the children with PCA had at least one positive result in the SPTs. However, the classification of the 12 children with teenage asthma in to the non-PCA group may have biased the results. In

the supplementary analyses, exclusion from the analyses of those children with teenage asthma but without PCA did not change the results. Further, when only the children with no asthma at any time formed the non-PCA group, the results remained almost the same.

6.2. Long-term outcome of early childhood wheezing in comparison with other long-term studies

Wheezing symptoms and asthma

In the present study, asthma was present in 40 % of the children in their teenage years after experiencing wheezing requiring hospitalization in infancy. Similar figures, 30-40%, for self-reported wheezing in teenage years after wheezing at the age of less than three years of age, have been reported in population-based cohorts (Morgan et al. 2005, Matricardi et al. 2008). In the previous follow-up studies, it was found that after wheezing requiring hospitalization in infancy, the asthma prevalence has been 17-39 % when the children are teenagers (Gurwitz et al. 1981, Noble et al. 1997, Wennergren et al. 1997, Sigurs et al. 2005).

In the present cohort, the occurrence of asthma was 51 % at pre-school age, and 40 % both at early school age and in teenage years. During the follow-up, 25 % of the cases had asthma at all three follow-up visits, i.e. from pre-school age until they were in their teens. Our results are in accordance with the Tucson birth cohort, in which about 25 % of the children with wheezing at age less than three years were wheezing still both at age six and at age 11 years (Morgan et al. 2005). The persistence of asthma has not been evaluated in other studies. In most hospital-based cohorts, unlike in the present study, the asthma prevalence has decreased between the start of school and the teen years (Wennergren et al. 1992 and 1997, Noble et al. 1997, Korppi et al. 1994, Hyvärinen et al. 2005), and increased only in one study (Sigurs et al. 2000 and 2005). In the Swedish and Finnish cohorts followed up until adulthood, the asthma prevalence increased again in the period between the teenage years and adolescence (Goksör et al. 2005, Piippo-Savolainen et al. 2006).

Atopy at early teenage

In Eastern Finland, the prevalence of SPT positivity to at least one aeroallergen at age 7-16 years has been 43-52 % (Remes and Korppi 1996, von Hertzen et al. 2006). In the present selected cohort, allergic sensitization in teenage years was more common: 63% of the children were sensitized to at least one aeroallergen, and 90-95 % of the children with teenage asthma or who had PCA had at least one positive SPT reaction compared to 45-51% of those without teenage asthma or PCA, respectively. In two other hospital-based cohorts, the sensitization figures in teenage years have been lower, 44 % to 50 % (Noble et al. 1997, Sigurs et al. 2005). These studies included only infants admitted at less than 12 months of age, compared to the age range of 1 to 23 months in the present study. Thus, because there is an increasing number of wheezing children at age 1-2 years who are atopic, probably more of these subjects were recruited into the present study.

Lung function and bronchial hyper-reactivity

In the present study, the mean values in FVS, measured over 11 years after severe early wheezing, were within the normal limits compared to gender-specific and height-related reference values for Finnish children. However, as many as 33 % of the children had one or more abnormal values in the FVS. Furthermore, BHR measured by two methods, was common, being present in 27 % of cases to exercise and in 46 % to methacholine. These values were rather similar to that of 37 % in another hospital-based study with a corresponding design (Wennergren et al. 1997).

In the population-based birth cohorts, forced expiratory flows have been lower in children with a history of LRTIs and/or wheezing compared to children with no such history (Stein et al. 1999, Morgan et al. 2005, Håland et al. 2009). In hospital-based studies after an episode of wheezing in infancy either due to RSV (Sigurs et al. 2005) or any respiratory virus (Noble et al. 1997), when compared to matched controls, lowered forced expiratory flows and BHR have been reported in teenage years. In addition, in line with the figures of present study, in the Finnish and Swedish bronchiolitis cohorts followed-up until adulthood, abnormal lung function parameters in the FVS were seen in 31-36 % of the study subjects, compared with 11-16 % in the control subjects, (Piippo-Savolainen et al. 2004, Goksör et al. 2008).

6.3. Early-life predictive factors for asthma 11 years after hospitalization for wheezing in comparison with other long-term studies

6.3.1. Age on admission, parental history of asthma and atopy, and environmental factors in comparison with other studies

Age and parental history of asthma

In the Tucson birth cohort, recurrence of wheezing became more common with increasing age at the first wheezing episode (Taussig et al. 2003). Likewise, children with teenage asthma and PCA were older (> 12 months) on admission, compared to the children who remitted in the present study. An increasing number of asthma risk factors become involved between the age 1-2 years (Jartti et al. 2009), which probably explains the association between age on admission and later asthma. However, in the study of Wennergren et al., hospitalization for wheezing at a young age (< six months) was associated with asthma at age 10 years (Wennergren et al. 1997), and Sigurs et al. included only young infants, mostly less than six months of age, and RSV bronchiolitis was still a risk factor for asthma at age 13 years (Sigurs et al. 2005). These results point to either the presence of a common underlying factor, probably genetic, involved in both severe bronchiolitis and asthma, or an interaction of an early viral infection with the developing immune system and allergic sensitization (Sigurs et al. 2005, Juntti et al. 2005 and 2009).

In most earlier long-term population-based cohorts (Taussig et al. 2003, Matricardi et al. 2008) and in hospital-based cohorts (Sigurs et al. 2005, Goksör et al. 2008, Piippo-Savolainen et al. 2006), a family history of asthma and atopy, in particular asthma in the parents, has been predictive for asthma. In the present study, there was a tendency for parental and maternal asthma, but not atopy in parents, to be associated with teenage asthma and PCA in children after wheezing in infancy. The statistically non-significant result was probably due to insufficient power of the present study, since asthma in parents was rare. Atopy, in contrast, was common in parents, permitting a reliable statistical assessment.

Environmental factors

In the Tucson birth cohort, maternal smoking, recorded at one year of age, was associated with transient wheezing at less than three years of age, and also with persistent wheezing until age six (Martinez et al. 1995), but exposure to environmental tobacco smoke was not detected as a risk factor for persistent wheezing in a longer follow-up. In the birth cohort from Germany, parental smoking in infancy was not a risk factor for wheezing at age 13 (Matricardi et al. 2008). However, the Finnish and Swedish follow-up studies until adulthood have stressed the role of exposure to environmental tobacco smoke (Piippo-Savolainen et al. 2006, Goksör et al. 2007), especially maternal smoking during pregnancy (Goksör et al. 2007) as important risk-factors for asthma and impaired lung function. In the Swedish cohort, postnatal exposure to environmental tobacco smoke seemed to act through increasing the risk of active smoking in adolescence, whereas pre-natal tobacco smoke exposure seemd to influence the development of BHR (Goksör et al. 2007). In the present study, exposure to environmental tobacco smoke in infancy was common, 46 %, and carried a slightly increased risk of asthma and PCA. Active smoking was very common in Finnish and Swedish cohorts followed up until adulthood; 27-28 % of the cases smoked, which complicates the evaluation of the role of early exposure to environmental tobacco smoke. None of the children in their teenage years reported active smoking in the present study.

The three-year follow-up of the present cohort revealed that exposure to a furry pet at home before the index episode of wheezing in infancy was related to less asthma (Reijonen et al. 2000), but at age six the protective effect was no longer significant (Kotaniemi-Syrjänen et al. 2002). In the present study when the children were in their teenage years, PCA was more common in children with no exposure to dogs (28%) in infancy than in exposed children (5%). However, after adjusting for confounding factors such as parental atopy and personal early atopy, the difference was not significant, suggesting “a healthy pet owner effect”, i.e. there is less pet keeping in atopic families. However, in accordance with the present results, exposure to furry pets in infancy has not increased the asthma risk at age 10-13 years (Wennergren et al. 1997, Sigurs et al. 2005) or in adolescence (Goksör et al. 2007, Piippo-Savolainen et al. 2007a) after

hospitalization for wheezing in infancy, as was also reported in the German birth cohort (Matricardi et al. 2008). Furthermore, early exposure to cats or dogs did not increase sensitization to these allergens at any phase of the present follow-up.

6.4. Viruses related to the index episode of wheezing in comparison with other long term studies

RSV

In the present study, an RSV aetiology of the index episode of wheezing was associated with a relatively better outcome than in those with other or no viral findings: Teenage asthma was present in 20 % and PCA in 8 % of the children after RSV-related early wheezing, which was less than the values of 48 % and 32 %, respectively, if the wheezing had been attributed either to some other or to no viral findings. However, compared to the 4-5 % asthma prevalence in the non-selected child population in eastern Finland (Remes and Korppi 1996, Lai and the ISAAC steering committee 2009), the risk of teenage asthma was still four-fold, and the risk of PCA was two-fold after hospitalization for RSV induced early wheezing. In accordance to our results, other hospital-based prospective long term follow-ups have reported more favourable outcomes after RSV-induced early wheezing than after wheezing with other or no viruses, but in some cases no differences have been detected in asthma prevalence (Wennergren et al. 1997, Hyvärinen et al. 2005, Piippo-Savolainen et al. 2007b). In the Tucson birth cohort, infants with RSV-LRTI during the first three years of life were more likely to suffer from wheezing up to the age of 11 years, but no longer at the age of 13 (Stein et al. 1999). In the study of Sigurs et al., hospitalization for RSV bronchiolitis at less than age one year was a risk factor for asthma at least up to 13 years of age. However, a reduction in risk ratios was seen with increasing age (Sigurs et al. 1995, 2000 and 2000), pointing to a disappearance of the effect of early RSV infection with increasing age, which is in accordance with conclusions of a recent review (Perez-Yarza et al. 2007) and with the Tucson birth cohort study (Stein et al.1999).

In the present study, RSV-positive cases were as likely to be sensitized to any aeroallergen in teenage years as were non-RSV-cases. Other studies have reported also similar (Stein et al. 1999, Korppi et al. 2004) or an even lower (Juntti et al. 2003) prevalence in SPT positivity in RSV cases. In contrast, in the study of Sigurs et al.,

sensitization to common aeroallergens was more frequent in the RSV group than in the control group (Sigurs et al. 2005). However, in comparison with the population data (von Hertzen et al. 2006), the prevalence of atopic sensitization in the control group was low, 28 %, and not very high (50%), in the RSV cases (Sigurs et al. 2005).

Rhinovirus

There are no population-based long-term follow-up studies on the association between early rhinovirus-induced wheezing and subsequent asthma, since molecular viral diagnostics for rhinoviruses were not available before the 1990s. In the childhood origins of asthma cohort, a birth cohort consisting of infants with at least one atopic parent, it was demonstrated that rhinovirus-induced viral wheezing not requiring hospitalization was a significant predictor of asthma at age six years (Jackson et al. 2008). In another high-risk cohort, wheezing induced by rhinovirus alone or in combination with RSV at less than one year of age was associated with wheezing at age five years, but only in children sensitized at less than two years of age (Kusel et al. 2007). The authors concluded that viral infections interact with atopy in infancy to promote subsequent asthma.

In the present cohort at early school age, asthma was more common after rhinovirus-induced wheezing compared to the wheezing caused by RSV or other viruses (52% vs. 15 %) (Kotaniemi-Syrjänen et al. 2003b). In the present study, teenage asthma (58%) and PCA (47%) were still more common after rhinovirus aetiology of early wheezing compared to other or no viral aetiology (33% and 17%, respectively teenage asthma and PCA), but the differences were no longer statistically significant. In the present cohort, we noted that rhinovirus aetiology of early wheezing was more common among infants aged more than 12 months, among infants with early atopic dermatitis and among infants with elevated eosinophil markers on admission. Thus, rather than being an independent risk factor for asthma, rhinovirus-induced wheezing in infancy is probably a marker identifying those children prone to wheeze due early respiratory allergy, ongoing eosinophil airway inflammation or even early structural changes in the airways.

6.5. History of atopic dermatitis, serum total IgE and early sensitization to allergens in comparison with other studies

In the present study, early atopic dermatitis and elevated serum total IgE, assessed at the time of the index episode of wheezing in infancy, predicted teenage asthma and PCA. Similarly, atopic dermatitis and elevated serum total IgE at age one were predictive for persistent wheezing in the Tucson birth cohort (Martinez et al. 1995). In the study of Wennergren et al., atopic diseases in early childhood were not predictive for asthma at age 10 (Wennergren et al. 1997). In other hospital-based long term follow-up studies, the role of early atopy on asthma at age 10-13 years has not been evaluated, or has been studied poorly (Gurwitz et al. 1981, Noble et al. 1997, Wennergren et al.1997, Sigurs et al. 2005, Goksör et al. 2006).

In the present study, early sensitization to inhaled allergens was rare, being present in 18 % of the infants, but highly predictive for teenage asthma and PCA. Sensitization to aeroallergens at the time of admission was rare, being exhibited only in infants aged more than 12 months. In order to evaluate the role of early sensitization to cat or dog allergens as predictors of teenage asthma and PCA, the data of SPTs at the median age of 1.5 years were combined to the serum sIgE data obtained in infancy. About a third of children with early teenage asthma or PCA were sensitized to cat or dog allergens, compared to less than 10 % of non-asthmatics. Early sensitization to cats was a significant predictor of teenage asthma, and sensitization to dog was predictive of PCA. Our findings are in accordance with the findings from the German birth cohort, which reported that early sensitization to perennial allergens, including cat dander, was a strong risk factor for persistent wheezing in children with a history of early wheezing (Matricardi et al. 2008). In the Finnish post-bronchiolitis cohort, which was followed up until adulthood, sensitization to inhaled allergens at age less than three years was predictive for asthma until the teenage years, but not thereafter, but in contrast to the present study, no associations were seen between sensitization to furry pets and later asthma (Piippo-Savolainen et al. 2007a).

In the present study, sensitization to food allergens was common, being present in 47 % of the infants, but predictive of teenage asthma or PCA only when serum sIgE to individual food allergens were present. Sensitization to cow′s milk predicted teenage asthma and wheat was a strong predictor of both, teenage asthma and PCA.

Sensitization to egg white, which had been predictive for asthma when the children started school (Kotaniemi-Syrjänen et al. 2003a), was no longer significant. In another Finnish cohort, IgE-mediated clinical cow′s milk allergy was a risk factor for school age asthma and allergic sensitization (Saarinen et al. 2005). A detection limit was applied in the present study, and low to moderate sIgE rises might be found in infancy without any clinical consequences (Kulig et al. 1999). In the German birth cohort study, higher concentration limits were applied, and early sensitization to food allergens in infancy predicted the persistence of wheezing in the univariate analyses, but no longer in a stepwise model including also early sensitization to perennial allergens (Matricardi et al. 2008). Consequently, the presence of low concentrations of serum sIgE to food allergens early in life probably reflects atopic diathesis before there are any clinical manifestations or aeroallergen sensitization (Kulig et al. 1999). In the present study, about a third of infants with serum sIgE to food allergens and/or to wheat had also serum sIgE to inhalant allergens, but by their teenage years about 80 % of the children displaying serum sIgE to food in infancy and all of the 11 wheat-positive children in infancy had become sensitized also to aeroallergens.

6.6. Responses to bronchodilating agents during the index episode of wheezing as predictors of later asthma

Increased BHR, when measured shortly after wheezing in infancy, was related to the development of later asthma in infants with an atopic predisposition (Saga et al. 2001). In the present study, we were not able to confirm the hypothesis that future asthmatics respond better than future non-asthmatics to inhaled bronchodilators during acute bronchial obstruction at age less than 24 months. However, we observed that oxygen saturation was higher after albuterol inhalations in children with later persistent asthma compared to the situation in children who did not develop persistent asthma, but lower after placebo and racemic epinephrine inhalations. Thus, there might be a subset of infants whose airways are susceptible to reversible smooth muscle contraction which will progress later to asthma. However, the absolute differences in oxygen saturations between children with PCA and no PCA were small, and not clinically relevant.

In asthma, the smooth muscle constriction is related to basement membrane thickening, epithelial damage and eosinophilic airway inflammation, and these changes

may ocur even in young children and infants (Barbato et al. 2006, Saglani et al. 2007). In a Norwegian study, an early bronchodilator response, as evaluated by tidal lung function measurements in asymptomatic children less than two years of age, was associated with recurrent bronchial obstruction, but not with allergic sensitization or atopic dermatitis (Lodrup Carlsen et al. 2004). The authors concluded that recurrent bronchial obstruction in infancy may be more closely associated with lung function abnormalities, like BHR, than with asthma at school age, which the authors believed was more closely linked with allergic sensitization (Lodrup Carlsen et al. 2004). However, the early bronchodilator responses with regard to later asthma status of the children were not, unfortunately, reported in that study. In the present study, asthma status was assessed three times over an 11 year-period after early wheezing, but the evaluation of early bronchodilator responses was based solely on clinical findings. Therefore, more follow-up studies with the appropriate methods for the measurement of bronchodilating response in infants are needed to study whether there is a significant link between early-life responses to bronchodilating agents and later asthma.

6.7. Eosinophilic inflammation during the index episode of wheezing as a prediction of later asthma

It is unclear which age is critical for the development of eosinophilic inflammation and permanent structural changes in the airways of wheezing children. In recent biopsy studies, evidence was found that the age from one to three years is probably the most critical period, since no changes were seen even in those children with a history of severe wheezing younger than 12 months, but they were demonstrated in wheezing children by three years of age (Saglani et al. 2007). Accordingly, in the present study, eosinophils and their activity markers measured in blood and nasopharyngeal aspirates in severely wheezing infants were higher in children aged more than 12 months. Further, high levels of eosinophil activity markers were also related to other asthma risk factors, such as atopy and wheezing with rhinovirus, which became more common with increasing age.

Based on the results from birth cohort studies, blood eosinophilia in early life has been included as a minor risk factor in the algorithms for starting asthma therapy in young children with repeated wheezing (Gastro-Rodriguez et al. 2000, Guilbert et al. 2004). In

hospital-based follow-up studies, as seen also in the earlier phases of the present cohort, both blood eosinophilia (Kotaniemi-Syrjänen et al.2002, Ehlenfield et al. 2000) and elevated serum ECP (Reijonen et al 1997a) have predicted the continuation of wheezing and asthma symptoms between five to seven years of age. In the few long-term follow-ups available, neither blood eosinophilia nor elevated serum ECP documented at less than 24 months of age, were associated with asthma at age 10 years (Wennergren et al. 1997). Likewise, no association was seen between blood eosinophilia in infancy and later asthma in a Finnish post-bronchiolitis cohort followed-up until adulthood, but the lack of an eosinopenic response to infection in infancy was a significant risk factor for asthma in adulthood (Piippo-Savolainen et al. 2007c). In the present study, blood eosinophilia and elevated serum and nasopharyngeal ECP at less than 24 months of age predicted PCA, but elevated nasopharyngeal ECP predicted both teenage asthma and PCA. However, in the multivariate analyses, only nasopharyngeal ECP was significantly associated with PCA, but not anymore with teenage asthma.

Although there is evidence that inflammatory markers in the nasal epithelium may reflect airway pathology (Frischer et al. 2000), samples obtained from the upper airways allow only an indirect assessment of the situation in the lower airways. In addition, the methods are poorly standardized. However, nasopharyngeal aspirates are easy to obtain and routinely used for viral studies, and we collected samples by aspirating mucus from the nasopharynx, near to the glottis. Since there were no reference values available for NPA-ECP, the concentrations were expressed per weight of mucus sample, and the value 815.0 ng/g resulted from the ROC curves was applied. Though ECP in respiratory secretions may reflect lower airway inflammation better than the corresponding values in blood or serum, only a few studies are available on nasal, nasopharyngeal or sputum ECP as risk factors of asthma in children with early-life wheezing. In the study of Sigurs *et al.,* nasal ECP was not associated with bronchial symptoms two years after RSV bronchiolitis (Sigurs et al.1994), but did predict recurrent wheezing after bronchiolitis for at least four months in the present cohort (Reijonen et al. 1997b).

In the present study, eosinophils and the eosinophil markers during wheezing in infancy were non-sensitive but rather specific predictors of teenage asthma and PCA, with sensitivities varying from 13 % to 58% and specificities from 73% to 93%. When

combined, these markers identified 61% of the children with teenage asthma and 75% of the PCA cases. Elevated serum ECP did not detect additional teenage asthmatics or PCA cases better than elevated blood eosinophils and NPA-ECP, but in fact more than one third of the PCA cases were recognized by elevated nasopharyngeal ECP values alone. This means that NPA-ECP identified different patients than detected by S-ECP or blood eosinophils.

6.8. Neutrophil activation in infancy, measured by serum MPO, as a risk factor for later asthma

The results of the present study revealed that serum levels of MPO, measured on admission and thereafter three times when the infants were healthy, was highest during acute wheezing, but this elevation occurred equally in future asthmatics and non-asthmatics. After the acute infection, the serum MPO value decreased, and was constantly lower thereafter, showing no connection to anti-inflammatory treatment or future asthma. Thus, the high serum MPO concentrations measured during acute infection most probably reflected a reaction to inflammation, and correspondingly, the subsequent measurements reflected the recovery of inflammation. Accordingly, there are higher serum MPO levels in asthmatics with an acute infection compared to the corresponding values in asthmatics with no acute infection (Tauber et al. 1999). In addition, the serum MPO value was higher in children with symptomatic asthma, when compared to children with episodic or non-symptomatic asthma (Carlsen et al. 1997, Kalayci et al. 2000), suggesting an involvement of neutrophilis in asthmatic inflammation, possibly related to viruses. In the study of Sigurs et al., serum MPO increased during RSV bronchiolitis, and decreased within a few weeks thereafter, and MPO at any level was not predictive for subsequent wheezing or asthma in the two-year follow-up (Sigurs et al. 1994). In accordance, serum MPO at any level in any measurement in infancy was not able to predict either the presence or absence of teenage asthma or PCA in the present study. Thus, MPO appears to be a marker of acute infection, and unlike eosinophils, not useful in the prediction of the development of atopic asthma after wheezing in infancy.

6.9. Risk factors for lung function abnormalities and bronchial hyper-reactivity in comparison with other studies

In the population-based Tucson birth cohort study, LRTI caused by RSV in infancy was predictive for decreased forced expiratory flows at age 11 years (Stein et al. 1999). Further, Sigurs et al. found that infants hospitalized for RSV bronchiolitis at less than 12 months of age, had lower FEV_1/FVC and maximal expiratory flows at 75 % of FVC compared to controls at age 13 (Sigurs et al. 2005). In addition, FEV_1/FVC and $MEF_{25,}$ were lower in subjects with a history of RSV bronchiolitis compared to controls in the Finnish post-bronchiolitis cohort followed up until adulthood (Korppi et al. 2004b). In the present study, in contrast, early wheezing related to RSV seemed to be associated with a restrictive pattern of lung function, as reflected by the significantly decreased FVC values in conjunction with the normal FEV_1 values and even elevated FEV_1 / FVC values. In the present cohort, subjects with teenage asthma or PCA, in contrast, had high FVC values and low FEV_1/FVC values compared to non-asthmatics, including most RSV positive cases. The FVC values were within normal limits in both groups, which obscures the clinical significance of the findings. However, in the German birth cohort study, FVC growth velocities were higher among children with asthma than among children without asthma until the age of 12-14 years (Strunk et al. 2006), and the FVC growth velocity pattern comparable to the subjects in the German study could explain our findings. On the other hand, the evidence of restriction found in the present study, as well as similar findings in other studies, may reflect pre-morbid structural changes in infants susceptible to severe RSV infection (Taussig et al. 2003).

The presences of atopic dermatitis and serum sIgE to inhalant allergens in early life were independent risk factors for BHR. These findings are in accordance with a recent birth cohort study from Germany, in which BHR at school age was significantly associated with sensitisation to perennial allergens in early life (Illi et al. 2006). In contrast, Wennergren et al. did not find any association between early atopy and BHR at the age of 10 years in children who had been hospitalized for wheezing in infancy (Wennergren et al. 1997), but in line with the present study, maternal smoking during pregnancy was a significant predictor of BHR in adolescence (Goksör et al. 2007). Although there is some evidence that in utero exposure to tobacco smoke may increase BHR which may continue through childhood (Cook et al. 1998), the presence of many

factors like early-life passive smoking and later active smoking complicate the interpretation of the results (Cook et al. 1998, Lodrup Carlsen et al. 2001). In the present study, the association between maternal smoking during pregnancy and BHR in teenage years was significant even after adjustment for passive smoking in infancy, and none of the children were smokers at the time of the study visit. However, in contrast to the Finnish follow-up study which continued until adulthood, no associations were seen between exposure to environmental tobacco smoke in infancy and abnormal lung function (Piippo-Savolainen et al. 2006).

7. SUMMARY AND CONCLUSIONS

In the present prospective follow-up study after hospitalization for wheezing at less than two years of age, teenage asthma was common, being present in 40 % of the children. Persistent childhood asthma, i.e. asthma at all three follow-up visits at pre-school age, at school age and in teenage years was present in 25 % of the children followed-up for 11 years. Most of the children with asthma were sensitized to at least one aeroallergen.

Compared to 4-5 % asthma prevalence in the child population in Eastern Finland, the prevalences of both, teenage asthma and persistent childhood asthma were elevated after both RSV- and rhinovirus-related hospitalization for wheezing in infancy. RSV aetiology of early wheezing was related to a better long-term outcome than wheezing with rhinoviruses or other viruses, though the difference did not reach statistical significance.

When tested in the teenage years, lung function assessed by FVS was within normal limits compared to gender- specific and height-related reference values for Finnish children. Children with teenage asthma or children with persistent childhood asthma had higher FVC values and lower FEV_1/FVC values than had similar aged children with no asthma or persistent asthma. Further, children with RSV-related early wheezing had lower FVC values and higher FEV_1/FVC values compared to other children raising the possibility that the difference might have been related to the susceptibility to early wheezing. However, lung function in infancy was not measured, and though only minor evidence was found that the children who would have later persistent asthma may respond better than the children without future persistent asthma to bronchodilators during the acute viral wheeze in infancy, the overall clinical responses to bronchodilators did not differ between these groups.

Non-specific early markers of atopy, atopic dermatitis in infancy and elevated serum total IgE were predictive of teenage asthma and persistent asthma. It was rare for there to be any allergen-specific IgE to inhalant allergens at the time of the index episode of wheezing, but if present, it was highly predictive for teenage asthma and persistent asthma as well as bronchial hyper-reactivity in the teenage years. The presence of

allergen-specific IgE to food allergens was common, and sensitization to cow's milk was predictive of teenage asthma and sensitization to wheat highly predictive for both teenage asthma and persistent childhood asthma. The present study failed to show any associations between family history of asthma or atopy and teenage asthma and persistent childhood asthma.

Early sensitization to cats and dogs was predictive for later asthma. However, simple early exposure to cats and dogs in contrast, was not related to any increased risk of atopic sensitization or the subsequent development of asthma in childhood. Exposure to environmental tobacco smoke in infancy was not related to abnormal lung function or bronchial hyper-reactivity at age 12 years, but maternal smoking during pregnancy increased the risk of bronchial hyper-reactivity.

With the selected cut-off points, elevated blood eosinophils and elevated eosinophil cationic protein in serum and in nasopharyngeal aspirate were found to be non-sensitive, but rather specific markers in recognizing wheezing infants at risk of chronic asthma. Elevated levels of eosinophil cationic protein in nasopharyngeal samples seemed to identify children at risk, and these children were not found by the other two markers. In addition, elevated levels of nasopharyngeal eosinophil cationic protein in infancy were an independent risk factor of persistent childhood asthma, which may suggest that eosinophil cationic protein, when present in respiratory secretions, is better at reflecting the airway pathology in children at high risk of chronic asthma than can be achieved by monitoring blood eosinophils or eosinophil cationic protein in serum.

In conclusion, teenage asthma is common after hospitalization for wheezing in infancy. The most important risk factors for both teenage asthma and persistent childhood asthma are early atopic dermatitis and early sensitization to inhalant allergens as well as early sensitization to wheat. Elevated markers of eosinophil activity in infancy predict later asthma, in particular active asthma lasting from early childhood at least until the teenage years. The level of eosinophil cationic protein in respiratory secretions was elevated in infants who would later develop asthma, pointing to the presence of airway eosinophilia in a subgroup of children at age 1-2 years. Rhinovirus-induced early wheezing serves as a marker identifying children with an increasing number of asthma risk factors, though it is not an independent risk factor for asthma.

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