KUOPION YLIOPISTON JULKAISUJA D. LÄÄKETIEDE 359
KUOPIO UNIVERSITY PUBLICATIONS D. MEDICAL SCIENCES 359

ANNE KOTANIEMI-SYRJÄNEN

Wheezing Requiring Hospitalisation in Infancy- Outcome at Early School Age

Viral Aetiology of Wheeze and Predictive Factors for Outcome

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium 2, Kuopio University Hospital, on Saturday 18th June 2005, at 12 noon

Department of Paediatrics
Kuopio University Hospital
and University of Kuopio

KUOPION YLIOPISTO
KUOPIO 2005

Distributor:
Kuopio University Library
P.O. Box 1627
FIN-70211 KUOPIO
FINLAND
Tel. +358 17 163 430
Fax +358 17 163 410
www.uku.fi/kirjasto/julkaisutoiminta/julkmyyn.html

Series Editors:
Professor Esko Alhava, M.D., Ph.D.
Department of Surgery

Professor Raimo Sulkava, M.D., Ph.D.
Department of Public Health and General Practice

Professor Markku Tammi, M.D., Ph.D.
Department of Anatomy

Author's address:
Department of Paediatrics
Kuopio University Hospital
P.O. Box 1777
FIN-70211 KUOPIO
FINLAND

Supervisors:
Docent Matti Korppi
Department of Paediatrics
University of Kuopio

Tiina Reijonen, M.D., Ph.D.
Department of Paediatrics
University of Kuopio

Reviewers:
Docent Marjo Renko
Department of Paediatrics
University of Oulu

Docent Merja Kajosaari
Department of Paediatrics
University of Helsinki

Opponent:
Professor Jussi Mertsola
Department of Paediatrics
University of Turku

ISBN 951-781-499-2
ISBN 951-27-0276-2 (PDF)
ISSN 1235-0303

Kopijyvä
Kuopio 2005
Finland

Kotaniemi-Syrjänen, Anne. Wheezing requiring hospitalisation in infancy – outcome at early school age. Viral aetiology of wheeze and predictive factors for outcome. Kuopio University Publications D. Medical Sciences 359. 2005. 97 p.
ISBN 951-781-499-2
ISBN 951-27-0276-2 (PDF)
ISSN 1235-0303

ABSTRACT

Wheezing requiring hospitalisation in infancy carries a risk for recurrent bronchial obstructions and subsequent development of asthma. However, identification of the at-risk children is still a challenge due to heterogeneity of the group of wheezing infants.

One hundred children with wheezing requiring hospital admission at the age of 1-23 months were enrolled into this prospective follow-up study in 1992-1993. On admission, baseline data were charted and blood specimens and nasopharyngeal aspirates collected. As an emergency treatment, racemic adrenalin and nebulised salbutamol were administered.

In 1999, median 6.3 years (range 5.3-7.2) after the index hospitalisation, 82 children took part in the reinvestigation at the median age of 7.2 years (range 5.6-8.8). The reinvestigation included clinical examination, parental interview on symptoms and medications, baseline pulmonary function measurements, exercise challenge, and skin prick tests (SPT) against common inhalant allergens.

In 2000-2002, supplementary studies on viral aetiology and specific immunoglobulin E antibodies were performed in frozen samples obtained during the index episode of wheezing.

At early school age, 40% of children were regarded as having asthma, and 30-49% of children had clinical atopic manifestations. Skin tests were positive in 46% of children. Supplementary viral studies revealed rhinoviruses in 33%, respiratory syncytial virus (RSV) in 29%, enteroviruses in 12%, and coronaviruses in none of the children on admission.

The clinical pictures of RSV and rhinovirus infection-associated wheezing did not differ significantly with regard to the clinical symptom scores or in relation to the response to the emergency treatment. However, oxygen saturation values were lower in RSV-positive children on admission ($p=0.027$). Rhinovirus-associated wheezing was characterized by the age of >12 months on admission, the presence of atopic dermatitis in infancy, and/or eosinophil activation during the index episode of wheezing. Rhinovirus-induced wheezing was found to be predictive for the development of asthma (OR 4.14; 95% CI 1.02-16.77).

In addition, recurrent wheezing (OR 4.29; 95% CI 1.02-17.99), atopic dermatitis (OR 4.11; 95% CI 1.42-11.84), elevated total serum IgE (>60 kU/l) (OR 3.41; 95% CI 1.03-11.33), and blood eosinophilia (>0.45 $x10^9$/l) (OR 7.02; 2.26-21.79) in infancy were predictive for development of asthma. Serum food-specific IgE of >0.70 kU/l (OR 2.97; 95% CI 1.10-7.97), inhalant allergen-specific IgE of >0.35 kU/l (OR 9.75; 95% CI 1.91-49.71), and specific IgE of >0.35 kU/l against egg white (OR 2.85; 95% CI 1.01-8.02), and/or wheat (OR 4.93; 95% CI 1.16-20.89), and sensitisation to common food or inhalant allergens by SPTs (OR 5.22; 95% CI 1.79-15.22) in early childhood were also predictive of early school-age asthma.

In conclusion, asthma at early school age is present in 40% of children after wheezing requiring hospital admission in infancy. Wheezing outside RSV epidemics, recurrent symptoms, early evidence of atopy or sensitisation to common food and inhalant allergens, and blood eosinophilia or a rhinoviral finding during acute wheezing increase the risk for persistence of symptoms and the subsequent development of asthma.

National Library of Medicine Classification: WD 300, WF 150, WF 553, WS 280
Medical Subject Headings: asthma; etiology; allergens; antigens, viral; hypersensitivity; respiratory sounds; hospitalization; infant; child; forecasting; prognosis; follow-up studies

Kotaniemi-Syrjänen, Anne. Varhaislapsuuden sairaalahoitoinen hengenahdistus – virusetiologia ja kouluiän ennusteeseen vaikuttavat tekijät. Kuopion yliopiston julkaisuja D. Lääketiede 359. 2005. 97 s.
ISBN 951-781-499-2
ISBN 951-27-0276-2 (PDF)
ISSN 1235-0303

TIIVISTELMÄ

Varhaislapsuuden sairaalahoitoiseen hengenahdistukseen sisältyy riski toistuvaan hengenahdistusoireiluun ja astman kehittymiseen. Hengenahdistusoireilusta kärsivät lapset muodostavat kuitenkin varsin heterogeenisen ryhmän, ja onkin varsin haasteellista yrittää tunnistaa astmariskissä olevat lapset kaikkien ahtauttavaa hengitystietulehdusta sairastavien lasten joukosta.

Kaikkiaan 100 alle 2-vuotiasta lasta, joilla oli sairaalahoitoa vaativa ahtauttava alahengitystietulehdus, otettiin mukaan tähän prospektiiviseen seurantatutkimukseen vuosina 1992-1993. Sairaalantulovaiheessa taustatiedot kartoitettiin, otettiin verinäytteitä ja nenänielun imulimanäytteitä, sekä tutkittiin vastetta akuutin vaiheen lääkehoidoille (raseeminen adrenaliini ja salbutamoli).

Vuonna 1999, 6.3 vuoden (mediaani; vaihteluväli 5.3-7.2 vuotta) kuluttua sairaalahoidosta, 82 lasta otti osaa seurantakäynnille 7.2 vuoden (mediaani; vaihteluväli 5.6-8.8 vuotta) iässä. Seurantakäynnillä lapsille tehtiin kliininen tutkimus, vanhempia haastateltiin lasten oireista ja lääkityksistä, keuhkojen toimintaa tutkittiin spirometrilla, ja hengitysteiden supistumisalttiutta tutkittiin juoksutestillä. Lisäksi lapsille tehtiin ihopistokokeet tavallisimpia hengitettäviä allergeeneja kohtaan.

Vuosina 2000-2002 alkuvaiheen virustutkimuksia täydennettiin uusilla tutkimuksilla ja spesifisiä immunoglobuliini E-tyypin vasta-aineita määritettiin pakastetuista tulovaiheen seeruminäytteistä tavallisimpia allergeeneja kohtaan.

Varhaisessa kouluiässä 40%:lla lapsista oli astma, ja 30-49%:lla oli muu atooppinen sairaus. Ihopistotestit olivat positiiviset 46%:lla. Virustutkimukset paljastivat että tutkimukseen tulovaiheessa rinovirusinfektio oli 33%:lla, respiratory syncytial virus (RSV) 29%:lla ja enterovirus 12%:lla.

RSV- ja rinovirusinfektioiden kliinistä kuvaa verrattaessa todettiin, että eroja ei juuri ollut oireiden vaikeuden tai lääkevasteen osalta. Happisaturaatioarvot olivat kuitenkin matalammat RSV-positiivisilla lapsilla sairaalaantulovaiheessa ($p=0.027$). Rinovirusinfektioon liittyvä taudinkuva erosi RSV-infektiosta iän, atooppisen ihottuman ja eosinofiilien aktivaation osalta. Rinovirusinfektion laukaiseman vinkuvan hengitystietulehduksen todettiin ennustavan astman kehittymistä (oddsien suhde (OR) 4.14; 95% luottamusväli (LV) 1.02-16.77).

Myös hengenahdistusten toistuminen varhaislapsuudessa (OR 4.29; 95% LV 1.02-17.99), atooppinen ihottuma (OR 4.11; 95% LV 1.42-11.84), koholla oleva seerumin IgE ($\geq$60 kU/l) (OR 3.41; 95% LV 1.03-11.33), sekä veren eosinofilia (OR 7.02; 95% LV 2.26-21.79) ennakoivat astman kehittymistä. Lisäksi spesifinen IgE $\geq$0.70 kU/l ruoka-allergeeneja kohtaan (OR 2.97; 95% LV 1.10-7.97), spesifinen IgE $\geq$0.35 kU/l hengitettäviä allergeeneja kohtaan (OR 9.75; 95% LV 1.91-49.71), sekä spesifinen IgE of $\geq$0.35 kU/l kanamunan valkuaista (OR 2.85; 95% LV 1.01-8.02), ja/tai vehnää (OR 4.93; 95% LV 1.16-20.89) kohtaan ennustivat astman kehittymistä, samoin ihotestipositiivisuus tavallisille ruoka-aineille ja/tai pölyille varhaislapsuudessa (OR 5.22; 95% LV 1.79-15.22).

Yhteenvetona voidaan todeta että varhaislapsuudessa sairaalahoitoa vaatineen ahtauttavan alahengitystietulehduksen jälkeen astma on 40%:lla lapsista kouluikään tultaessa. Hengityksen vinkuminen RSV-epidemian ulkopuolella, oireiden toistuminen, atopia tai herkistyminen tavallisimmille ruoka-aineille tai pölyille varhaislapsuudessa, veren eosinofilia, sekä rinoviruslöydös ahtauttavan alahengitystietulehduksen taustalla liittyvät lisääntyneeseen astma-riskiin.

To Samuli, Siiri & Santeri

ACKNOWLEDGEMENTS

This clinical follow-up study was carried out in the Department of Paediatrics, Kuopio University Hospital, during the years 1992-1999. I want to thank Professor Kari Launiala, M.D., the former Head of the Department of Paediatrics, and Professor Raimo Voutilainen, M.D., for giving me the opportunity to participate in this long-term research project, and Docent Mikko Perkkiö, M.D., the Head of the Department of Paediatrics, for allowing me to perform the clinical phase of the study in the Department of Paediatrics, Kuopio University Hospital.

I want to express my deepest thanks to my principal supervisor, Docent Matti Korppi, M.D. for his humanity, never failing encouragement, immediate responses, fresh ideas, and constructive comments. I am also deeply grateful for Tiina M. Reijonen, M.D. for being my secondary supervisor. Her clear advice and practical aspect on the issues have helped me many times. These two very different personalities have composed the best combination of supervisors one ever can wish to have, and I am very grateful for their guidance.

I wish to acknowledge most sincerely my official referees, Docent Merja Kajosaari, M.D. and Docent Marjo Renko, M.D., for their constructive criticism and expert advice during the preparation of the final manuscript of this thesis.

I want to thank Kaj Korhonen, M.D. and Ritva-Liisa Väätäinen, R.N., for their kind assistance during the clinical phase of the study. I also want to thank Kaj Korhonen for his friendship, all his advice, and help in preparing the manuscripts.

I also want to express my deepest thanks to my co-authors Jarkko Romppanen, M.D. and Kari Savolainen, Ph.D., in the Department of Clinical Chemistry, Kuopio University Hospital, Aino Laatikainen, Ph.D., in the Department of Clinical Microbiology, Kuopio University Hospital, and Docent Raija Vainionpää, Ph.D., and Matti Waris, Ph.D., in the Department of Virology, University of Turku, for performing all laboratory work and giving their expert advice during manuscript preparation processes.

I express my thanks to Pekka Rikkonen, M.D., M.A., for revising the language of this thesis, and I also wish to express my gratitude to Allan Halme, M.Sc., for revising the language of one of the manuscripts in concern. I also wish to thank Pirjo Halonen, M.Sc. for her kind advice in statistical analyses.

I am also grateful for Mrs Liisa Korkalainen and Mrs Mirja Pirinen for all their help in many practical problems.

I want to express my sincere thanks to all the children and their parents for their co-operation; without them this study would never have been completed.

I express my thanks to all my colleagues in the Department of Paediatrics, Kuopio University Hospital with whom I have had the pleasure to work.

Above all, I am most grateful for my husband Samuli for his love and support during this work. I want to express my warmest thanks to my dear children Siiri and Santeri for being the joys of my life. I am also very grateful for my parents, Kaisu and Antero, for their care and encouragement.

The study was financially supported by Ida Montin's Foundation, Kerttu and Kalle Viik's Fund, the Foundation for Paediatric Research, the National Graduate School of Clinical Investigation, Kuopio University Hospital, and the University of Kuopio, which is gratefully acknowledged.

Vantaa, May 2005

Anne Kotaniemi-Syrjänen

ABBREVIATIONS

CF	Complement fixation
CI	Confidence interval
ECP	Eosinophil cationic protein
EIA	Enzyme immunoassay
FEIA	Fluoroenzyme-immunometric assay
FEV_1	Forced expiratory volume in 1 second
FVS	Flow-volume spirometry
GM-CSF	Granulocyte macrophage colony-stimulating factor
ICAM	Intercellular adhesion molecule
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
LR	Likelihood ratio
NPA	Nasopharyngeal aspirate
OR	Odds ratio
PCR	Polymerase chain reaction
RACS	Respiratory assessment change score
RAST	Radioallergosorbent test
RDAI	Respiratory distress assessment instrument
ROC	Receiver operating characteristic
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription-polymerase chain reaction
SARS	Severe acute respiratory syndrome
SPT	Skin prick test
TR-FIA	Time-resolved fluoroimmunoassay

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. Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Korppi M. Wheezing requiring hospitalization in early childhood: Predictive factors for asthma in a six-year follow-up. Pediatr Allergy Immunol 2002; 13: 418-425.

II. Kotaniemi-Syrjänen A, Reijonen TM, Romppanen J, Korhonen K, Savolainen K, Korppi M. Allergen-specific immunoglobulin E antibodies in wheezing infants: the risk for asthma in later childhood. Pediatrics 2003; 111: e255-e261.

III. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy – the first sign of childhood asthma? J Allergy Clin Immunol 2003; 111: 66-71.

IV. Korppi M, Kotaniemi-Syrjänen A, Waris M, Vainionpää R, Reijonen TM. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. Pediatr Infect Dis J 2004; 23: 995-999.

V. Kotaniemi-Syrjänen A, Laatikainen A, Waris M, Reijonen TM, Vainionpää R, Korppi M. Respiratory syncytial virus infection in children hospitalized for wheezing: Virus-specific studies from infancy to preschool years. Acta Pædiatr 2005; 94: 159-165.

CONTENTS

1. INTRODUCTION

Wheezing related to respiratory infections is common in infancy and early childhood; 20% of all children will suffer from wheezing during the first years of life (Morgan and Martinez 1992, Martinez *et al.* 1995), and 1-3% of all infants are admitted to hospital due to wheezing (Korppi *et al.* 1986, Phelan *et al.* 1990, Boyce *et al.* 2000). In particular, after hospitalisation for wheezing, recurrent episodes of wheezing and subsequent development of asthma are increased when compared with occurrence of wheezing and asthma in non-selected child populations (Noble *et al.* 1997, Sigurs *et al.* 2000 and 2005, Piippo-Savolainen *et al.* 2004). However, wheezing in infancy is a heterogeneous condition with different phenotypes and outcomes (Martinez *et al.* 1995, Martinez and Godfrey 2003), and consequently, identification of children at risk for long-term respiratory morbidity is difficult.

After early childhood wheezing, persistence of wheezing and subsequent development of asthma have been related to inherited susceptibility to asthma and atopy, and to the development of clinical allergic manifestations (Morgan and Martinez 1992, Martinez and Godfrey 2003). In addition, there is evidence that certain infections, respiratory syncytial virus (RSV) bronchiolitis in particular, contribute to later asthma (Sly and Hibbert 1989, Sigurs *et al.* 1995 and 2005). Furthermore, during the last decade, advances in methods of detection have elucidated the role of rhinoviruses as lower respiratory tract pathogens (Andréoletti *et al.* 2000, Juvén *et al.* 2000, Papadopoulos *et al.* 2000 and 2002), and as common agents found to trigger wheezing in asthmatic children (Duff *et al.* 1993, Johnston *et al.* 1995, Freymuth *et al.* 1999, Rakes *et al.* 1999, Rawlinson *et al.* 2003). Likewise, coronaviruses are well-known pathogens in respiratory infections in children (Isaacs *et al.* 1983), and also encountered in exacerbations of asthma in schoolchildren (Johnston *et al.* 1995). Recent evidence suggests that enteroviruses may also play a role in childhood respiratory morbidity (Ruohola *et al.* 2000, Jartti *et al.* 2002 and 2004b). However, the role of rhino-, entero- and coronaviruses as inducers of wheezing in infants and as predictors of outcome has not been fully established.

Although a majority of wheezing children who finally are diagnosed as asthmatics develop clinically evident atopic manifestations in the long run, they

may not have any symptoms of atopy in infancy (Paganelli *et al.* 1998, Martinez 1999). Total serum immunoglobulin E (IgE) has been used to determine subclinical atopy (Martinez 1999), and blood eosinophilia (Martinez 1999), and serum eosinophil cationic protein (ECP) (Koller *et al.* 1997) to determine susceptibility to respiratory allergy. However, all these commonly used markers are non-specific, and to point out the allergen-specific responses, skin prick tests (SPT) or radioallergosorbent tests (RAST), have had to be performed this far. SPTs are qualitative, and for technical reasons, only a restricted number of allergens are applicable in SPTs in young children. Both of these tests may also be susceptible to technical errors and variations due to either non-standardized or non-automated methods. Consequently, new approaches are needed to screen those prone to atopy or allergen-induced asthma among the large group of wheezing infants.

2. REVIEW OF THE LITERATURE

2.1. Wheezing disorders in early childhood

2.1.1. Incidence of wheezing in infancy

In early life, approximately 20% of all children will suffer from wheezing illnesses (Morgan and Martinez 1992, Martinez *et al.* 1995), most commonly diagnosed as acute viral bronchiolitis or wheezy bronchitis. In 1-3% of all infants, respiratory infection-related expiratory difficulties are severe enough to require hospitalisation (Korppi *et al.* 1986, Phelan *et al.* 1990, Boyce *et al.* 2000). In infants with underlying chronic cardiac or pulmonary problems, the risk of hospitalisation for wheezing is greater, even up to 39%, during viral respiratory infections (Boyce *et al.* 2000).

According to several longitudinal studies, infants and toddlers hospitalised for wheezing represent a group of children at risk for recurrent bronchial obstructions and subsequent development of asthma (Sly and Hibbert 1989, Noble *et al.* 1997, Sigurs *et al.* 2000 and 2005, Piippo-Savolainen *et al.* 2004). However, there is evidence that most episodes of wheezing in early life are of viral origin, and wheezing infants form a heterogeneous group with different phenotypes and outcomes (Martinez and Godfrey 2003). Consequently, it is still a challenge to identify the children at risk for persistent wheezing and asthma.

2.1.2. The role of respiratory viruses in early childhood wheezing

2.1.2.1. Respiratory syncytial virus (RSV)

RSV is the agent most commonly identified in infants hospitalised with acute bronchiolitis (McIntosh *et al.* 1993, Ruuskanen and Ogra 1993, Kneyber *et al.* 2000), especially in children aged between 2 to 6 months (Ruuskanen and Ogra 1993). Premature infants (Hall *et al.* 1979, Meert *et al.* 1990), and the children with chronic cardiorespiratory diseases, especially those with chronic lung disease of prematurity, are at greatest risk of severe disease requiring hospital admission (MacDonald *et al.* 1982, Groothuis *et al.* 1988, Navas *et al.* 1992, Boyce *et al.* 2000). Other risk factors for severe RSV lower respiratory illness include underlying immunosuppressive conditions (Navas *et al.* 1992,

Wendt and Hertz 1995, McCarthy *et al.* 1999, Madhi *et al.* 2001), lack of breast feeding in infancy (Wright *et al.* 1989, Holberg *et al.* 1991), *in utero* exposure to tobacco-smoke products or passive smoking in infancy (Holberg *et al.* 1991, Law *et al.* 2002), and crowded living conditions (Holberg *et al.* 1991, Law *et al.* 2002). However, most hospitalised children have no apparent underlying condition (Graham *et al.* 2002).

RSV is a large, enveloped, single-stranded RNA virus, classified in the genus *Pneumovirus*, belonging to the subfamily of *Pneumovirinae* of the family *Paramyxoviridae* (Brooks *et al.* 2004). There are two major groups of RSV – A and B – with antigenic differences in the structural proteins of the virus. The surface proteins F (fusion protein) and G (attachment protein) are the major antigenic determinants of the virus, inducing neutralizing and protective antibodies (Ruuskanen and Ogra 1993, Brooks *et al.* 2004).

In temperate climates, RSV infections occur yearly as winter epidemics, and almost all children encounter the virus by the age of 2 to 3 years (Glezen and Denny 1973, Glezen *et al.* 1986). In Finland, the RSV epidemics occur in two-year cycles, with a minor spring epidemic preceding the major autumn epidemic (Waris 1991).

In RSV bronchiolitis, the bronchial epithelium is colonised by virus which then replicates. Subsequent destruction of ciliated epithelial cells occurs by necrosis, and the peribronchiolar tissues are infiltrated by lymphocytes. Submucosal tissues become oedematous, and plugs consisting of mucus, fibrin, and cellular debris occlude the bronchioles (Brooks *et al.* 2004). The clinical picture of RSV bronchiolitis is characterized by low fever, rhinorrhea, cough, poor feeding, tachypnoea, expiratory distress and audible wheezing. In addition, intercostal retractions, expiratory wheezes and fine inspiratory crackles on auscultation, pulmonary hyperinflation, and impaired gas exchange are common clinical findings in infants with RSV bronchiolitis (Phelan *et al.* 1990, Ruuskanen and Ogra 1993). However, the diagnostic criteria for bronchiolitis as a clinical syndrome vary in different countries, leading to variation in the incidence figures (Ruuskanen and Ogra 1993).

In addition to bronchiolitis, infections with RSV have several other clinical presentations; central apnoea is one of the most common presentations of RSV infection in first two months of life (Kneyber *et al.* 1998). In older children, infection may present as croup-like symptoms (Castro-Rodríguez *et al.* 2001);

and pneumonia may occur together with bronchiolitis or as a separate syndrome (Martinez and Godfrey 2003). However, in most of the infants, RSV infection presents only as an upper respiratory illness (Henderson *et al.* 1979, Heikkinen *et al.* 1999). Nevertheless, based on lung function studies performed in infants with acute upper respiratory illness, it has been speculated that all infants with RSV infection may have lower airway involvement, although in most cases that would remain clinically unapparent (Martinez *et al.* 1990, Martinez and Godfrey 2003).

RSV-specific immunoglobulin G (IgG) antibody response can be detected in most infected children, reaching maximum values in 20 to 30 days after the onset of symptoms (Welliver *et al.* 1980, Meurman *et al.* 1984). In infants aged less than 1 year, the RSV-specific IgG responses have been lower than in older children (Meddens *et al.* 1990). RSV-specific IgG appears to decline to low levels in one year after the primary infection, but reach again high levels within 5 to 7 days after RSV re-infection (Welliver *et al.* 1980).

There is evidence that immune mechanisms play an important role in the development of RSV bronchiolitis (Ruuskanen and Ogra 1993, Graham *et al.* 2002). RSV infection induces incomplete immunity to disease, even after multiple infections. The primary infection most commonly occurs at age 6 weeks to 6 months, when there are still maternally derived IgG antibodies present in the circulation. However, bronchiolitis is rare in infants aged less than 6 weeks, who have the highest serum concentrations of maternally derived antibodies (Ruuskanen and Ogra 1993), and there is evidence that high titres of maternal RSV antibodies can be protective against severe RSV-associated illness (Glezen *et al.* 1981, Holberg *et al.* 1991). In addition, passive immunization to RSV by intravenous immunoglobulins prepared from donors with high titres of anti-RSV antibodies, or by humanized monoclonal antibodies against the F protein of RSV, has proven to be rather effective and safe in the prevention of severe RSV disease in high-risk infants (PREVENT Study Group 1997, IMpact-RSV Study Group 1998).

In vitro experiments have shown that RSV antibody complexes can stimulate human neutrophils and macrophages to produce arachidonic acid metabolites (Faden *et al.* 1983, Ananaba and Anderson 1991), which can cause bronchoconstriction. Moreover, it has been found that most RSV-infected patients have high levels of an arachidonic acid metabolite, leukotriene C_4, in

the respiratory tract during the acute phase of infection, and the levels in wheezing subjects appear to be significantly higher than in non-wheezing subjects (Garofalo *et al.* 1991, van Schaik *et al.* 1999).

2.1.2.2. Rhinoviruses

Human rhinoviruses are the most common cause of upper respiratory tract infections at all ages (Mäkelä *et al.* 1998, Brooks *et al.* 2004). By the age of two years, almost all children have experienced at least one rhinoviral infection (Blomqvist *et al.* 2002).

Rhinoviruses are non-enveloped, single-stranded RNA viruses, classified in the genus *Rhinovirus*, belonging to the family *Picornaviridae*. Human rhinoviruses include more than 100 different serotypes (Brooks *et al.* 2004).

In temperate zones, there are peaks in the incidence of rhinoviral infections in early fall and later spring (Brooks *et al.* 2004, Jartti *et al.* 2004b). In a recent study, rhinovirus was detected most often in those wheezing children aged under three years who were hospitalised outside RSV seasons (Heymann *et al.* 2004).

During rhinovirus infection, viral replication is in most cases limited to the surface epithelium of the nasal mucosa, although rhinoviruses are able to replicate at lower airway temperatures (Papadopoulos *et al.* 1999), and capable of infecting lower airways as well (Papadopoulos *et al.* 2000). Histopathologic changes related to rhinovirus infection include oedema and mild cellular infiltration in submucosa and surface epithelium (Brooks *et al.* 2004).

In children and adolescents, in addition to upper respiratory symptoms, rhinoviruses have been identified as possible causative agents in lower respiratory tract infections and wheezing illnesses (Table 1).

In infants hospitalised for bronchiolitis, rhinoviruses have been detected in approximately 20% of patients (Andréoletti *et al.* 2000, Papadopoulos *et al.* 2002). In children aged 2 years or more, rhinoviruses appear to be the most important viral findings associated with wheezing episodes and exacerbations of asthma, having been detected in 23-79% of cases in several studies (Horn *et al.* 1979, Duff *et al.* 1993, Johnston *et al.* 1995, Freymuth *et al.* 1999, Rakes *et al.* 1999, Rawlinson *et al.* 2003, Heymann *et al.* 2004). In children with cystic

fibrosis, rhinoviruses have also been found to cause exacerbations of the disease (Smyth *et al.* 1995).

It is not fully established how rhinoviruses trigger wheezing in susceptible persons, but several mechanisms have been proposed (Gern and Busse 1999, Papadopoulos *et al.* 2004). The ability of rhinoviruses to infect respiratory airway epithelium seems to depend on the density of viral receptors on the cell membrane (Gern and Busse 1999). Most, 90%, of the rhinovirus serotypes use intercellular adhesion molecule (ICAM) 1 receptor (Staunton *et al.* 1989), which is highly expressed in bronchial epithelial cells especially during asthmatic airway inflammation (Vignola *et al.* 1993). Consequently, high expression of ICAM-1 in bronchial epithelial cells would render asthmatic persons more susceptible to lower airway rhinovirus infections (Gern and Busse 1999). In addition, *in vitro* and *in vivo* studies indicate that rhinovirus infection promotes secretion of several cytokines like interleukin (IL) 6, IL-8, IL-11, and granulocyte macrophage colony-stimulating factor (GM-CSF) from epithelial cells (Subauste *et al.* 1995, Einarsson *et al.* 1996, Gern *et al.* 2000). IL-11 may have direct effects on bronchial hyperresponsiveness (Einarsson *et al.* 1996), and the other cytokines have profound effects on inflammatory cells that can potentiate asthma (Gern and Busse 1999).

Few studies have described the clinical characteristics of rhinovirus-associated wheezing in infants and young children. Kellner *et al.* (1989) carefully registered respiratory symptoms and signs in 60 children with rhinovirus and in 119 children with RSV infection. The patients were younger than 3 years old, and more than 90% were treated in hospital. Rhinoviruses were identified by culture, and RSV by culture or antigen detection in respiratory samples. A comparison of respiratory symptoms and signs, and the findings in the physical examination revealed that there was no characteristic clinical pattern with respiratory rhinovirus infection in young children (Kellner *et al.* 1989). Papadopoulos *et al.* (2002) assessed the severity of bronchiolitis associated with rhinovirus infection by clinical scoring, and found that rhinoviral finding, especially when associated with RSV infection, increased the severity of the disease. Rhinovirus was a single pathogen in only 12 cases, and therefore the clinical characteristics of rhinovirus infection could not be presented.

Table 1. Lower respiratory tract infections and wheezing illnesses with rhinoviral findings in children.

Reference	Study subjects	Number of subjects (samples)	Age (years)	Duration of the study (months)	Method(s) of detection	Rhinovirus detection rate
Horn *et al.* 1979	Children with wheezy bronchitis	22 (72)	5-15	17	Culture	33%
Kellner *et al.* 1988	Children with acute respiratory tract infection (9% outpatients, 91% hospitalised)	519	<1-11.5	21	Culture	12%
Duff *et al.* 1993	Children with wheezing (83% outpatients, 17% hospitalised)	20 70	<2 2-16	43	Culture	20% 23%
Johnston *et al.* 1995	Children with exacerbated asthma	96 (292)	9-11	13	Culture RT-PCR	29%
Freymuth *et al.* 1999	Children hospitalised for exacerbated asthma	32 (71) 43 (61)	<2 2-14	60	Culture RT-PCR	44% 51%
Rakes *et al.* 1999	Children with wheezing (80% outpatients, 20% hospitalised)	22 48	<2 2-16	14	Culture RT-PCR	41% 71%
Andréoletti *et al.* 2000	Infants hospitalised for bronchiolitis	84	≤1	4	RT-PCR	20%
Juvén *et al.* 2000	Children hospitalised for pneumonia	254	<1-16	36	Culture RT-PCR	24%
Jartti *et al.* [a] 2002	Children hospitalised for wheezing	132	<1-13	9	RT-PCR	23%

Reference	Study subjects	Number of subjects (samples)	Age (years)	Duration of the study (months)	Method(s) of detection	Rhinovirus detection rate
Papadopoulos *et al.* 2002	Infants hospitalised for bronchiolitis	118	≤1	12	RT-PCR	21%
Rawlinson *et al.* 2003	Children hospitalised for exacerbated asthma	179	<1-17	16	Culture RT-PCR	79%
Thumerelle *et al.* 2003	Children hospitalised for exacerbated asthma	82	2-15	9	RT-PCR	12%
Camara *et al.* 2004	Children with wheezing	74 58	<2 2-12	20	RT-PCR	20% 19%
Heymann *et al.* 2004	Children hospitalised for wheezing	79 54	<3 3-18	12	RT-PCR	32% 48%
Jartti *et al.* 2004b	Children hospitalised for wheezing	293	<1-15	19	RT-PCR Culture	24%
Jennings *et al.* 2004	Children hospitalised for acute respiratory tract infection	75	<3	5	Culture RT-PCR	15%

[a] A part of the study by Jartti *et al.* 2004b.
RT-PCR, reverse-transcription polymerase chain reaction

2.1.2.3. Other respiratory viruses

Influenza virus types A and B, parainfluenza virus types 1-3, and adenoviruses are also important causes of lower respiratory tract infections and hospital admissions in early childhood (Henrickson 1994). As regards wheezing infants, clinically most important of these viruses is parainfluenza virus type 3; an RNA virus which belongs to the family *Paramyxoviridae* (Brooks *et al.* 2004). It causes 10-15% of bronchiolitis in non-hospitalised children, and is second only to RSV as a cause of bronchiolitis and pneumonia requiring hospitalisation (Henrickson 2003).

Recently discovered human metapneumovirus - an RNA virus belonging to the family of *Paramyxoviridae* (Brooks *et al.* 2004) - has been found to cause a wide range of respiratory illnesses, clinical symptoms resembling those caused by RSV (van den Hoogen *et al.* 2001, Stockton *et al.* 2002). In hospitalised children with acute respiratory tract infection, wheezing, or exacerbated asthma, the virus has been identified only in <10% of patients (Jartti *et al.* 2002 and 2004b, Boivin *et al.* 2003, Rawlinson *et al.* 2003, Jennings *et al.* 2004). During an outbreak, metapneumovirus was identified in up to 21% of children hospitalised for acute respiratory tract infection; half of these having an underlying chronic disease (Døllner *et al.* 2004).

Human coronaviruses - RNA viruses belonging to the family *Coronaviridae* (Brooks *et al.* 2004) - are common pathogens encountered in respiratory infections at all ages (Isaacs *et al.* 1983). The two serogroups of human coronaviruses are 229E and OC43. In addition, a novel coronavirus, discovered in 2003 from patients with severe acute respiratory syndrome (SARS), appears to represent a new group of coronaviruses (Rota *et al.* 2003).

Enteroviruses (Ruohola *et al.* 2000) - RNA viruses belonging to the family of *Picornaviridae* (Brooks *et al.* 2004) - also seem to play a role in childhood respiratory morbidity. Due to diagnostic method limitations, limited data on lower respiratory tract infections and wheezing illnesses associated with corona- and enteroviruses have been available until more recently.

During an outbreak, human coronaviruses have been detected in up to 30% of non-hospitalised children aged ≤6 years with acute respiratory tract infection (Isaacs *et al.* 1983). In schoolchildren with exacerbations of asthma, the detection rate has been 13% (Johnston *et al.* 1995). In wheezing infants, human coronaviruses have been found rarely, only in up to 5% of patients

(Freymuth *et al.* 1999, Papadopoulos *et al.* 2002, Camara *et al.* 2004, Heymann *et al.* 2004).

Enteroviruses have been detected in up to 25% of infants and toddlers with acute upper respiratory tract infections (Ruohola *et al.* 2000). In infants and toddlers hospitalised for bronchiolitis or other wheezing illnesses, the detection rate has been only 2-12% (Andréoletti *et al.* 2000, Heymann *et al.* 2004). In older children with wheezing illnesses or exacerbations of asthma, enteroviruses have been identified in up to 16-29% of cases (Jartti *et al.* 2002 and 2004b, Rawlinson *et al.* 2003, Thumerelle *et al.* 2003).

2.1.2.4. Diagnostic approaches for viral infections

Viral isolation in cell cultures, antigen detection tests, and antibody assays are widely available for common respiratory viruses, like RSV, influenza A and B viruses, parainfluenza viruses and adenoviruses (McIntosh *et al.* 1993, Jartti *et al.* 2004b). For rhinoviruses, in contrast, viral isolation in cell cultures was the only available diagnostic method for a long time; the fact that there are over 100 serotypes of rhinoviruses has hampered the development of antigen and antibody assays (Hyypiä *et al.* 1998). The development of reverse-transcription polymerase chain reaction (RT-PCR) for detection of rhino- and enteroviruses (Halonen *et al.* 1995, Hyypiä *et al.* 1998, Lönnrot *et al.* 1999) and for coronaviruses (Myint *et al.* 1994) has allowed the re-evaluation of the role of these viruses in respiratory infections of young children.

Nowadays, there are RT-PCR methods available also for detection of RSV and human metapneumovirus (Jartti *et al.* 2002 and 2004b). In clinical use, the role of RT-PCR as a detection method for RSV is still to be established. In case of serological diagnosis of RSV infection, the determination of IgG antibody titre rises has yielded the most sensitive result (Meurman *et al.* 1984).

The known disadvantage of the polymerase chain reaction (PCR) methods is that improved sensitivity of viral detection may lead to over-diagnostics, as fewer virus particles are needed to yield a positive result by PCR than to achieve a clinically evident disease (Johnston *et al.* 1993, Jartti *et al.* 2004a). However, in a recent Finnish study on rhino-, entero- and coronavirus findings by RT-PCR in asymptomatic children (Nokso-Koivisto *et al.* 2002), the viral detection rate by RT-PCR was low, 5%, if there were no preceding, concurrent, or following respiratory symptoms reported.

2.1.3. Most common phenotypes of early childhood wheezing

Wheezing in early life is a heterogeneous condition with different phenotypes and prognoses for outcomes. According to the Tucson Children's Respiratory Study, children who wheeze before the age of 3 years can be classified as transient or persistent wheezers (Martinez *et al.* 1995), and the persistent wheezers can further be grouped by presence or absence of atopy-related risk factors (Martinez and Godfrey 2003).

Transient early wheezers compose approximately 60% of infants and young children who present with recurrent wheezing episodes during the first years of life. Children belonging to this group are not more likely to have a family history of asthma or to have a personal or family history of atopy than children who do not wheeze during the first six years of life (Martinez *et al.* 1995). Transient early wheezers tend to have symptoms mainly during viral infections, and will stop all wheezing by 3 to 6 years of age (Martinez and Godfrey 2003).

Approximately 40% of children with recurrent wheezing during the first three years of life, continue to have wheezing episodes at least up to the age of 6 years. Half of these persistent wheezers - atopic persistent wheezers - become sensitised to inhalant allergens, whereas in the other half - non-atopic persistent wheezers - wheezing symptoms are not associated with increased prevalence of allergic sensitisation but occur mainly during viral infections (Martinez and Godfrey 2003). The long-term prognosis of wheezing is better in non-atopic than in atopic persistent wheezers (Halonen *et al.* 1999).

Factors associated with transient early wheezing and non-atopic and atopic persistent wheezing are presented in Tables 2, 3, and 4, respectively.

Table 2. Risk factors and protective factors for transient early wheezing, according to Martinez and Godfrey (2003).

Factor	OR (95% CI)[a]	(Reference)
Smoke exposure		
Maternal prenatal smoking	2.3 (1.4-3.8)	(Stein *et al.* 1999a)
Maternal prenatal and postnatal smoking	2.08 (1.59-2.71)	(Strachan and Cook 1997)
Diminished lung function at birth	3.7 (0.9-15.5)	(Martinez *et al.* 1988)
Male sex	1.54 (1.37-1.74)	(Rusconi *et al.* 1999)
	1.5 (1.3-1.7)	(Sherriff *et al.* 2001)
Company of other children		
Older siblings at home	1.36 (1.19-1.57)	(Rusconi *et al.* 1999)
Day care attendance in infancy	1.71 (1.50-1.95)	(Rusconi *et al.* 1999)
Older siblings at home or day care attendance in infancy	1.4[b] (1.1-1.8)	(Ball *et al.* 2000)
Endotoxin exposure in infancy	1.56[b] (1.03-2.38)	(Park *et al.* 2001)
Exposure to cockroaches in infancy	1.76[b] (1.20-2.57)	(Gold *et al.* 1999)
Maternal age at birth		
>35 years	0.68 (0.53-0.86)	(Rusconi *et al.* 1999)
≤20 years	2.4 (1.8-3.1)[c]	(Martinez *et al.* 1992)
<20 years	2.5 (1.7-3.7)[d]	(Sherriff *et al.* 2001)
Feeding		
Bottle feeding in infancy	1.4 (1.2-1.6)	(Sherriff *et al.* 2001)
Bottle feeding in infancy and sharing a room	3.29 (1.8-6.0)	(Wright *et al.* 1989)
Breast feeding for ≥6 months	0.82 (0.68-0.97)	(Rusconi *et al.* 1999)

[a] Compared with children without wheezing.

[b] Relative risk

[c] Compared with infants of mothers aged >30 years.

[d] Compared with infants of mothers aged >25 years.

Table 3. Factors associated with non-atopic persistent wheezing, according to Martinez and Godfrey (2003).

Factor	OR (95% CI)	(Reference)
RSV lower respiratory illness in infancy	4.3 (2.2-8.7)[a]	(Stein *et al.* 1999b)
	3.2 (2.0-5.0)[b]	(Stein *et al.* 1999b)
Atopy-unrelated bronchial hyperresponsiveness in infancy		
Doubling the concentration of histamine in a challenge test	0.52 (0.37-0.75)[c]	(Palmer *et al.* 2001)
Prematurity		
One week increase in length of gestation	0.90 (0.84-0.97)[c]	(Rona *et al.* 1993)
Onset of wheezing at the age of 0-1 years	2.6[d]	(Halonen *et al.* 1999)

[a] For frequent wheeze (>3 episodes of wheezing in the preceding year). Children with no lower respiratory tract illnesses before the age of 3 years as a reference group.

[b] For infrequent wheeze (up to 3 episodes of wheezing in the preceding year). Children with no lower respiratory tract illnesses before the age of 3 years as a reference group.

[c] Comparisons made within the cohort.

[d] Relative risk; compared with children with atopic persistent wheezing; calculated from the numeral data presented in the reference study. Data for calculations of 95% confidence intervals were not applicable.

Table 4. Factors associated with atopic persistent wheezing, according to Martinez and Godfrey (2003).

Factor	OR (95% CI)[a]	(Reference)
Family history of asthma		
Maternal asthma	4.1 (2.1-7.9)	(Martinez *et al.* 1995)
Maternal asthma or chronic obstructive airway disease	3.27 (2.59-4.13)	(Rusconi *et al.* 1999)
Atopic eczema during the first year of life	2.4 (1.3-4.6)	(Martinez *et al.* 1995)
Sensitisation to inhalant allergens		
Preceded by sensitisation to any allergen early in life	10.12 (3.81-26.88)	(Illi *et al.* 2001)
Persistent eosinophilia	1.70 (1.18-2.43)	(Karakoc *et al.* 2002)
Eosinophil activation during viral infections		
Serum ECP of ≥8 μg/l	2.7[b]	(Reijonen *et al.* 1997b)[c]
Serum ECP of ≥16 μg/l	3.9[b]	(Reijonen *et al.* 1997b)[c]
Onset of wheezing at the age of 2-3 years	4.1[b]	(Halonen *et al.* 1999)

[a] Children without wheezing as a reference group.

[b] Relative risk; compared with children with transient early wheezing. Calculated from the numeral data presented in the reference study. Data for calculations of 95% confidence intervals were not applicable.

[c] The study cohort is the same as that presented in this thesis.

2.2. Long-term outcome of early childhood wheezing requiring hospital admission

Especially after hospital admission for wheezing in infancy, recurrent episodes of wheezing and subsequent development of asthma are common. According to earlier studies, 40-60% of children with a history of hospitalisation for wheezing at age <2 years suffer from recurrent episodes of wheezing at 2-3 years of age, (Table 5), and 25-59% of children wheeze persistently or have childhood asthma at 3-6 years of age (Table 6). The prevalence of school-age asthma has been 15-39% (occasionally even up to >70%), in children followed up to 6-14 years of age (Table 7), and even in early adulthood, 30-41% subjects with a history of hospital admission for wheezing in infancy have had symptoms and clinical findings suggestive of asthma (Piippo-Savolainen *et al.* 2004). In comparison, in non-selected school-aged populations in developed countries, the occurrence of asthma is clearly lower, 4-19% (Remes *et al.* 1996, ISAAC Steering Committee 1998). However, the reported prevalence rates of asthma vary a lot, the overall rates in developed countries having been highest in New Zealand and Australia (15-19%), and lowest in Finland and Italy (4-6%) (ISAAC Steering Committee 1998).

After RSV-infection associated lower respiratory tract illness (including bronchiolitis and/or pneumonia) requiring hospitalisation in infancy, the later occurrence of recurrent wheezing has been 40-44% in toddlers and preschoolers (Welliver *et al.* 1986, Osundwa *et al.* 1993, Sigurs *et al.* 1995), and the prevalence of school-age asthma 5-28% (occasionally up to 71%) (Pullan and Hey 1982, Hall *et al.* 1984, Sly and Hibbert 1989, Sigurs *et al.* 2000 and 2005).

In children with hospital admission for wheezing in infancy, the school-age occurrence of atopic manifestations (other than asthma) has been reported to be 11-45% (Murray *et al.* 1992, Wennergren *et al.* 1997, Sigurs *et al.* 2000 and 2005). In non-selected schoolchildren, the prevalence of allergic rhinoconjunctivitis varies from 6 to 24% and the prevalence of atopic eczema from 3 to 19% in developed countries (ISAAC Steering Committee 1998). In Finland, the prevalence of allergic rhinoconjunctivitis is 13-23%, and that of atopic eczema 13-17% (ISAAC Steering Committee 1998).

Table 5. Wheezing requiring hospital admission in infancy - outcome at the age of 2 to 3 years.

Reference	Study type	Number of index children with wheezing	Number of controls	Age (months) on admission	Age (years) at the follow-up	Prevalence of recurrent wheezing at the follow-up
Carlsen *et al.* 1987	Cohort study Prospective	51	0	median 6	2	60%
Korppi *et al.* 1993	Cohort study Prospective	76	0	<24	2.5-3	58%
Osundwa *et al.* 1993	Case-control Retrospective	70	70	<12 (mean 3.8)	2	Index group: 44% Controls: 13%
Sigurs *et al.* 1995	Case-control Prospective	47	93	<12 (mean 3.8)	2.7-3.5 (mean 3)	Index group: 40% Controls: 9%

Table 6. Wheezing requiring hospital admission in infancy - outcome at the age of 3 to 6 years.

Reference	Study type	Number of children with wheezing	Age (months) on admission	Age (years) at the follow-up	Prevalence of persistent wheezing or asthma at the follow-up
Webb *et al.* 1985	Cohort study Prospective	81	<12 (mean 3.6)	3.5-4.7 (mean 3.9)	59%
Welliver *et al.* 1986	Cohort study Prospective	38	<6	4	42%
Wennergren *et al.* 1992	Cohort study Prospective	94	<24 (median 10)	3.3-6.3	47%
Kuikka *et al.* 1994	Cohort study Prospective	68	<24	4.5-6.2	25%
Reijonen *et al.* [a] 2000	Cohort study Prospective	89	<24 (mean 10.6)	3.2-5.1 (median 4.0)	51%

[a] The study cohort is the same as that presented in this thesis.

Table 7. Wheezing requiring hospital admission in infancy - outcome at the age of 6 to 14 years.

Reference	Study type	Number of index children with wheezing	Number of controls	Age (months) on admission	Age (years) at the follow-up	Prevalence of asthma at the follow-up	Definition of asthma
Gurwitz *et al.* 1981	Cross-sectional Retrospective	48	0	<24 (mean 7.6)	mean 10.4	17%	Asthma diagnosed by a pediatrician
Sly and Hibbert 1989	Cohort study Prospective	35	0	<12 (mean 2.8)	6	71%	Based on a history and/or physical findings consistent with asthma
Korppi *et al.* 1994	Case-control Prospective	62	52	<24	8.0-9.5	Index group: 15% Controls: 2%	Wheezing on ≥3 occasions and/or need for continuous or intermittent antiasthmatic medication
Noble *et al.* 1997	Case-control Prospective	61	47	<12 (mean 3.7)	mean 10.1	Index group: 39% Controls: 13%	Based on a history of current respiratory symptoms and prescribed drugs
Wennergren *et al.* 1997	Cohort study Prospective	92	0	<24 (median 10)	10	30%	Based on the frequency of symptoms and the need for treatment during the last 12 months
Sigurs *et al.* 2000	Case-control Prospective	47	93	<12 (mean 3.8)	7.1-8.1 (mean 7.5)	Index group: 23% Controls: 2%	≥3 episodes of wheezing verified by a physician, with symptoms during the preceding year
Sigurs *et al.* 2005	Case-control Prospective	46	92	<12 (mean 3.8)	13.0-14.0 (median 13.4)	Index group: 28% Controls: 3%	≥3 episodes of wheezing verified by a physician, with symptoms during the preceding year

2.3. Prediction of long-term outcome of early childhood wheezing requiring hospital admission

2.3.1. Predictive factors for school-age asthma

In several prospective follow-up studies, a variety of characteristics and markers have been evaluated as predictive factors for recurrent wheezing and subsequent development of asthma. Those characteristics that have been found to predict school-age asthma after wheezing requiring hospital admission are presented in Table 8.

Table 8. Wheezing requiring hospital admission in infancy - factors associated with school-age asthma.

Factor	OR (95% CI)	(Reference)
Hospital admission for bronchiolitis	4.43 (1.63-12.0)[a]	(Noble *et al.* 1997)
Hospital admsission for RSV bronchiolitis	12.7 (3.4-47.1)[a]	(Sigurs *et al.* 2000)
	10.1 (3.4-29.8)[a]	(Sigurs *et al.* 2005)
Parental asthma	4.7 (1.6-13.9)[a]	(Sigurs *et al.* 2005)
Elevated serum IgE in infancy		
Serum IgE of >48 μg/l	8.4[b]	(Korppi *et al.* 1994)
Serum IgE of >144 μg/l (60 kU/l)	4.6[b]	(Korppi *et al.* 1994)
Onset of wheezing at the age of <6 months	2.3[b]	(Wennergren *et al.* 1997)
Intense obstructive disease in early childhood	2.5[b]	(Wennergren *et al.* 1997)
Passive smoking in infancy	2.0[b]	(Wennergren *et al.* 1997)
Development of atopic manifestations	3.7[b]	(Wennergren *et al.* 1997)

[a] Children with transient early wheezing or without early childhood wheezing as a reference group.

[b] Relative risk; compared with children with transient early wheezing. Calculated from the numeral data presented in the reference study. Data for calculations of 95% confidence intervals were not applicable.

These predictors of asthma are in line with the risk factors for persistence of early childhood wheezing, presented by Martinez and Godfrey (2003) (Tables 3 and 4), implying that both non-atopic and atopic forms of asthma are seen in schoolchildren with a history of wheezing requiring hospitalisation in infancy. However, mainly due to different study designs, there exists variation in identified predictors of asthma between the studies.

When compared to controls without a history of wheezing in infancy, hospital admission for RSV bronchiolitis has been found to predict school-age asthma up to the age of 14 years (Sigurs *et al.* 2005), and hospital admission for bronchiolitis of non-specified origin has found to increase the risk of asthma

even up to young adulthood (Piippo-Savolainen *et al.* 2004). However, when the school-age outcome has been compared between RSV-positive and RSV-negative children with wheezing requiring hospital admission, RSV infection has not been found to further increase the risk of asthma (Korppi *et al.* 1994, Wennergren *et al.* 1997). Mainly due to limitations in methods of viral detection, there are no earlier long-term follow-up data available on the role of rhinoviruses, enteroviruses, and coronaviruses as predictors of recurrent wheezing or subsequent development of asthma.

Likewise, data on RSV seropositivity and the later development of asthma or bronchial reactivity are scarce. In a three-year follow-up of non-hospitalised infants, no association with the presence of RSV-specific IgG and the development of recurrent wheezing was found (Sigurs *et al.* 1995). On the other hand, in another study, the RSV-IgG positivity rate at early childhood correlated closely with attacks of wheezing during infancy (Forster *et al.* 1996). In school-aged children, no relationship between the presence of IgG antibodies against RSV and bronchial hyperreactivity was found (Backer *et al.* 1993).

Age <6 months at onset of wheezing has been found to predict school-age asthma after wheezing requiring hospitalisation in infancy (Wennergren *et al.* 1997). On the other hand, in a study of non-hospitalised children, onset of wheezing at the age of >18 months was predicitive for school-age asthma (Foucard and Sjöberg 1984). In addition, in early retrospective data of hospitalised wheezing children, the age of ≥12 months is presented as a risk factor for school-age asthma (Boesen 1953).

Although elevated levels of serum IgE in infancy (Korppi *et al.* 1994) as well as the development of atopic manifestations later in childhood (Wennergren *et al.* 1997) have been found to predict school-age asthma among hospitalised wheezing infants, data on early sensitisation to allergens and its role in the development of asthma are scarce.

SPTs are commonly performed to determine IgE-mediated allergic responses. According to an Italian study, 30% of children aged 5 months to 4 years who were referred to an outpatient clinic due to respiratory symptoms, were sensitised to at least one allergen, most commonly to house dust mites (Silvestri *et al.* 1996). In a Turkish study, 73% of asthmatic children aged <3 years tested positively to house dust mites, 52% to pollens, and 10% to milk (Emin *et al.* 2004). In wheezing infants requiring hospitalisation, emergency

medication usually prevents skin testing during an acute phase of the disease. Consequently, there is shortage of studies on the role of SPT positivity in wheezing infants as a predictive factor for asthma. In a French study, skin test positivity to inhalant allergens (present in 25% of recurrently wheezing or asthmatic infants) was significantly associated with the development of childhood asthma during the follow-up of 2 years (Delacourt *et al.* 1994).

Studies on the occurrence of food and inhalant allergen-specific IgE in wheezing or asthmatic infants are presented in Table 9.

Follow-up data on the role of allergen-specific IgE antibodies as predictors of outcome in wheezing infants are, as well, few: in a French study, most of the infants were already diagnosed as having asthma (Delacourt *et al.* 1994), and in a Dutch study, infants were referred to the outpatient clinic due to suspicion of asthma (Wever-Hess *et al.* 1999). In both studies, the assays applied to determine specific IgE antibodies gave the results only as either positive or negative - not as concentrations - and neither study reported specific IgE for individual allergens (Delacourt *et al.* 1994, Wever-Hess *et al.* 1999). The follow-up time in both studies was no more than 2 years. Delacourt *et al.* (1994) found that specific IgE antibodies to inhalant allergens were rare, and thus, not predictive of asthma, whereas Wever-Hess *et al.* (1999) found specific IgE to inhalant allergens to be highly predictive for development of asthma.

Table 9. Occurrence of specific IgE to food and inhalant allergens in wheezing or asthmatic infants.

Reference	Study type	Study subjects	Age (months)	Number of index subjects	Number of controls	Specific IgE to	Positivity rate
Duff *et al.* 1993	Case-control Cross-sectional	Wheezing children (most outpatients)	<24	23	10	Inhalants	9% in wheezing children 0% in controls
						Mites	9% in wheezing children
Delacourt *et al.* 1994	Cohort study Prospective	Children hospitalised for wheezing or infantile asthma	1-25	30 wheezers 37 asthmatics	NA	Inhalants	10% in wheezing children 5% in asthmatics
Price *et al.* 1995	Case-control Cross-sectional	Wheezing children and children with atopic dermatitis	<24	27 wheezers 19 with atopic dermatitis	13	Foods	4% in wheezing children 84% in children with atopic dermatitis 0% in controls
Rakes *et al.* 1999	Case-control Cross-sectional	Wheezing children (most outpatients)	<24	23	17	Inhalants	0% in children 0% in controls
Wever-Hess *et al.* 1999	Cohort study Prospective	Children with symptoms suggestive of asthma	<24	231	NA	Foods Inhalants Foods and inhalants	20% 8% 5%
Camara *et al.* 2004	Case-control Cross-sectional	Wheezing children	<24	74	30	Foods	9% in wheezing children 11% in controls
						Inhalants	5% in wheezing children 0% in controls
						Mites	5% in wheezing children
						Dog	1% in wheezing children

NA, not applicable.

Eosinophils are known to play a central role in the pathophysiology of asthma (Holgate 1997), and they may contribute in the pathophysiology of wheezing in early childhood. During acute viral respiratory infection, eosinopenia has been considered as a normal response (Garofalo *et al.* 1994, Martinez *et al.* 1998), and in children with wheezing lower respiratory tract infection, blood eosinophilia or normal eosinophil counts have been predictive of persistent wheezing (Martinez *et al.* 1998, Ehlenfield *et al.* 2000, Øymar *et al.* 2001). In addition, elevated levels of eosinophil activation products like ECP in serum or respiratory tract secretions have been predictive of subsequent recurrent wheezing in infants hospitalised for wheezing (Reijonen *et al.* 1997b, Reijonen *et al.* 1997c, Pifferi *et al.* 2001). However, it is known that in addition to eosinophils, also neutrophils are able to secrete ECP, and thus it has been speculated that in wheezing infants, lower respiratory tract infections with neutrophilia, rather than eosinophilic airways inflammation, would result in elevated levels of ECP (Azevedo *et al.* 2001).

In fact, little is known about the role of eosinophils or their activation products as predictors of school-age asthma in hospitalised wheezing infants: Ehlenfield *et al.* (2000) found that eosinophilia (i.e. blood eosinophil count over median) during RSV bronchiolitis predicted the presence of wheezing at 7 years of age. However, the authors made no efforts to evaluate the occurrence of clinical asthma. Wennergen *et al.* (1997) found that blood eosinophil count of $\geq$300 cells/mm^3 during the acute episode of wheezing in infancy was predictive of atopy but not of asthma at the age of 10 years.

2.3.2. Clinical implications

In recent years, algorithms based on personal atopic findings and the parental history of asthma have been developed for clinical use, in order to facilitate the prediction of later asthma in young wheezing children. The algorithms introduced by Martinez (1999) and Castro-Rodríguez *et al.* (2000) are presented in Table 10. These algorithms have thus far included blood eosinophilia ($\geq$4-5%) as the only laboratory marker (Martinez 1999, Castro-Rodríguez *et al.* 2000).

Table 10. Algorithms to define children at risk for asthma.

Reference	**Martinez 1999**	**Castro-Rodríguez *et al.* 2000**
Major criteria	1. Hospitalisation for bronchiolitis/severe wheezing 2. ≥3 wheezing lower respiratory illnesses during previous 6 months 3. Parental history of asthma 4. Atopic dermatitis	1. Parental history of asthma (physician-diagnosed) 2. Atopic dermatitis (physician-diagnosed)
Minor criteria	1. Rhinorrhea apart from colds 2. Wheezing apart from colds 3. Eosinophilia (≥5%) 4. Male sex	1. Allergic rhinitis (physician-diagnosed) 2. Wheezing apart from colds 3. Eosinophilia (≥4%)
Prediction of asthma	Very high risk of persistent wheezing: Major criteria 1. or 2. + any other major criteria or Major criteria 1. or 2. + two minor criteria	Loose index: Any wheezing at age <3 years + ≥1 of the two major criteria or Any wheezing at age <3 years + ≥2 of the three minor criteria Stringent index: Recurrent wheezing at age <3 years + ≥1 of the two major criteria or Recurrent wheezing at age <3 years + ≥2 of the three minor criteria

3. AIMS OF THE STUDY

The aims of the study were to evaluate the early school-age outcome of infantile wheezing requiring hospital admission, to clarify asthma-predictive factors present in early childhood, and to assess the occurrence and the role of certain respiratory virus infections in infants hospitalised for wheezing.

The specific aims were:

1. To assess the occurrence and the role of rhino-, entero- and coronavirus infections in infants hospitalised for wheezing.

2. To evaluate the role of RT-PCR as a supplementary method for RSV detection, and to assess the occurrence and the role of RSV-specific IgG antibodies in early childhood.

3. To clarify the clinical characteristics of rhinovirus infection-associated wheezing, compared with RSV bronchiolitis.

4. To evaluate the occurrence and the role of food- and inhalant allergen-specific IgE antibodies in infants hospitalised for wheezing.

5. To assess the occurrence of asthma and atopy at early school age in children hospitalised for wheezing in infancy.

6. To identify the asthma-predictive factors present in early childhood in children requiring hospitalisation for wheezing in infancy, paying special attention to the role of respiratory viruses, RSV-specific serum IgG antibodies, and allergen-specific serum IgE antibodies.

4. SUBJECTS AND METHODS

4.1. Acute-phase study in 1992 to 1993

4.1.1. Study subjects

In 1992-1993, 100 children, aged 1 to 23 months (median 10 months), were recruited into this study during a period of 22 months, from January 1st 1992 to November 2nd 1993 (Reijonen *et al.* 1996). The inclusion criterion was respiratory infection-related wheezing resulting in hospital treatment (the index episode) in the Department of Paediatrics, Kuopio University Hospital (Reijonen *et al.* 1995). The exclusion criteria were a history of a chronic cardiorespiratory disease, regular medication for any pulmonary disease, or acute threatening respiratory failure (Reijonen *et al.* 1995).

4.1.2. Collection of baseline data

The baseline data were obtained by interviewing the parents at enrolment. A structured questionnaire included questions on the duration of dyspnoea before the admission (at 1 day accuracy), the history of physician-diagnosed episodes of wheezing, the presence of atopic dermatitis in infancy, the family history of physician-diagnosed asthma and atopy, maternal smoking during pregnancy, passive smoking during infancy, and pet contacts at home or at day care during infancy (Reijonen *et al.* 1995 and 1996).

During the index episode of wheezing, age on admission, and the duration of hospitalisation were also registered (at 1 day accuracy).

4.1.3. Laboratory studies

During the index episode of wheezing, total serum immunoglobulin E (IgE), blood eosinophil count, and serum eosinophil cationic protein (ECP) were determined, as published earlier (Reijonen *et al.* 1996 and 1997b). The total serum IgE concentration of $\geq$60 kU/l (Saarinen *et al.* 1982), the blood eosinophil count of $\geq$0.45 cells x 10^9/l (Eisen 1980), and the serum ECP concentration of $\geq$16 μg/l (Peterson *et al.* 1991) were considered as elevated.

Seven common respiratory viruses, that is RSV, adenoviruses, influenza A and B viruses, and parainfluenza type 1, 2 and 3 viruses, were studied by antibody and antigen assays. On admission, nasopharyngeal aspirates (NPA)

were taken by suctioning a mucus specimen via nostrils with a disposable extractor (Reijonen *et al.* 1997b and 1997c). Direct detection of viral antigens by time-resolved fluoroimmunoassay (TR-FIA) was available for RSV, parainfluenza viruses type 1, 2 and 3, influenza A and B viruses, and adenoviruses (Reijonen *et al.* 1997b and 1997c). Complement fixation (CF) serology was studied for the same seven respiratory viruses in paired sera taken 6 weeks apart; four-fold or greater increase in titres was defined as positive (Reijonen *et al.* 1997b). A part of each NPA and convalescent serum sample were frozen for later viral studies.

4.1.4. Emergency treatment

In all patients, a clinical baseline evaluation was performed shortly after hospitalisation, and the emergency medication was studied in a prospective trial published earlier (Reijonen *et al.* 1995). Supplementary viral studies in 2000-2002 allowed retrospectively a virus-specific aspect on the baseline clinical findings and responses to treatment.

Emergency treatment in brief; the patients were randomised to get either inhaled salbutamol or racemic adrenalin, and intramuscular adrenalin was given to all patients 60 minutes later. The repeated clinical evaluations every 15 minutes consisted of the measurements of respiratory rate, heart rate and transcutaneous oxygen saturation, and assessment of respiratory distress assessment instrument (RDAI) scores. The RDAI score is based on two respiratory variables, wheezing (0-8 points) and retractions (0-9 points); a good inter-observer agreement has been documented earlier (Lowell *et al.* 1987, Klassen *et al.* 1991), and the agreement was excellent in the comparison of the investigators (Reijonen *et al.* 1995). Responses to inhaled emergency treatment were assessed by respiratory assessment change scoring (RACS). The scores were calculated by subtracting RDAI scores that were assessed at the start of the study and at 15 minutes intervals until 90 minutes, and by combining the changes in RDAI and changes in respiratory rate (Lowell *et al.* 1987, Reijonen *et al.* 1995).

4.2. Follow-up

Since the index episode of wheezing, several study visits had been organised in order to follow up the respiratory status and allergic manifestations in the children (Reijonen *et al.* 2000). The ongoing medication was registered at the follow-up visits, including the exact data on cromone and steroid maintenance medication for asthma. The need for and the type of medication were assessed by a paediatric allergist on a clinical basis independently of the investigators.

SPTs were performed for the first time at the 8-month follow-up visit, at the median age of 1.5 years (Reijonen *et al.* 2000). The allergens (ALK SPT extracts, ALK Laboratories, Copenhagen, Denmark) tested were the food allergens cow's milk, egg white, fish, and wheat, the outdoor inhalant allergens birch, timothy grass and mugwort pollens, and the indoor inhalant allergens cat and dog epithelial danders and home dust mites *Dermatophagoides (D) pteronyssinus* and *D. farinae*.

4.3. Follow-up visit in 1999

4.3.1. Study subjects

Eighty-two children, 61 boys and 21 girls, attended the follow-up study visit in 1999, from January to March. The age of the children varied from 5.6 to 8.8 years (median 7.2 years), and the time from the index episode of wheezing ranged from 5.3 to 7.2 years (median 6.3 years).

4.3.2. Collection of follow-up data and clinical examinations

A structured questionnaire was used to record the symptoms suggestive of asthma associated with exercise, infections or allergens. In addition, physician-diagnosed (ever in life) allergic rhinitis, allergic conjunctivitis and atopic dermatitis were recorded. Allergic diseases were classified as current if there had been clinical manifestations during the preceding 12 months.

The allergens tested by SPTs were the outdoor allergens birch, common alder, timothy grass, meadow grass and mugwort pollens, and spores of *Cladosporium (C) herbarum*, and the indoor allergens cat and dog epithelial dander and home dust mites *D. pteronyssinus* and *D. farinae*.

The concentration of a non-standardised extract of *C. herbarum* spores was 1:20 weight/volume. Other allergen extracts were standardized, the concentrations being 10 histamine equivalent points. Histamine hydrochloride (10 mg/ml) was used as a positive and 50% glycerol as a negative control. Wheals with a mean diameter of at least 3 mm were regarded as positive (EAACI 1993), and no reactions were allowed in negative controls.

4.3.3. Flow-volume spirometry and the exercise challenge test

The exercise challenge test was performed in all 82 children. The baseline pulmonary function was examined by flow-volume spirometry (FVS) (Medikro, Kuopio, Finland), and forced expiratory volume in 1 second (FEV_1) was the parameter used in challenge tests. First, the children were carefully instructed on how to perform the test. Thereafter, the measurements were repeated at least three times, and accepted if the FEV_1 variation was less than 5%, and the printed graphic curves were appropriate and equal in shape. The highest FEV_1 value was used in later comparisons. The baseline pulmonary testing was followed by exercise that constituted of free running outdoors for 8 minutes at the heart rate of 80% or more of the predicted maximum. The heart rate was monitored by telemetry (Polar Sport Tester, Polar Elektro Ltd, Kempele, Finland) at 1-minute intervals. FVS was performed 10 minutes after the exercise. FEV_1 changes were calculated as follows: [(pre-exercise FEV_1 - post-exercise FEV_1)/ pre-exercise FEV_1] x 100%.

The children's breathing sounds were auscultated before the baseline lung function testing and immediately after the exercise. Symptoms, and abnormal auscultatory findings, if present, were recorded.

The exercise challenge test was regarded as positive if there was auscultatory wheezing present after the exercise and/or a 15% or greater fall in FEV_1 (Carlsen 2001).

4.3.4. Definition of asthma

Asthma was considered to be present if 1) the child had suffered from at least 2 episodes of wheezing and/or prolonged cough apart from infection for at least 4 weeks during the preceding 12 months, as reported by parents, and there was auscultatory wheezing present after the exercise and/or a 15% or greater fall in FEV_1 after the exercise (Remes *et al.* 1996), or 2) the child had ongoing

continuous inhaled steroid or cromone medication prescribed by a paediatrician for clinical asthma.

4.4. Supplementary laboratory studies in 2000 to 2002

4.4.1. Determination of allergen-specific IgE antibodies

In 2000, frozen serum samples, obtained during the index episode of wheezing in 1992-1993, were available for the determination of allergen-specific IgE antibodies (Phadiatop Combi panel) in 80 children who participated in the follow-up visit in 1999. The determination of specific IgE antibodies was performed by an automated *in vitro* screening method, UniCAP fluoroenzyme-immunometric assay (FEIA) (Pharmacia, Uppsala, Sweden) (Paganelli *et al.* 1998).

According to the manufacturer, the specific IgE concentrations of 0.35 kU/l to 0.69 kU/l are comparable to RAST class 1, the concentrations of 0.70 kU/l to 3.49 kU/l to RAST class 2, and the concentrations of ≥3.50 kU/l to RAST classes 3 to 4.

The presence of IgE antibodies to the mixtures of inhalant and food allergens was screened first, the concentrations of ≥0.35 kU/l being detectable in both assays. In occasions with detectable antibodies, further determinations were performed to assess individual allergen-specific IgE antibody concentrations. The detection limit was the same (0.35 kU/l) as for the allergen mixtures. The other cut-off concentrations proposed by the manufacturer (≥0.70 kU/l and ≥3.50 kU/l) were also explored in the present analyses.

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, mugwort pollen, cat dander, dog dander, horse dander, house dust mite *D. pteronyssinus*, and spores of the mould *C. herbarum*.

4.4.2. Studies for rhino-, entero- and coronaviruses

In 2000, viral studies were supplemented by detection of rhino-, entero- and coronaviruses in frozen NPA specimens, obtained in 1992-1993 and stored at -40°C. An adequate amount of NPA for rhino-, entero- and coronavirus studies

was available for 81 children, 66 of whom also took part in the follow-up visit in 1999.

An RT-PCR assay was used for direct detection of rhinoviruses and enteroviruses in NPA samples, as described recently in more detail (Lönnrot *et al.* 1999). In brief; prior to the PCR test, the nucleic acids from the samples were isolated by the "High Pure Viral Nucleic Acid Kit" (Roche, cat no 1 858 874) according to the manufacturer's instructions. The primers were from the conserved region of the rhinovirus and enterovirus genomes (a biotinylated positive-sense primer with map-position 454-473; a negative-sense primer with map-position 548-568). The PCR products were detected by agarose gel electrophoresis and ethidium bromide staining. The results were confirmed in a liquid-phase hybridisation assay using the oligonucleotide probes with a maximum difference between rhinoviruses (a samarium-labelled probe with map-position 528-544) and enteroviruses (an europium-labelled probe with map-position 534-549) to differentiate the rhinovirus and enterovirus amplicons. The probes could be detected simultaneously by time-resolved fluorometry (Halonen *et al.* 1995, Santti *et al.* 1997).

The NPA samples were also tested for sequences of human coronavirus strains 229E and OC-43 by an RT-PCR assay similar to that for rhinoviruses and enteroviruses (Ruohola *et al.* 2000). The primers and probes were modified either from the primers and nucleotide sequences described earlier (Kamahora *et al.* 1989, Myint *et al.* 1994), or from those available in GenBank.

4.4.3. Studies for RSV

In 2002, RSV studies were supplemented by detection of RSV genome in NPAs obtained during the index episode of wheezing in 1992-1993 and frozen at -40°C. An adequate amount of NPA for detection of RSV genome was available for 61 children.

Detection of RSV genome was performed by in-house RT-PCR of the F (fusion) protein gene as follows: RNA was extracted from 250 µl of NPA using High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany) and eluted in a 60-µl volume according to the manufacturer's instructions. RNA was reverse transcribed and amplified in a 50-µl single-tube reaction containing 50 mM Tris-HCl (pH 8.4), 50 mM NaCl, 4.5 mM $MgCl_2$, 2 mM dithiothreitol, 200 nM of each primer, 600 nM deoxynucleoside triphosphates (Amersham

Biosciences, Uppsala, Sweden), 4 U of RNase inhibitor (RNAsin, Promega, Madison, WI), 2 U of DNA polymerase (DynaZyme, Finnzymes, Espoo, Finland), 40 U of reverse transcriptase (M-MLV RNaseH$^-$, Promega), and 5 µl of extracted RNA.

The primers RSVF-F1 (TGA ACA GTT TAA CAT TAC CAA GTG A) and RSVF-F2 (CCA CGA TTT TTA TTG GAT GC) amplify a conserved sequence of RSV F gene with an amplicon size of 181 base pairs. The amplification program included an RT step at 42°C for 45 min, denaturation at 95°C for 7 min, 40 cycles of the following: at 95°C for 1 min, at 58°C for 1 min, at 72°C for 1 min, respectively; final elongation step at 72°C for 10 min, and cooling at 4°C until further processed. PCR products were analysed by electrophoresis in 2% agarose gel with ethidium bromide staining. Each analysis included two positive controls (cDNA from cells infected with RSV group A or B strains), one RNA extraction blank for every 11 aspirates, and one PCR blank.

In 2001, RSV-specific IgG antibodies were studied in frozen convalescent sera obtained at the 6-week, 1-year and 3-year follow-up visits. An adequate amount of serum for determination of specific IgG antibodies was available for 90 children who took part in the 6-week follow-up, for 77 children who completed the 1-year follow-up, and for 72 children who completed the 3-year follow-up. Sixty-three children with complete IgG data available took part in the follow-up visit in 1999.

RSV-specific IgG antibodies were determined by enzyme immunoassay (EIA) (RSV-IgG-ELISA, IBLHamburg). According to the manufacturer's instructions, the absorbance values of the patient sera were quantitated (U/ml) from the reference curve of the standard sera. The values below 10 U/ml (the concentration of the cut-off standard) were interpreted as negative and the values higher than 150 U/ml (the concentration of the highest standard) were expressed as >150 U/ml.

4.5. Ethics

The study was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent was obtained from the parents of the children in 1999. In addition, an informed oral consent had been obtained from the parents at enrolment (Reijonen *et al.* 1995).

4.6. Statistical analyses

The data were analysed using SPSS/PC+ 9.0 software (SPSS Inc. Chicago, IL, USA). Statistical significances of the differences between the groups were assessed with chi-squared test for proportions supplemented by odds ratios (OR) with 95% confidence intervals (CI). Fisher's exact test was used when the expected frequency for any cell was <5.

Mann-Whitney U-test was used for the univariate statistical analyses of continuous data. Logistic regression was used for adjusted multivariate analyses of the dichotomous outcome data, and linear regression for adjusted multivariate analyses of the continuous outcome data. The logistic regression analysis was used to calculate the adjusted ORs and related 95% CIs.

Two-tailed tests were used in all analyses. P-values less than 0.05 were considered as statistically significant.

The kappa (κ) statistics was applied to compare agreement between allergen-specific IgE and other markers and characteristics of atopy. The κ-value of >0.80 indicates good agreement; 0.61 to 0.80 substantial agreement; 0.41 to 0.60 moderate agreement; 0.21 to 0.40 fair agreement, and $\leq$0.20 poor agreement (Petrie and Sabin 2000).

Sensitivity and specificity were calculated by using routine equations. Positive likelihood ratios (LR+) were calculated as LR+ = sensitivity/(1-specificity). LR+ values of $\geq$3 are considered to have moderate effects on pre-test probability, and LR+ values of $\geq$10 (or of $\geq$5) significant effects (Sackett *et al.* 1998). Receiver operating characteristic (ROC) analysis was performed in order to determine the optimal cut-off concentrations for specific IgE antibodies as predictors of asthma.

5. RESULTS

5.1. Supplementary viral findings related to the index episode of wheezing (III)

Viral findings related to the index episode of wheezing are presented in Table 11.

Table 11. Viral findings in 100 infants hospitalised for wheezing.

Viral findings related to the index episode of wheezing[a]	All children (n=100)	Age on admission (months) 1-5 (n=28)	6-11 (n=36)	12-23 (n=36)
RSV	29	18	9	2
as a single viral finding	21	12	9	-
in mixed viral infections[b]	8	6	-	2
Rhinovirus	27	2	8	17
as a single viral finding	22	1	7	14
in mixed viral infections[b]	5	1	1	3
Enterovirus	10	2	3	5
as a single viral finding	5	-	2	3
in mixed viral infections[b]	5	2	1	2
Other viruses	9	3	5	1
No viral findings	31	6	12	13

[a] RSV was studied by TR-FIA in NPAs and by CF antibodies in paired sera in 100 children (Reijonen 1997a), and by RT-PCR in frozen NPAs in 61 children. Rhino-, entero- and coronaviruses were studied by RT-PCR in frozen NPAs in 81 children. Other common respiratory viruses were studied by TR-FIA in NPAs and by CF antibodies in paired sera in 100 children (Reijonen 1997a).

[b] Mixed viral findings include 1 RSV-rhinovirus co-infection, 4 RSV-enterovirus co-infections, and 1 rhinovirus-enterovirus co-infection. The rest of the mixed viral findings were co-infections with other common respiratory viruses (Reijonen 1997a).

Antigen and/or antibody assays performed during the index episode of wheezing in 1992-1993 were positive for RSV in 25 (25%) of the 100 children. When supplemented by RT-PCR results, 4 additional RSV-positive cases were found, resulting in a total of 29 (29%) children with RSV infection on admission (Table 11). RSV infection was most common in children younger than 6 months: only 7% of the children hospitalised with RSV were 12 months old or older.

In all, RT-PCR was performed for 61 children, of whom 12 children had earlier been tested positive for RSV by antigen or CF assays. Of those 12 children, 2 had been tested positive both by the antigen assay and the CF assay, 8 by the antigen assay only and 2 by the CF assay only. RT-PCR for RSV found all the 10 cases detected by antigen assay and 2 of the 4 cases identified by CF assay, and in addition, 4 cases not identified by the antigen or CF assays. In all, 16 of the 61 children tested positive for RSV by the antigen assay, CF assay and/or RT-PCR. In case of RT-PCR, this means a sensitivity of 83% and a specificity of 92%. When compared to antigen detection alone, the sensitivity of RT-PCR was 100% and the specificity 92%.

Rhinoviruses were identified in 27 (33%) of the 81 specimens analysed (Table 11). Twenty-five (93%) of the rhinovirus-positive children were 6 months or more of age ($p=0.003$ vs. children under 6 months of age). Rhinovirus infections were most common in the age group of 12 to 17 months, present in 65% of the children at that age. Enterovirus infection was found in 10 children with no significant age dependence, and coronavirus infection was found in no child.

In most (81%) of the 27 cases with rhinovirus identified, there were no other viral findings. Enterovirus infections were mixed in 5 (50%) cases.

The recruitment period in 1992-1993 occurred between two RSV infection epidemics; the peak incidence of RSV infections appeared at the beginning of the year 1992, representing an ongoing winter epidemic having started at the end of the year 1991, and was followed by a minor spring epidemic in 1993. In contrast, the peak incidence of rhinovirus infections occurred at the end of the year 1992, between the major and minor RSV epidemics.

5.2. RSV-specific IgG from infancy to preschool age (V)

In sera obtained at the 6-week follow-up, 41 (46%) of the 90 children had RSV-specific IgG antibodies (of $\geq$10 U/ml). A positive RSV finding on admission (in 26 (29%) of the 90 children) was followed by a positive IgG finding in 17 cases, and by a negative finding in 9 children, 5 of whom were younger than 6 months on admission. Moreover, there were 24 children with negative RSV results on admission, but a positive specific IgG finding (of $\geq$10 U/ml) 6 weeks later. These children were regarded as having had an earlier RSV infection

before the index episode of wheezing. It is probable that in most of these 24 children RSV infection had been mild or even subclinical, as only 3 (13%) of them had a history of an earlier physician-diagnosed wheezing episode. Moreover, RSV-specific IgG antibodies were not of maternal origin in most cases as only 2 (8%) of the 24 children were younger than 6 months. Forty (44%) of the 90 children had no findings of acute or past RSV infection on admission.

The RSV-specific IgG antibody concentrations, as well as the number of children with positive IgG findings, rose with age (Table 12). At <1 year of age, 43% of children had RSV-specific IgG (of ≥10 U/ml) in sera; at age 1 to 2 years, 66% of children, and at age ≥3 years, 92% of children had detectable RSV-specific IgG in serum samples, respectively.

Table 12. Concentrations of RSV-specific IgG antibodies by age.

	Age (months)				
RSV-specific IgG concentration (U/ml)	**1-5 (n=14)**	**6-11 (n=33)**	**12-23 (n=77)**	**24-35 (n=34)**	**≥36 (n=72)**
<10	7 (50%)	20 (61%)	33 (43%)	5 (15%)	6 (8%)
10-99	7 (50%)	11 (33%)	27 (35%)	8 (24%)	16 (22%)
≥100	0	2 (6%)	17 (22%)	21 (62%)	50 (69%)
Median	<10 U/ml	<10 U/ml	14 U/ml	122 U/ml	145 U/ml

5.3. Comparison of the clinical pictures of RSV and rhinovirus-associated wheezing (IV)

Baseline data, collected on admission, with respect to rhinovirus or RSV aetiology are presented in Table 13. The children with rhinovirus infection were older than those with RSV infection; the medians were 13 months (range 2-23) and 5 months (1-22), respectively ($p<0.001$). Five children in the rhinovirus group, compared with none in the RSV group, had experienced an earlier episode of physician-diagnosed wheezing ($p=0.051$). In addition, children with rhinovirus infection presented more frequently with atopic dermatitis, blood eosinophilia, and/or elevated serum ECP (Table 13). The groups did not differ with regard to total serum IgE (Table 13).

Table 13. Baseline data of the 50 study children with either rhinovirus or RSV infection aetiology.

Baseline data	Rhinovirus (n=26)	RSV (n=24)	p
Age ≥12 months	17 (65%)	2 (8%)	<0.001[#]
Gender (boys/girls)	18/8	13/11	0.273[#]
Atopic dermatitis	14 (54%)	4 (17%)	0.006[§]
Total serum IgE (kU/l) [median (range)][a]	38 (1-154)	3 (1-241)	0.261[§]
Total serum IgE of ≥60 kU/l[a]	8 (31%)	3 (14%)	0.763[§]
Blood eosinophil count ($x10^9$/l) [median (range)][b]	0.500 (0.028-1.432)	0.094 (0.006-0.613)	0.019[§]
Blood eosinophil count of ≥0.45 $x10^9$/l[b]	14 (54%)	2 (9%)	0.045[§]
Serum ECP (μg/l) [median (range)][c]	7.3 (<1.9-116.6)	2.0 (<1.9-12.5)	0.038[§]
Serum ECP of ≥16 μg/l[c]	9 (35%)	0 (0%)	0.002[¤]

[a] Data available for 26 rhinovirus-positive and 22 RSV-positive children.

[b] Data available for 25 rhinovirus-positive and 22 RSV-positive children.

[c] Unpublished data.

[#] Chi-squared test.

[§] Logistic regression adjusted for age (<12 or ≥12 months) and gender, rhinovirus vs. RSV group as a reference.

[¤] Fisher's exact test.

The table includes only those RSV-positive children who were also tested for rhinoviruses (by RT-PCR), and were rhinovirus negative.

The findings of the clinical examination on admission are presented in Table 14.

Table 14. Results of the clinical examination on hospital admission in the 50 study children with either rhinovirus or RSV infection aetiology.

Variable	Rhinovirus (n=26)	p#	RSV (n=24)
Respiratory rate/min [median (range)]	49 (36-80)	0.938	53 (36-72)
Heart rate/min [median (range)][a]	161 (132-180)	0.001	146 (112-172)
Oxygen saturation (%) [median (range)]	95 (90-98)	0.027	92 (87-97)
RDAI score [median (range)]	9 (2-14)	0.537	8 (4-14)

[a] Data available for 26 children with rhinovirus and 23 children with RSV infection.

[#] Age-adjusted linear regression; viral aetiology as an explanatory variable. The table includes only those RSV-positive children who were also tested for rhinoviruses (by RT-PCR), and were rhinovirus negative.

Oxygen saturation values were lower in children with RSV infection. Mild hypoxemia (<94%) was present in 15 (63%) of RSV and in 7 (27%) of rhinovirus positive cases (age-adjusted p=0.2), but severe hypoxemia (<90%) was present in only one patient with RSV infection. Instead, heart rates were higher in rhinovirus-positive cases. There were no significant differences in respiratory rate and RDAI scores (describing the severity of wheezing and retractions) between the two viral groups (Table 14).

There were no differences between the RSV and rhinovirus groups in the clinical measurements at 90 minutes after the emergency treatment. The median respiratory rates were 50/min (range 26-62) and 44/min (28-66), and the median oxygen saturations were 93% (89-96) and 93% (90-100), respectively. The median RDAI scores at 90 minutes were 5 (range 0-9) and 3 (0-11) in the RSV and rhinovirus groups, respectively. These data suggest that, on average, RSV- and rhinovirus-associated wheezing respond similarly to emergency therapy. Likewise, the RACS from 0 to 90 minutes (Table 15), as well as from 0 to 15, 30, 45, 60 and 75 minutes (data not shown), were rather similar in the two etiological groups. In addition, no differences were found between RSV- and rhinovirus-positive cases in RACS for the subgroups of emergency treatment (racemic adrenalin or salbutamol inhalations) (Table 15).

Table 15. RACS in children with either rhinovirus or RSV infection aetiology, by emergency treatment.

RACS from 0 to 90 min	Rhinovirus (n=24)	RSV (n=24)
Total RACS [median (range)]	5 (-1 - 17)	4 (-2 - 13)
RACS by emergency treatment [median (range)]		
Racemic adrenalin / i.m. adrenalin[a]	5 (0 - 17)	3 (-2 - 13)
Salbutamol / i.m. adrenalin[a]	5 (-1 - 12)	5 (0 - 12)

[a] Data available for 12 children in both rhinovirus and RSV groups.
There were no statistically significant differences between the rhinovirus and RSV groups.
The table includes only those RSV-positive children who were also tested for rhinoviruses (by RT-PCR), and were rhinovirus negative.

The children with RSV infection were treated in hospital <1 to 7 days (median 2 days). The respective time in children with rhinovirus infection was <1 to 8 days (median 1 day) (age-adjusted p=0.7). The children with RSV infection had suffered from dyspnoea <1 to 7 days (median 1 day) at home before admission, compared with <1 to 3 days (median 1 day) in the children with rhinovirus infection (age-adjusted p=0.068).

5.4. Allergen-specific IgE findings related to the index episode of wheezing (II)

Allergen-specific IgE findings related to the index episode of wheezing are presented in Table 16.

Almost half (46%) of the children were sensitised (specific IgE of ≥0.35 kU/l) to food allergens whereas only 18% were sensitised to inhalant allergens. All children, except one, with specific IgE to inhalant allergens were over 12 months of age. Sensitisation to food allergens was more evenly distributed; 35% of the children under and 65% of those over 12 months were sensitised. Concerning food allergens, the sensitisation was most frequent to egg white (39%), followed by cow's milk (28%), peanut (18%), and wheat (15%), and concerning inhalant allergens, to dog (10%) and cat (9%) dander. Sensitisation to birch pollen (4%) and the house dust mite *D. pteronyssinus* was rare (3%). All but two children (97%) with specific IgE to inhalant allergens had specific IgE also to food allergens; the corresponding figure for specific IgE to inhalant allergens was 32% among the food allergen positive children.

Table 16. Specific IgE antibodies to food and inhalant allergens in wheezing infants during the index episode of wheezing.

	All children	Age		
		<12 months		≥12 months
Specific IgE to	**(n=80)**	**(n=49)**	**p[#]**	**(n=31)**
Food allergens				
≥0.35 kU/l	37 (46%)	17 (35%)	0.009	20 (65%)
≥0.70 kU/l[a]	31 (39%)	14 (29%)	0.019	17 (55%)
≥3.50 kU/l[a]	14 (18%)	6 (12%)	0.120	8 (26%)
Inhalant allergens				
≥0.35 kU/l	14 (18%)	1 (2%)	<0.001	13 (42%)
≥0.70 kU/l[a]	12 (15%)	1 (2%)	<0.001[§]	11 (35%)
≥3.50 kU/l[a]	6 (8%)	1 (2%)	0.030[§]	5 (16%)

[a] Unpublished data.

[#] Chi-squared test.

[§] Fisher's exact test.

When associations between allergen-specific IgE antibodies and other markers or characteristics on entry were analysed, significant associations were to be seen with elevated total serum IgE, elevated serum ECP, blood eosinophilia, and the presence of atopic dermatitis (Table 17).

Table 17. Specific IgE to food and inhalant allergens with regard to the presence of atopic dermatitis, elevated total serum IgE, eosinophilia, and/or elevated serum ECP on entry.

		Specific IgE of ≥0.35 kU/l to					
		Food allergens			Inhalant allergens		
Baseline data		**(n=37)**	**p**	**κ**	**(n=14)**	**p**	**κ**
Atopic dermatitis	(n=23)	15	0.031	0.225	9	0.001	0.344
Total serum IgE of ≥60 kU/l	(n=17)	14/36[a]	0.002	0.326	8[a]	<0.001	0.396
Blood eosinophil count of ≥0.45 x10^9 cells/l	(n=26)	16/35[b]	0.036	0.230	10/13[b]	0.001	0.374
Serum ECP of ≥16 μg/l	(n=15)	12	0.004	0.266	8	<0.001	0.453

[a] Data were available for 77 children.

[b] Data were available for 78 children.

All but one of the κ-values were <0.40, indicating only fair agreement between the presence of allergen-specific IgE antibodies and other markers or characteristics on entry. The family history of atopy or asthma had no association with allergen-specific IgE antibodies. Interestingly, only 4 (16%) of the 25 infants with a furry pet at home or at day care had specific IgE to inhalant allergens. Among the 20 RSV positive children, there were only 4 (20%) children with specific IgE to food allergens and one (5%) child with specific IgE to inhalant allergens. Thus, RSV identification was associated with non-detectable concentrations of specific IgE to food (16/20 cases; p=0.007) or to inhalant (19/20 cases; p=0.17) allergens.

5.5. Outcome at early school age (I)

Eighty-two children of the original study group of 100 children were re-examined in 1999. The baseline characteristics of the 18 drop-outs did not differ from the participants of the study (data not shown).

Asthma was considered to be present in 33 children (40%). Thirty of them used either cromones (n=18) or inhaled steroids (n=12) as maintenance medication for asthma. Despite the ongoing medication, 25 (83%) children (14 of whom were on cromones, and 11 on inhaled steroids) had suffered from repeated episodes of wheezing, prolonged cough, or both during the preceding 12 months, and the exercise challenge test was regarded as positive in 8 (27%) cases. The 3 children with no ongoing maintenance medication for asthma had suffered from repeated episodes of wheezing or prolonged cough during the preceding 12 months, and all of them had positive results in the exercise challenge test.

In 49 children asthma was considered as not to be present at early school age. Among them, 12 (24%) children had suffered either from parent-reported wheezing episodes or from prolonged cough during the preceding 12 months, but none of them had a positive exercise challenge test. Likewise, two children with positive exercise challenge reported no symptoms suggestive of asthma, and were considered as non-asthmatics.

Among allergic manifestations at early school age (Table 18), there was a significant association between asthma and allergic rhinitis and/or allergic conjunctivitis, and in addition, SPT positivity was more common in asthmatics.

In contrast, atopic dermatitis was common both in asthmatic and non-asthmatic children, and had no such association.

Table 18. Atopic manifestations/findings and asthma at early school age.

	Asthma				
Atopic manifestations/findings	**Present (n=33)**	**Not present (n=49)**	**p**	**OR**	**95% CI**
Allergic rhinitis	22 (67%)	8 (16%)	<0.001	10.25	3.60-29.22
Allergic conjunctivitis	19 (58%)	6 (10%)	<0.001	9.73	3.24-29.17
Atopic dermatitis	20 (61%)	20 (41%)	0.079	2.23	0.91-5.49
Any positive SPT reactions[a]	24 (73%)	14 (29%)	<0.001	6.67	2.49-17.86
to indoor allergens[b]	23 (70%)	11 (22%)	<0.001	7.95	2.92-21.61
to outdoor allergens[c]	18 (55%)	10 (20%)	0.001	4.68	1.76-12.42

[a] SPT reactions were defined as positive if the wheal had a mean diameter of ≥3 mm.

[b] There were 29 positive SPT reactions to cat dander, 25 to dog dander, 6 to *D. pteronyssinus*, and 3 to *D. farinae*.

[c] There were 19 positive SPT reactions to birch pollen, 19 to common alder pollen, 16 to meadow grass pollen, 15 to timothy grass pollen, 4 to *C. herbarum,* and 1 to mugwort pollen.

p-values were determined by the chi-squared test supplemented by ORs and 95% CIs.

5.6. Predictive factors for early school age asthma (I, II, III, V)

5.6.1. The role of age and gender (I)

Neither age on admission nor sex were significantly predictive for early school-age asthma: 34% (17/50) of the children aged <12 months and 50% (16/32) of those aged ≥12 months on admission were considered as asthmatics (p=0.15), and 44% (27/61) of boys and 29% (6/21) of girls had asthma (p=0.21).

5.6.2. The role of family history of asthma and atopy, and the role of personal history of wheezing and atopy in infancy (I)

Neither the family history of asthma or atopy (including only the diagnoses made by a physician), nor the maternal history of asthma or atopy were predictive for later childhood asthma (Table 19). The history of an earlier physician-diagnosed episode of wheezing, atopic dermatitis in infancy, and total serum IgE of ≥60 kU/l were significantly predictive for later asthma.

Table 19. Baseline data on the family history of asthma and atopy and the personal history of wheezing and atopy at the index episode of wheezing, with regard to asthma at early school age.

	Asthma				
Baseline data	**Present (n=33)**	**Not present (n=49)**	**p**	**OR**	**95% CI**
Family history of asthma[a]	10 (30%)	9 (18%)	0.136	2.34	0.76-7.17
Maternal history of asthma[a]	3 (9%)	3 (6%)	0.613	1.58	0.27-9.32
Family history of atopy[a]	21 (64%)	25 (51%)	0.209	1.83	0.71-4.68
Maternal history of atopy[a]	10 (30%)	17 (35%)	0.498	0.71	0.26-1.92
An earlier episode of wheezing in infancy[a]	8 (24%)	3 (6%)	0.047	4.29	1.02-17.99
Atopic dermatitis	15 (45%)	8 (16%)	0.009	4.11	1.42-11.84
Total serum IgE ≥60 kU/l[b]	11 (33%)	6 (12%)	0.045	3.41	1.03-11.33

[a] Physician-diagnosed.

[b] The samples were obtained for 78 children; asthma was present in 30 of them.

p-values, ORs and 95% CIs were determined by logistic regression adjusted for sex and age (<12 or ≥12 months) on entry to the study. Analyses have been done separately for each factor.

5.6.3. The role of early sensitisation to allergens (I, II)

As presented in Table 20, specific IgE of ≥0.35 kU/l to the mixture of inhalant allergens, but not to the mixture of food allergens, was significantly associated with school-age asthma. However, among individual food allergens, specific IgE of ≥0.35 kU/l to egg white and to wheat were associated with later asthma. In contrast, cases with cow´s milk-specific IgE of ≥0.35 kU/l were almost equally common in both later asthmatics and non-asthmatics.

When a cut-off concentration of 0.70 kU/l was applied, specific IgE to the mixture of food allergens associated significantly with school-age asthma (food-specific IgE of ≥0.70 kU/l in infancy in 18 (56%) of the 32 children regarded as asthmatics at early school age) independently of age and sex (p=0.031; OR 2.97; 95% CI 1.10-7.97). Further, at the level of 3.50 kU/l (food-specific IgE of ≥3.50 kU/l in infancy in 10 (31%) of the 32 children regarded as asthmatics at early school age), the respective OR was even higher, 4.23 (95% CI 1.16-15.50; p=0.029). For cow's milk-specific IgE even the higher cut-off concentrations of 0.70 to 3.50 kU/l were not predictive of asthma (data not shown). Thus, specific IgE to cow's milk did not predict later asthma at any level.

Table 20. Allergen-specific IgE antibodies in infancy with regard to asthma at early school age.

	Asthma					
	Present	Not present				
Specific IgE of ≥0.35 kU/l to	(n=32)	(n=48)	LR+	p	OR	95% CI
Food allergens	20 (63%)	17 (35%)	1.76	0.075	2.48	0.91-6.74
Egg white	18 (56%)	13 (27%)	2.08	0.048	2.85	1.01-8.02
Cow's milk	12 (38%)	10 (21%)	1.80	0.253	1.85	0.65-5.28
Wheat	9 (28%)	3 (6%)	4.50	0.030	4.93	1.16-20.89
Peanut	8 (25%)	6 (13%)	2.00	0.471	1.61	0.44-5.85
Inhalant allergens	11 (34%)	3 (6%)	5.50	0.006	9.75	1.91-49.71
Cat	5 (16%)	2 (4%)	3.75	0.257	2.94	0.46-18.90
Dog	5 (16%)	3 (6%)	2.50	0.522	1.74	0.32-9.49

p-values, ORs and 95% CIs were determined by logistic regression with adjustment for sex and age (<12 or ≥12 months) on entry. Analyses have been performed separately for each factor. LR+ values were calculated as LR+ = sensitivity/(1-specificity). Allergens with <5 positive cases were not included in analyses.

Sensitisation to individual inhalant allergens was rather rare, and no significant association was seen between asthma and specific IgE to any individual inhalant allergen at any concentration level.

When evaluated as an asthma-predicting marker, specific IgE of ≥0.35 kU/l to the mixture of food allergens had a moderate sensitivity (63%) and specificity (65%), whereas specific IgE of ≥0.35 kU/l to the mixture of inhalant allergens was less sensitive (34%) but very specific (94%). The LR+ values were 1.76 and 5.50, respectively (Table 20). Among individual allergens, specific IgE of ≥0.35 kU/l to wheat had the highest specificity (94%), with a LR+ of 4.50.

ROC-analysis revealed no substantially better cut-off concentrations for the mixture of inhalant allergens, or for any individual inhalant or food allergen (data not shown) than the detection concentration. However, for the mixture of food allergens, the optimal cut-off concentration of specific IgE was higher, close to 0.70 kU/l (LR+ 2.08).

In addition, specific IgE combined with other atopic manifestations was evaluated as an asthma-predictive marker. In the present cohort, atopic dermatitis was the only clinically evident atopic manifestation on admission in infancy. It was found that the combination of atopic dermatitis and food-specific IgE of ≥0.70 kU/l gave the highest LR+, 5.00 (unpublished data) (p=0.014) (Table 21). Since specific IgE to the mixture of inhalant allergens was rarely detectable in infancy, and, whenever present, highly predictive for asthma at the detection level, any combinations with atopic dermatitis or use of other than

detection concentrations in analyses gave no additional advantages (data not shown).

Table 21. Specific IgE to food allergens, combined with the presence of atopic dermatitis in infancy, with regard to asthma at early school age.

	Asthma					
	Present	Not present				
Combination	**(n=32)**	**(n=48)**	**LR+**	**p**	**OR**	**95% CI**
Specific IgE to food allergens						
of ≥0.35 kU/l and atopic dermatitis	12 (38%)	3 (6%)	2.00	0.004	8.13	1.97-33.54
of ≥0.35 kU/l or atopic dermatitis	23 (72%)	22 (46%)	1.57	0.070	2.53	0.93-6.87
Specific IgE to food allergens						
of ≥0.70 kU/l and atopic dermatitis	10 (31%)	3 (6%)	5.00	0.014	6.15	1.45-26.03
of ≥0.70 kU/l or atopic dermatitis	23 (72%)	18 (38%)	1.92	0.009	3.79	1.40-10.24

p-values, ORs and 95% CIs were determined by logistic regression with adjustment for sex and age (<12 or ≥12 months) on entry. Analyses have been done separately for each factor.
LR+ values were calculated as LR+ = sensitivity/(1-specificity).

In addition, positive SPT reactions in early childhood were significantly associated with early school-age asthma (Table 22).

Table 22. Skin test reactivity 8 months after the index episode of wheezing with regard to asthma at early school age.

	Asthma					
	Present	Not present				
Positive SPT reactions to	**(n=31)**	**(n=46)**	**LR+**	**p**	**OR**	**95% CI**
Food allergens	8 (26%)	2 (4%)	5.94	0.012	7.65	1.50-39.04
Inhalant allergens	14 (45%)	6 (13%)	3.46	0.002	5.49	1.81-16.69
Indoor allergens	11 (35%)	6 (13%)	2.72	0.020	3.67	1.18-11.35
Outdoor allergens	5 (16%)	1 (2%)	7.42	0.036	8.65	0.96-78.15
Food or inhalant allergens	15 (48%)	7 (15%)	3.18	0.002	5.22	1.79-15.22

p-values were determined by the chi-squared test or Fisher's exact test, supplemented by ORs and 95% CIs.
LR+ values were calculated as LR+ = sensitivity/(1-specificity).

With regard to the subsequent development of asthma, any positive SPT reactions to food allergens (in 13% of the children) during the first 3 years of life gave a LR+ of 5.94, and any positive SPT reactions to inhalant allergens (in

26% of the children) during the first 3 years of life gave a LR+ of 3.46 (unpublished data).

5.6.4. The role of eosinophil activation during the index episode of wheezing (I)

Blood eosinophil count of ≥0.45 x10^9/l was predictive for later asthma (Table 23). However, serum ECP of ≥16 μg/l was not predictive for early school age asthma.

Table 23. Baseline data on eosinophil activation at the index episode of wheezing with regard to asthma at early school age.

	Asthma					
Baseline data	**Present**	**Not present**	**LR+**	**p**	**OR**	**95% CI**
Blood eosinophil count of ≥0.45 x10^9/l	18/31 (58%)	8/48 (17%)	3.48	0.0008	7.02	2.26-21.79
Serum ECP of ≥16 μg/l	9/32 (28%)	6/49 (12%)	2.30	0.1739	2.35	0.69-8.05

p-values, ORs and 95% CIs were determined by logistic regression adjusted for sex and age (<12 or ≥12 months) on entry to the study. Analyses have been done separately for each factor.
LR+ values were calculated as LR+ = sensitivity/(1-specificity).

5.6.5. The role of viral findings related to the index episode of wheezing (III, V)

Table 24 shows the occurrence of asthma at early school age in children with and without RSV infection on hospital admission for wheezing. Hospitalisation for RSV infection was associated with an 8% to 50% risk for early school-age asthma, depending on age on admission. However, hospitalisation with no evidence of RSV was associated with a higher risk, 45% to 57%. After adjustment for atopy (atopic dermatitis and/or elevated total serum IgE) and eosinophilia on admission, asthma was significantly less common in children with RSV infection on admission compared to those without. That concerned the whole study group, as well as the subgroup of 1 to 11 month-old children.

Table 24. Occurrence of school-age asthma in children hospitalised for wheezing with or without RSV.

Hospital admission for wheezing at the age of		Asthma at early school age in children with RSV[a] on admission	without RSV[b] on admission	Adjusted[c] p
1-5 months	(n=20)	1/13	4/7	0.054
6-11 months	(n=30)	2/8	10/22	0.324
12-23 months	(n=32)	1/2	15/30	0.944
1-11 months	(n=50)	3/21	14/29	0.049
1-23 months	(n=82)	4/23	29/59	0.034

[a] RSV identified by antigen detection, CF assay or RT-PCR.

[b] RSV not identified by antigen detection, CF assay or RT-PCR.

[c] Analyses were performed by logistic regression analysis with adjustment for atopic dermatitis, total serum IgE of ≥60 kU/l and the blood eosinophil count of ≥0.45 x10^9/l on admission (as a combined variable).

The table includes data of the 82 children who took part in the follow-up visit at early school age.

When the RSV-specific IgG antibody concentrations were analysed in relation to later asthma, no association was found between RSV-specific IgG antibody concentrations and asthma in any age group (Table 25).

Table 25. Age on seroconversion for RSV and later occurrence of asthma.

First positive specific IgG measurement for RSV[a] at the age of		Asthma present at early school age (n=25) In each age group	Cumulative frequency
1-5 months	(n=5)	1 (4%)	1 (4%)
6-11 months	(n=10)	2 (8%)	3 (12%)
12-23 months	(n=23)	12 (48%)	15 (60%)
≥24 months	(n=21)	10 (40%)	25 (100%)

[a] RSV-specific IgG of ≥10 U/ml.

There were 4 children with RSV-specific IgG of <10 U/ml in every measurement. There were no statistically significant differences between later childhood asthmatics and non-asthmatics in appearance of RSV-specific IgG antibodies in early childood.

The analyses were performed by logistic regression with adjustment for atopic dermatitis, total serum IgE of ≥60 kU/l and the blood eosinophil count of ≥0.45 x10^9/l on admission (as a combined variable).

The table includes data of the 63 children who had complete IgG data available and who had taken part in the follow-up visit at early school age.

Among the 66 children with frozen NPAs and follow-up data available, rhinoviruses were identified in 25 (38%), and enteroviruses in 9 (14%) children. Asthma was considered to be present in 27 (41%) of the 66 children at early school age (Table 26).

Table 26. Rhino- and enterovirus findings related to the index episode of wheezing with regard to the presence of asthma at early school age.

	Asthma	
Viral findings related to the index episode of wheezing	**Present (n=27)**	**Not present (n=39)**
Rhinovirus	15 (56%)	10 (26%)
as a single viral finding	14 (52%)[#]	6 (15%)
Enterovirus	2 (7%)	7 (18%)
as a single viral finding	0 (0%)	5 (13%)

[#] p=0.047; LR+ 3.37; OR 4.14; 95% CI 1.02-16.77 vs. rhinovirus-negative cases, determined by logistic regression with adjustment for age (<12 or ≥12 months), sex and atopic dermatitis on entry to the study.

Asthma was present in 15 (60%) of the rhinovirus positive and in 2 (22%) of the enterovirus positive cases (Table 26). As single viral identifications, rhinoviruses were associated with early school-age asthma, independently of age, sex and atopic dermatitis on entry to the study. If the mixed rhinovirus infections were also included in the analyses, the risk for asthma remained increased (OR 2.29) but the statistical significance was lost. In contrast, enteroviruses had no association with later asthma. In total, rhinoviruses were identified in frozen NPAs in over half (56%) of the later asthmatics. The figure varied from 25% to 75% in the four different age groups, from the youngest (1-5 months) to the oldest (18-23) group, respectively.

5.6.6. The role of environmental factors in infancy (I)

Tobacco smoke exposure and pet contacts in early childhood are evaluated as predictive factors for asthma in Table 27. No association was found with exposure to smoke or with early pet contacts and later childhood asthma.

Table 27. Baseline data on tobacco smoke exposure and furry pet contacts in infancy with regard to asthma at early school age.

	Asthma					
Baseline data	**Present (n=33)**	**Not present (n=49)**	**LR+**	**p**	**OR**	**95% CI**
Passive smoking during infancy	16 (48%)	23 (47%)	1.03	0.60	1.28	0.51-3.25
Maternal smoking during pregnancy	9 (27%)	11 (22%)	1.21	0.48	1.46	0.51-4.19
A furry pet at home/day care during infancy	8 (24%)	17 (35%)	0.70	0.36	0.62	0.22-1.72

p-values, ORs and 95% CIs were determined by logistic regression adjusted for sex and age (<12 or ≥12 months) on entry to the study. Analyses have been done separately for each factor.
LR+ values were calculated as LR+ = sensitivity/(1-specificity).

5.7. Specific IgE antibodies in infancy and allergic manifestations at early school age (II)

A significant association was found between allergic rhinitis and specific IgE of ≥0.35 kU/l to the mixture of inhalant allergens (p=0.003; OR 12.40, 95% CI 2.35-65.42), and specific IgE of ≥0.35 kU/l to the mixture of food allergens (p<0.001; OR 7.29, 95% CI 2.34-22.71) in infancy. However, no such association was found with the later occurrence of atopic dermatitis (data not shown). When specific IgE in infancy was compared with SPT reactivity at school age, all 14 children with specific IgE to inhalant allergens (p<0.001), and 26 (70%) of those 37 with specific IgE to food allergens (p=0.002) had positive SPT results. On the other hand, there were 9 (24%) SPT positive children with specific IgE neither to food nor to inhalant allergens in infancy.

6. DISCUSSION

6.1. Design of the study

This clinical study was a prospective follow-up of children with wheezing severe enough to require hospitalisation in infancy, at the age of <2 years. The recruitment period lasted for almost two years, and most of the children were enrolled between the two RSV outbreaks. The follow-up lasted for median 6.3 years, continuing up to early school age, median 7.2 years of age. Despite the long follow-up, the coverage of the study was good - the proportion of drop-outs was only 18%, and the basic characteristics of the drop-outs did not differ from those of the participating children. In fact, such long-term follow-ups with similar study designs are rare, comparable only with two earlier cohorts (Korppi *et al.* 1994, Wennergren *et al.* 1997).

The study design did not include healthy non-wheezing controls. However, when focused on predictive factors within the study group, the analyses do not necessitate a control group (Wennergren *et al.* 1997). On the other hand, the lack of a control group can be regarded as a shortcoming when such figures like prevalence of asthma are considered, as those figures have to be compared with the figures estimated in population-based studies (Wennergren *et al.* 1997). However, there are two recent studies on the prevalence of asthma at school age in the non-selected populations of our area (Timonen *et al.* 1995, Remes *et al.* 1996), and as the criteria for asthma in the present study were, with minor modifications, similar to the criteria applied in those population-based studies, the figures of prevalence are mutually comparable.

The criteria for the presence of asthma were rather strict and based on the clinical evaluation; the patients either had to have both recurrent subjective symptoms suggestive of asthma and an objective, experimental evidence of asthma, or they had to be on continuous maintenance medication for asthma. However, the definition of paediatric asthma and its severity, in particular, varies between different countries and even between different centres in the same country, leading to varying diagnostic criteria in different studies (Samet *et al.* 2001).

As the study cohort represents a selected, hospitalised child population, the conclusions cannot be generalized to concern all wheezing children, only the most severe cases. However, among wheezing children, those who need

hospital admission are at greatest risk for subsequent wheezing respiratory tract illnesses (Sly and Hibbert 1989, Noble *et al.* 1997, Sigurs *et al.* 2000 and 2005, Piippo-Savolainen *et al.* 2004). Consequently, identification of those at-risk children who might benefit from therapeutic or other preventive measures is important.

6.2. Viral findings in relation to previous studies

In most of the earlier prospective follow-up studies on wheezing requiring hospitalisation in infancy, the study subjects have been recruited only during RSV epidemics, and consequently, in those studies, the occurrence of RSV-positive findings have been clearly higher, 63-100% (Webb *et al.* 1985, Welliver *et al.* 1986, Sly and Hibbert 1989, Sigurs *et al.* 1995), than that of 29% in the present study. On the other hand, in studies with a recruitment period of at least 12 months or more, the RSV-positivity rate has been lower, 29-53% (Korppi *et al.* 1993, Wennergren *et al.* 1992, Papadopoulos *et al.* 2002), resembling the respective figure of the present study.

The rhinovirus-positivity rate was up to 33% in the present study. The finding is in accordance with the detection rates of 20-32% in infants and toddlers hospitalised for wheezing illnesses (Andréoletti *et al.* 2000, Papadopoulos *et al.* 2002, Heymann *et al.* 2004). Enteroviruses were found in 12% of wheezing hospitalised infants in the present study, consistent with similar findings by Andréoletti *et al.* (2000). Coronaviruses were identified in none of the study children, consistent with 0-5% detection rates in wheezing hospitalised infants and toddlers published earlier (Andréoletti *et al.* 2000, Papadopoulos *et al.* 2002, Heymann *et al.* 2004). Human metapneumovirus was detected in only 1 child (unpublished finding), supporting the earlier findings that the newly defined virus causes only a minority of hospitalisations in wheezing infants and toddlers (Jartti *et al.* 2004b, Jennings *et al.* 2004).

In addition to the length of the recruitment period, the viral detection rates are dependent on age of the study subjects. RSV is the most common viral finding in children under the age of 6 to 12 months (Webb *et al.* 1985, Welliver *et al.* 1986, Sly and Hibbert 1989, Sigurs *et al.* 1995), whereas rhinovirus becomes more prevalent as a triggerer of wheezing in older age groups (Rakes *et al.* 1999, Heymann *et al.* 2004). Consistent with earlier findings, in the present

study, RSV was the most common viral finding in children younger than 6 months on admission, and rhinovirus most prevalent from 6 to 12 months' age on.

The data published on subclinical RSV infections and/or appearance of RSV-specific IgG antibodies in young children are not abundant. In the present study, 35% of the RSV bronchiolitis cases diagnosed by antigen, RT-PCR or CF assays had no detectable RSV-specific IgG antibodies 6 weeks later, and over half of them were younger than 6 months. The result is, however, consistent with earlier findings, as it is known that the presence of maternal antibodies affects infants' ability to develop an immune response to RSV (Meurman *et al.* 1992), and a substantial proportion of hospitalised infants experiencing their first RSV infection do not show a rise in antibody titres (Cox *et al.* 1998), as antibody titres are age-dependent, showing marked increase after 6 to 9 months of age (Harsten *et al.* 1989, Cox *et al.* 1998). Consequently, lack of antibodies is not always an indication of no previous exposure (Cox *et al.* 1998).

The finding that approximately 85% of all children were seropositive for RSV by 3 years of age is in line with earlier studies (Harsten *et al.* 1989, Cox *et al.* 1998). However, some of the early RSV infections (not inducing IgG antibodies in significant amounts) may have been missed in the present study since one third of children were recruited into the study during their second year of life (Reijonen 1997a).

Against the earlier findings by Papadopoulos *et al.* (2002), RSV and rhinoviruses were found in different patients in the present study cohort since there was only one infant with RSV-rhinovirus co-infection. That allowed the comparison of the clinical characteristics of RSV bronchiolitis and rhinovirus infection-associated wheezing. In contrast with the findings by Papadopoulos *et al.* (2002), rhinovirus infection was not associated with increased severity of the disease: rhinovirus-positive infants had higher oxygen saturation values on admission, and there were no significant differences in the clinical scores regarding the severity of the wheezing illness or the response to the emergency treatment. However, the rhinovirus-positive patients were older than those with RSV infection, and their disease was associated with clinical atopy, and eosinophil activation during infection. Consequently, the results of the comparison suggest that rhinovirus-associated wheezing and RSV bronchiolitis,

though having rather similar clinical characteristics, may have different pathogenetic bases.

6.3. Outcome of wheezing requiring hospital admission in infancy in comparison with earlier studies

After wheezing requiring hospital admission in infancy, the school-age prevalence of asthma has most commonly varied between 15% to 39% (Korppi *et al.* 1994, Noble *et al.* 1997, Wennergren *et al.* 1997, Sigurs *et al.* 2000 and 2005), and the prevalence of atopic manifestations from 11% to 45% (Murray *et al.* 1992, Wennergren *et al.* 1997, Sigurs *et al.* 2000 and 2005). In the present study, asthma was present in 40% of the children, and atopic manifestations or findings in 30% to 49% of the children at early school age. The occurrence of asthma and atopy in the present study are consistent with that of earlier studies, when taken into account the fact that the children in the present study were a bit younger at the follow-up than the schoolchildren in the other long-term studies. However, even the youngest children of the present study were nearly 6 years of age during the follow-up visit, and at that age, recurrent wheezing can be regarded as real asthma, not anymore as a transient respiratory disorder (Martinez *et al.* 1995, Holgate 1997).

However, when the occurrence of asthma and atopy in the present follow-up study are compared with the prevalence of asthma and atopy in Finnish population based-studies, the occurrence of asthma can be seen as 8- to 10-fold (Timonen *et al.* 1995, Remes *et al.* 1996), and the occurrence of atopic manifestations as 1.5- to 3.5-fold (ISAAC Steering Committee 1998).

6.4. Prediction of outcome of wheezing requiring hospital admission in comparison with earlier studies

6.4.1. The role of recurrence of wheezing, the family history of asthma and atopy, and the personal atopic manifestations and findings in infancy

In the present study, several characteristics, markers and environmental factors present in early childhood were evaluated as possible predictive factors for the outcome. Consistent with findings of Wennergren *et al.* (1997), recurrent

wheezing in infancy predicted development of asthma, and by early school age, most asthmatic children had developed atopic manifestations or findings. In addition, personal atopy in infancy, like the presence of atopic dermatitis or elevated serum total IgE, was found to predict early school-age asthma in the present cohort, in accordance with earlier findings (Korppi *et al.* 1994, Martinez *et al.* 1995). However, in contrast with earlier findings (Mrazek *et al.* 1999, Sigurs *et al.* 2005), neither the family history of asthma nor the family history of atopy were predictive of asthma in the present follow-up. Power calculations, which were performed retrospectively for non-significant results, implied that the power of the study was too low, 20-24%, to show the possible predictive effects of the family history of atopy or asthma.

6.4.2. The role of early sensitisation to allergens by specific IgE

Early sensitisation to food allergens determined by specific serum IgE antibodies, was common, present in 46% of infants, and thus predictive for asthma only when elevated levels of specific IgE antibodies were seen. In contrast, the presence of inhalant allergen-specific IgE antibodies was much more infrequent, found in 18% of infants of whom almost all were aged ≥12 months, but highly predictive of asthma even at the detection level, as also suggested by Wever-Hess *et al.* (1999) and Martinez and Godfrey (2003). In addition, in the present study, specific IgE antibodies to both food and inhalant allergens in infancy significantly predicted allergic rhinitis and SPT reactivity.

Sensitisation to individual allergens by specific IgE was only predictive with regard to egg white and wheat, not for instance to cow's milk, although specific IgE antibodies were most commonly seen against egg white (39%) and cow's milk (28%). Yunginger *et al.* (2000) have stressed the role of egg-specific IgE as a predictive factor in the development of inhalant allergies. On the other hand, in food allergy, confirmed by elimination-challenge tests, the diagnostic concentrations of specific IgE have varied from 6 kU/l for egg white to 32 kU/l for cow's milk (Sampson and Ho 1997), and thus, low to moderate IgE rises to cow's milk might be found in infancy even without clinical consequences (Kulig *et al.* 1999).

Consequently, IgE responses to food allergens may rather reflect the atopic reactivity in general than specific contribution to the development of atopic symptoms or later asthma. Moreover, the clinically significant concentrations for

specific IgE seem to depend on the tested allergens, the age of the child, and the clinical questions in consideration (Sampson and Ho 1997). Nevertheless, allergen-specific IgE antibodies may be of help in discovering subclinical atopy in children.

In all, allergen-specific IgE antibodies were found more frequently in the present study than in earlier studies where food-specific IgE antibodies have been present in 4-20% of wheezing or asthmatic infants (Price *et al.* 1995, Wever-Hess *et al.* 1999, Camara *et al.* 2004), and inhalant allergen-specific IgE antibodies in <5-10% of wheezing infants (Duff *et al.* 1993, Delacourt *et al.* 1994, Rakes *et al.* 1999, Camara *et al.* 2004). In addition, the occurrence of egg- and cow's milk-specific IgE resembles the respective findings in children with atopic dermatitis (positivity rate 30-33%) (ETAC Study Group 1998) rather than the figures in asthmatic children (positivity rate 2-11%) (Tridon *et al.* 1999) or children at risk for atopy (positivity rate 4-14%) (Sigurs *et al.* 1994, Bruno *et al.* 1995).

In addition to serum specific IgE antibodies, SPT positivity indicates IgE-mediated responsiveness to allergens. In the present study, SPT positivity to food allergens was present in 13% of the wheezing children during the first 3 years of life, and to inhalant allergens in 26% of the children. SPT positivity to any of the tested allergens was present in 29% of the children. These figures are close to the findings of an Italian study group (Silvestri *et al.* 1996), but clearly lower than the figures in asthmatic children presented in a Turkish study (Emin *et al.* 2004). In contrast to aforementioned studies made in Mediterranean climate, sensitisation to house dust mites is rare in Nordic countries (Sigurs *et al.* 1994). In agreement with the findings of a Dutch study group, (Delacourt *et al.* 1994), SPT positivity to inhalant allergens in early childhood was highly predictive for later childhood asthma also in the present study.

6.4.3. The role of viral findings and eosinophil activation during wheezing respiratory tract illnesses

In previous follow-up studies with non-wheezing controls, RSV bronchiolitis has been found to be predictive for persistent wheezing and development of asthma up to teenage (Sigurs *et al.* 2000 and 2005). However, when viral aetiology of wheeze has been compared within the study cohorts, RSV has had

a minor role as a predictor of school-age asthma (Korppi *et al.* 1994, Wennergren *et al.* 1997). Consistent with these earlier studies, the later occurrence of asthma in RSV-positive wheezing infants was 3- to 4-fold when compared to the prevalence of asthma in non-selected schoolchildren (Timonen *et al.* 1995, Remes *et al.* 1996), but within the study cohort, RSV infection did not further increase the risk of development of asthma. In addition, in accordance with findings of Sigurs *et al.* (1995), the presence of RSV-specific IgG antibodies in early childhood was not predictive of persistent wheezing or the development of asthma.

In contrast, rhinoviral aetiology for wheezing was predictive of later childhood asthma, even if age, gender and the presence of atopic dermatitis were taken into account. Due to limitations of rhinovirus detection methods, there are virtually no earlier follow-up data on the role of rhinoviruses as predictors of persistent wheezing or asthma in wheezing infants. However, asthma exacerbations in children are often triggered by rhinovirus infections (Freymuth *et al.* 1999, Rawlinson *et al.* 2003), suggesting that rhinoviruses mostly induce wheezing symptoms in susceptible subjects, and thus identification of rhinoviruses may imply the presence of asthmatic inflammation in bronchi even in infants. Consequently, at least in atopic children, early childhood wheezing may actually be the first sign of asthma (Martinez *et al.* 1995).

During viral respiratory infections, blood eosinopenia is considered as a normal response (Garofalo *et al.* 1994, Martinez *et al.* 1998). In the present study, blood eosinophilia during infection-associated wheezing was found to be highly predictive for later childhood asthma, consistent with earlier findings stressing the role of eosinophilia in the recurrence of wheezing (Martinez *et al.* 1998, Ehlenfield *et al.* 2000). However, in contrast with the findings by Pifferi *et al.* (2001), elevated levels of serum ECP during the episode of wheezing requiring hospital admission were not predictive of persistent wheezing or asthma in the long run, maybe due to the non-specific nature of the serum ECP as a marker of inflammation (Azevedo *et al.* 2001).

6.4.4. The role of environmental factors

Smoking was as common in families of later asthmatic as in families of later non-asthmatic children. In disagreement with previous findings (Wennergren *et al.* 1997, Csonka *et al.* 2000), passive smoking or maternal smoking during

pregnancy did not significantly predict later childhood asthma. The non-significant findings of smoke exposure in the present study are probably due to a highly selected, relatively small cohort, not allowing other than atopy-related risk factors to play a significant role in predicting asthma. In addition, though parental smoking was common, nearly 50%, in the present study, smoking indoors is rare in Finland nowadays, particularly in families with young children (Timonen *et al.* 1995). That may lessen the straight exposure to tobacco smoke, and may partly explain the non-significant results.

Furry pet contacts in infancy did not lead to sensitisation to animal dander very often. In fact, there seemed to be a trend toward a lower risk for development of asthma in children with pet contacts in early childhood. Although the finding was not statistically significant, it supports the current concept (Hesselmar *et al.* 1999), that pet contacts in infancy are not necessarily harmful even for children at risk. If immunologic tolerance develops instead of atopic sensitisation, such contacts may even be asthma-preventing, as was seen in children with dog exposure in early life in a recent study (Remes *et al.* 2001). On the other hand, atopic families are often advised not to keep furry pets at home, which may have biased the results, leading to underestimation of the role of pet contacts (Hesselmar *et al.* 1999, Reijonen *et al.* 2000).

6.5. Methodological aspects

6.5.1. Strengths of the study design

In the present study, versatile clinical and laboratory data related with allergy were carefully registered on admission. The clinical evaluation during acute-phase of the study was prospective, took place shortly after hospitalisation (often outside office hours), and was performed by study doctors especially trained to assess respiratory problems in young children. The doctors registered objective measurements like respiratory rate and oxygen saturation (Reijonen *et al.* 1995), and for the assessment of wheezing and retractions, a standardized RDAI scoring was applied (Lowell *et al.* 1987). In paired comparisons, the study doctors' ratings did not differ for any patient more than 1-2 units in the 17-point scale, the kappa values indicating an excellent agreement (Reijonen *et al.* 1995).

The viral studies were extensive; seven conventional respiratory viruses had been studied already on entry to the study in infancy (Reijonen *et al.* 1997c), and the findings were later supplemented by rhino-, entero- and coronavirus studies. RT-PCR used in the present study has been shown to be more sensitive than conventional virus isolation for rhino- and enteroviruses (Hyypiä *et al.* 1998, Lönnrot *et al.* 1999). In a study by Lönnrot *et al.* (1999), 30 most common enterovirus serotypes among 64 known human serotypes were tested and they all gave a positive signal by RT-PCR. Nine known rhinovirus serotypes, among 101 known serotypes, and 20 clinical isolates of unknown serotypes were tested and the RT-PCR assay amplified all tested rhinoviruses.

Since rhinoviruses were rarely involved in mixed viral infections, it was possible to study their specific effect on the outcome of the children.

The in-house RT-PCR for RSV was, and TR-FIA is known to be, a sensitive and specific method for RSV infection in young children (McIntosh *et al.* 1993). In addition, the present study is one of the few studies where RT-PCR has been used in diagnosing RSV infection in addition to conventional methods (Paton *et al.* 1992). When compared to antigen detection and virus isolation in culture, the sensitivity and the specificity of RT-PCR have been over 90% (Paton *et al.* 1992). The 83% to 100% sensitivity and 92% specificity, found in the present study, mean a likelihood ratio of >10 for a positive test result, which has significant value in diagnostic decision making (Sackett *et al.* 1998).

An automated *in vitro* screening method, UniCAP FEIA, which allows detection of allergen-specific serum IgE antibodies quantitatively, was applied in the present study. The accuracy of the UniCAP assay has been good compared with earlier, non-automated laboratory methods like the CAP System RAST (Paganelli *et al.* 1998). The storage of serum samples as frozen for several years, as was done in the present study, has not significantly interfered with the results (Paganelli *et al.* 1998). The detection concentration for specific IgE, 0.35 kU/l, has been used as a threshold for a positive result by several other study groups (Sigurs *et al.* 1994, ETAC Study group 1998, Tridon *et al.* 1999). Other investigators have preferred higher cut-off concentrations, like 0.70 kU/l (Kulig *et al.* 1999, Lau *et al.* 2000, Camara *et al.* 2004). However, the different cut-off concentrations for specific IgE have rarely been evaluated as predictive factors for atopic disease (Sampson and Ho 1997). In the present study, optimal cut-off concentrations for specific IgE were evaluated by ROC

analyses; the analyses preferred no other cut-off concentrations than the detection level for any allergen-specific IgE.

A large panel of specific IgE antibodies to several common allergens, including 6 individual food and 8 inhalant allergens, was investigated as a predictor of asthma. Such large panels of allergens in combination with automated quantitative methods for determining specific IgE antibodies have rarely been available. In addition, SPTs against several food and inhalant allergens were performed in early childhood.

6.5.2. Shortcomings of the study design

RT-PCR studies were performed 6 to 8 years after the acute episode, and therefore, good-quality frozen NPA specimens obtained on entry into the study were available only for 60% to 80% of the children. On the other hand, antibody and antigen assays for RSV were performed in all cases.

RSV-specific IgG antibodies were measured only in convalescent and follow-up sera, and only a limited number of good-quality frozen nasopharyngeal and serum samples were available for later viral studies by PCR and the immune assay. An optimal design would consist of viral studies in nasopharyngeal samples and in paired sera at all infection episodes during the follow-up time.

We did not have a control group with no respiratory infection and/or with respiratory infection with no wheezing. In asymptomatic children with no preceding or concurrent respiratory symptoms, rhinoviruses have been detected in 12-13% of the NPAs by RT-PCR (Johnston *et al.* 1993, Nokso-Koivisto *et al.* 2002). The overall detection rate of rhino-, entero- and coronaviruses by RT-PCR has been low, 5%, if no preceding, concurrent, or following respiratory symptoms have been reported (Nokso-Koivisto *et al.* 2002). In the present study, the viral detection rate by RT-PCR in wheezing children was over 8-fold, 44% altogether.

Due to emergency medication during the index hospitalisation for wheezing, SPTs could not have been performed on entry, and the data on SPT positivity were obtained for the first time at 8 months after the hospital admission for wheezing. Therefore the analyses comparing specific IgE and SPTs related to the index episode of wheezing were not possible to perform.

The used exercise challenge test was positive only in one third of asthmatics. At the age of 5 to 8 years, multiple FVS measurements carry a risk of bad

compliance resulting in technically unacceptable measurements. To avoid that, FVS was performed only once after the exercise. However, FVS, when the adequacy of flow-volume curve can be checked, gives more reliable information than e.g. peak expiratory flow and pocket-sized FEV_1 meters. In addition, wheezing on auscultation after the exercise was also regarded as an objective evidence of asthma. In the present study, the exercise challenge was the only challenge test performed, which can be considered as a shortcoming as well. On the other hand, the exercise challenge test is commonly used, quite easy to perform, and mimics the every-day activities of children, and as a diagnostic approach for asthma, it is known to be a very specific test (Carlsen 2001).

In addition, inclusion of children on maintenance therapy carries the bias that children cured of their asthma have been registered as asthma cases. In most children with asthma in the present study, the ongoing anti-inflammatory medication was effective enough to control bronchial inflammation and hyperresponsiveness. Nevertheless, more than 80% of children on maintenance were still symptomatic, having suffered from repeated wheezing or prolonged cough during the preceding 12 months, implying active asthma. In addition, the asthma-treatment policy of our hospital was to check the need of maintenance medication twice a year in young children (Korhonen *et al.* 1999). Thus, there is a high probability that all asthma cases in the present study were real, though possibly mild, asthmatics.

Among the non-asthmatic children, 12 children had been symptomatic during the preceding year. However, neither including these 12 children in the asthma group for analyses, nor excluding them from analyses, changed the main results of the present study. In addition, if a lower limit, a 10% decrease in FEV_1, was applied instead of a 15% decrease, for a positive exercise challenge test result (Carlsen 2001), no more than 1 of these 12 children would have been regarded as asthmatic. Thus, the results of the alternative analyses did not support abandoning the used criteria for the presence of asthma, which have been approved earlier in population-based studies in our area (Timonen *et al.* 1995, Remes *et al.* 1996).

7. SUMMARY AND CONCLUSIONS

In the present study, covering a 22-month period between two RSV epidemics from the beginning of 1992 to the end of 1993, 29% of all hospitalised wheezing infants had RSV infection. An additional 27% of the children were regarded as having been infected with RSV before that hospital admission, as demonstrated by RSV-specific IgG antibody measurements. By the age of 3 years, 85% of the study subjects showed seroconversion to RSV. The concentrations of RSV-specific IgG antibodies were related to age, but were not predictive of school-age asthma. RSV infection on admission did not increase the risk of asthma over the risk attributed to hospitalisation for wheezing.

In addition to RSV, rhinoviruses were commonly found to induce wheezing even in children under the age of 2 years. Especially the infants with atopic dermatitis or blood eosinophilia were likely to wheeze during rhinovirus infection. Rhinovirus-induced wheezing leading to hospitalisation seemed to predict the development of asthma. The association with atopy suggests that there actually might be active asthma-like bronchial inflammation even in infants wheezing during rhinovirus infection.

Specific IgE antibodies to food allergens were found to be common, and specific IgE to inhalant allergens rather rare in infants hospitalised for wheezing. Specific IgE antibodies to the mixture of inhalant allergens, to egg white, and to wheat, were found to be predictive of later asthma even at the detection level. Detection of specific IgE antibodies in wheezing infants may, thus, facilitate the early diagnosis of asthma, especially in cases with no clinically evident atopic manifestations. Specific IgE to the mixture of food allergens, however, was not associated with school-age asthma at the detection level of 0.35 kU/l, whereas concentrations of $\geq$0.70 kU/l were predictive.

In addition, recurrent wheezing in infancy, presence of atopic dermatitis or elevated levels of total serum IgE in infancy, blood eosinophilia during infection-associated wheezing, and SPT positivity to common food or inhalant allergens in early childhood were also found to predict later childhood asthma in infants requiring hospitalisation for wheezing.

In conclusion, when hospitalisation for wheezing occurs in infancy, more than every third child will suffer from asthma at early school age. In accordance with algorithms presented earlier, recurrence of wheezing in early childhood, atopic dermatitis in infancy, and blood eosinophilia imply an increased risk for later childhood and school-age asthma. In addition, wheezing outside RSV epidemics, detection of rhinoviruses by RT-PCR during acute wheezing illnesses, as well as SPT positivity to common food or inhalant allergens or detection of specific IgE antibodies against egg white, wheat, or inhalant allergens in infancy may facilitate the identification of children at risk for persistent wheezing and the development of asthma.

REFERENCES

Ananaba GA, Anderson LJ. Antibody enhancement of respiratory syncytial virus stimulation of leukotriene production by a macrophage cell line. J Virol 1991; 65: 5052-5060.

Andréoletti L, Lesay M, Deschildre A, Lambert V, Dewilde A, Wattré P. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. J Med Virol 2000; 61: 341-346.

Azevedo I, de Blic J, Vargaftig BB, Bachelet M, Scheinmann P. Increased eosinophil cationic protein levels in bronchoalveolar lavage from wheezy infants. Pediatr Allergy Immunol 2001; 12: 65-72.

Backer V, Ulrik CS, Bach-Mortensen N, Glikmann G, Mordhorst C-H. Relationship between viral antibodies and bronchial hyperresponsiveness in 495 unselected children and adolescents. Allergy 1993; 48: 240-247.

Ball TM, Castro-Rodríguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000; 343: 538-543.

Blomqvist S, Roivainen M, Puhakka T, Kleemola M, Hovi T. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. J Med Virol 2002; 66: 263-268.

Boesen I. Asthmatic bronchitis in children. Prognosis for 162 cases, observed 6-11 years. Acta Pædiatr 1953; 42: 87-96.

Boivin G, De Serres G, Côté S, Gilca R, Abed Y, Rochette L, Bergeron MG, Déry P. Human metapneumovirus infections in hospitalized children. Emerg Infect Dis 2003; 9: 634-640.

Boyce TG, Mellen BG, Mitchel EF Jr, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. J Pediatr 2000; 137: 865-870.

Brooks GF, Butel JS, Morse SA, eds. Jawetz, Melnick, & Adelberg's Medical Microbiology. 23rd edition. New York: McGraw-Hill, 2004.

Bruno G, Cantani A, Ragno V, Milita O, Zirulo G, Businco L. Natural history of IgE antibodies in children at risk for atopy. Ann Allergy Asthma Immunol 1995; 74: 431-436.

Camara AA, Silva JM, Ferriani VPL, Tobias KRC, Macedo IS, Padovani MA, Harsi CM, Cardoso MRA, Chapman MD, Arruda E, Platts-Mills TAE, Arruda LK. Risk factors for wheezing in a subtropical environment: Role of respiratory viruses and allergen sensitization. J Allergy Clin Immunol 2004; 113: 551-557.

Carlsen KH, Larsen S, Ørstavik I. Acute bronchiolitis in infancy. The relationship to later recurrent obstructive airways disease. Eur J Respir Dis 1987; 70: 86-92.

Carlsen K-H. Exercise-induced asthma in children and adolescents and its relationship to sports. In: Naspitz CK, Szefler SJ, Tinkelman DG, Warner JO, eds. Textbook of pediatric asthma. An international perspective. London: Martin Dunitz, 2001, p. 211-222.

Castro-Rodríguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med 2000; 162: 1403-1406.

Castro-Rodríguez JA, Holberg CJ, Morgan WJ, Wright AL, Halonen M, Taussig LM, Martinez FD. Relation of two different subtypes of croup before age three to wheezing, atopy, and pulmonary function during childhood: a prospective study. Pediatrics 2001: 107: 512-518.

Cox MJ, Azevedo RS, Cane PA, Massad E, Medley GF. Seroepidemiological study of respiratory syncytial virus in São Paulo State, Brazil. J Med Virol 1998; 55: 234-239.

Csonka P, Kaila M, Laippala P, Kuusela A-L, Ashorn P. Wheezing in early life and asthma at school age: Predictors of symptom persistence. Pediatr Allergy Immunol 2000; 11: 225-229.

Delacourt C, Labbé D, Vassault A, Brunet-Langot D, de Blic J, Scheinmann P. Sensitization to inhalant allergens in wheezing infants is predictive of the development of infantile asthma. Allergy 1994; 49: 843-847.

Døllner H, Risnes K, Radtke A, Nordbø SA. Outbreak of human metapneumovirus infection in Norwegian children. Pediatr Infect Dis J 2004; 23: 436-440.

Duff AL, Pomeranz ES, Gelber LE, Price GW, Farris H, Hayden FG, Platts-Mills TAE, Heymann PW. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. Pediatrics 1993; 92: 535-540.

EAACI. The European Academy of Allergology and Clinical Immunology. Position paper: Allergen standardization and skin tests. Allergy 1993; 48 Suppl 14: 48-82.

Ehlenfield DR, Cameron K, Welliver RC. Eosinophilia at the time of respiratory syncytial virus bronchiolitis predicts childhood reactive airway disease. Pediatrics 2000; 105: 79-83.

Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. J Clin Invest 1996; 97: 915-924.

Eisen AH. Eosinophilia. In: Bierman CW, Pearlman DS, eds. Allergic diseases of infancy, childhood and adolescence. Philadelphia: W.B. Saunders, 1980, p. 761-762.

Emin O, Nermin G, Ulker O, Gokcay G. Skin sensitization to common allergens in Turkish wheezy children less than 3 years of age. Asian Pac J Allergy Immunol 2004; 22: 97-101.

ETAC Study Group. Allergic factors associated with the development of asthma and the influence of cetirizine in a double-blind, randomised, placebo-controlled trial: first results of ETAC (Early Treatment of the Atopic Child). Pediatr Allergy Immunol 1998; 9: 116-124.

Faden H, Kaul TN, Ogra PL. Activation of oxidative and arachidonic acid metabolism in neutrophils by respiratory syncytial virus antibody complexes: possible role in disease. J Infect Dis 1983; 148: 110-116.

Forster J, Tacke U, Krebs H, Streckert HJ, Werchau H, Bergmann RL, Schulz J, Lau S, Wahn U. Respiratory syncytial virus infection: its role in aeroallergen sensitization during the first two years of life. Pediatr Allergy Immunol 1996; 7: 55-60.

Foucard T, Sjöberg O. A prospective 12-year follow-up study of children with wheezy bronchitis. Acta Pædiatr Scand 1984; 73: 577-583.

Freymuth F, Vabret A, Brouard J, Toutain F, Verdon R, Petitjean J, Gouarin S, Duhamel J-F, Guillois B. Detection of viral, Chlamydia pneumoniae and Mycoplasma pneumoniae infections in exacerbations of asthma in children. J Clin Virol 1999; 13: 131-139.

Garofalo R, Welliver RC, Ogra PL. Concentrations of LTB_4, LTC_4, and LTE_4 in bronchiolitis due to respiratory syncytial virus. Pediatr Allergy Immunol 1991; 2: 30-37.

Garofalo R, Dorris A, Ahlstedt S, Welliver RC. Peripheral blood eosinophil counts and eosinophil cationic protein content of respiratory secretions in bronchiolitis: relationship to severity of disease. Pediatr Allergy Immunol 1994; 5: 111-117.

Gern JE, Busse WW. Association of rhinovirus infections with asthma. Clin Microbiol Rev 1999; 12: 9-18.

Gern JE, Vrtis R, Grindle KA, Swenson C, Busse WW. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. Am J Respir Crit Care Med 2000; 162: 2226-2231.

Glezen WP, Denny FW. Epidemiology of acute lower respiratory disease in children. N Engl J Med 1973; 288: 498-505.

Glezen WP, Paredes A, Allison JE, Taber LH, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. J Pediatr 1981; 98: 708-715.

Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child 1986; 140: 543-546.

Gold DR, Burge HA, Carey V, Milton DK, Platts-Mills T, Weiss ST. Predictors of repeated wheeze in the first year of life. The relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. Am J Respir Crit Care Med 1999; 160: 227-236.

Graham BS, Rutigliano JA, Johnson TR. Respiratory syncytial virus immunobiology and pathogenesis. Virology 2002; 297: 1-7.

Groothuis JR, Gutierrez KM, Lauer BA. Respiratory syncytial virus infection in children with bronchopulmonary dysplasia. Pediatrics 1988; 82: 199-203.

Gurwitz D, Mindorff C, Levison H. Increased incidence of bronchial reactivity in children with a history of bronchiolitis. J Pediatr 1981; 98: 551-555.

Hall CB, Kopelman AE, Douglas RG Jr, Geiman JM, Meagher MP. Neonatal respiratory syncytial virus infection. N Engl J Med 1979; 300: 393-396.

Hall CB, Hall WJ, Gala CL, MaGill FB, Leddy JP. Long-term prospective study in children after respiratory syncytial virus infection. J Pediatr 1984; 105: 358-364.

Halonen M, Stern DA, Lohman C, Wright AL, Brown MA, Martinez FD. Two subphenotypes of childhood asthma that differ in maternal and paternal influences on asthma risk. Am J Respir Crit Care Med 1999; 160: 564-570.

Halonen P, Rocha E, Hierholzer J, Holloway B, Hyypiä T, Hurskainen P, Pallansch M. Detection of enteroviruses and rhinoviruses in clinical specimens by PCR and liquid-phase hybridization. J Clin Microbiol 1995; 33: 648-653.

Harsten G, Prellner K, Löfgren B, Heldrup J, Kalm O, Kornfält R. Serum antibodies against respiratory tract viruses: A prospective three-year follow-up from birth. J Laryngol Otol 1989; 103: 904-908.

Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N Engl J Med 1999; 340: 260-264.

Henderson FW, Collier AM, Clyde WA Jr, Denny FW. Respiratory-syncytial-virus infections, reinfections and immunity. A prospective, longitudinal study in young children. N Engl J Med 1979; 300: 530-534.

Henrickson KJ. Lower respiratory viral infections in immunocompetent children. Adv Pediatr Infect Dis 1994; 9: 59-96.

Henrickson KJ. Parainfluenza viruses. Clin Microbiol Rev 2003; 16: 242-264.

Hesselmar B, Åberg N, Åberg B, Eriksson B, Björkstén B. Does early exposure to cat or dog protect against later allergy development? Clin Exp Allergy 1999; 29: 611-617.

Heymann PW, Carper HT, Murphy DD, Platts-Mills TAE, Patrie J, McLaughlin AP, Erwin EA, Shaker MS, Hellems M, Peerzada J, Hayden FG, Hatley TK, Chamberlain R. Viral infections in relation to age, atopy, and season of admission among children hospitalised for wheezing. J Allergy Clin Immunol 2004; 114: 239-247.

Holberg CJ, Wright AL, Martinez FD, Ray CG, Taussig LM, Lebowitz MD, Group Health Medical Associates. Risk factors for respiratory syncytial virus-associated lower respiratory illnesses in the first year of life. Am J Epidemiol 1991; 133: 1135-1151.

Holgate ST. The cellular and mediator basis of asthma in relation to natural history. Lancet 1997; 350 Suppl II: sii5- sii9.

van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RAM, Osterhaus ADME. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001; 7: 719-724.

Horn MEC, Reed SE, Taylor P. Role of viruses and bacteria in acute wheezy bronchitis in childhood: a study of sputum. Arch Dis Child 1979; 54: 587-592.

Hyypiä T, Puhakka T, Ruuskanen O, Mäkelä M, Arola A, Arstila P. Molecular diagnosis of human rhinovirus infections: comparison with virus isolation. J Clin Microbiol 1998; 36: 2081-2083.

Illi S, von Mutius E, Lau S, Nickel R, Niggemann B, Sommerfeld C, Wahn U, the Multicenter Allergy Study Group. The pattern of atopic sensitization is

associated with the development of asthma in childhood. J Allergy Clin Immunol 2001; 108: 709-714.

The IMpact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics 1998; 102: 531-537.

ISAAC Steering Committee. The International Study of Asthma and Allergies in Childhood. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998; 351: 1225-1232.

Isaacs D, Flowers D, Clarke JR, Valman HB, MacNaughton MR. Epidemiology of coronavirus respiratory infections. Arch Dis Child 1983; 58: 500-503.

Jartti T, van den Hoogen B, Garofalo RP, Osterhaus ADME, Ruuskanen O. Metapneumovirus and acute wheezing in children. Lancet 2002; 360: 1393-1394.

Jartti T, Lehtinen P, Vuorinen T, Koskenvuo M, Ruuskanen O. Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children. J Med Virol 2004a; 72: 695-699.

Jartti T, Lehtinen P, Vuorinen T, Österback R, van den Hoogen B, Osterhaus ADME, Ruuskanen O. Respiratory Picornaviruses and respiratory syncytial viruses as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 2004b; 10: 1095-1101.

Jennings LC, Anderson TP, Werno AM, Beynon KA, Murdoch DR. Viral etiology of acute respiratory tract infections in children presenting to hospital. Role of polymerase chain reaction and demonstration of multiple infections. Pediatr Infect Dis J 2004; 23: 1003-1007.

Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, Lambden PR, Tyrrell DAJ, Holgate ST. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J Clin Microbiol 1993; 31: 111-117.

Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH, Tyrrell DAJ, Holgate ST. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. BMJ 1995; 310: 1225-1229.

Juvén T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, Eskola J, Saikku P, Ruuskanen O. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000; 19: 293-298.

Kamahora T, Soe LH, Lai MMC. Sequence analysis of nucleocapsid gene and leader RNA of human coronavirus OC43. Virus Res 1989; 12: 1-9.

Karakoc F, Remes ST, Martinez FD, Wright AL. The association between persistent eosinophilia and asthma in childhood is independent of atopic status. Clin Exp Allergy 2002; 32: 51-56.

Kellner G, Popow-Kraupp T, Kundi M, Binder C, Wallner H, Kunz C. Contribution of rhinoviruses to respiratory viral infections in childhood: a prospective study in a mainly hospitalized infant population. J Med Virol 1988; 25: 455-469.

Kellner G, Popow-Kraupp T, Kundi M, Binder C, Kunz C. Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. Acta Pædiatr Scand 1989; 78: 390-394.

Klassen TP, Rowe PC, Sutcliffe T, Ropp LJ, McDowell IW, Li MM. Randomized trial of salbutamol in acute bronchiolitis. J Pediatr 1991; 118: 807-811.

Kneyber MCJ, Brandenburg AH, de Groot R, Joosten KFM, Rothbarth PH, Ott A, Moll HA. Risk factors for respiratory syncytial virus associated apnoea. Eur J Pediatr 1998; 157: 331-335.

Kneyber MCJ, Steyerberg EW, de Groot R, Moll HA. Long-term effects of respiratory syncytial virus (RSV) bronchiolitis in infants and young children: a quantitative review. Acta Pædiatr 2000; 89: 654-660.

Koller DY, Wojnarowski C, Herkner KR, Weinländer G, Raderer M, Eichler I, Frischer T. High levels of eosinophil cationic protein in wheezing infants predict the development of asthma. J Allergy Clin Immunol 1997; 99: 752-756.

Korhonen K, Korppi M, Remes ST, Reijonen TM, Remes K. Lung function in school-aged asthmatic children with inhaled cromoglycate, nedocromil and corticosteroid therapy. Eur Respir J 1999: 13: 82-86.

Korppi M, Halonen P, Kleemola M, Launiala K. Viral findings in children under the age of two years with expiratory difficulties. Acta Pædiatr Scand 1986; 457-464.

Korppi M, Reijonen T, Pöysä L, Juntunen-Backman K. A 2- to 3-year outcome after bronchiolitis. Am J Dis Child 1993; 147: 628-631.

Korppi M, Kuikka L, Reijonen T, Remes K, Juntunen-Backman K, Launiala K. Bronchial asthma and hyperreactivity after early childhood bronchiolitis or pneumonia. An 8-year follow-up study. Arch Pediatr Adolesc Med 1994; 148: 1079-1084.

Kuikka L, Reijonen T, Remes K, Korppi M. Bronchial asthma after early childhood wheezing: a follow-up until 4.5-6 years of age. Acta Pædiatr 1994; 83: 744-748.

Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U, and the Multicenter Allergy Study Group. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. J Allergy Clin Immunol 1999; 103: 1173-1179.

Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, Wahn U, and the Multicentre Allergy Study Group. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Lancet 2000; 356: 1392-1397.

Law BJ, Carbonell-Estrany X, Simoes EAF. An update on respiratory syncytial virus epidemiology: a developed country perspective. Respir Med 2002; 96 Suppl B: S1-S7.

Lowell DI, Lister G, Von Koss H, McCarthy P. Wheezing in infants: the response to epinephrine. Pediatrics 1987; 79: 939-945.

Lönnrot M, Sjöroos M, Salminen K, Maaronen M, Hyypiä T, Hyöty H. Diagnosis of enterovirus and rhinovirus infections by RT-PCR and time-resolved fluorometry with lanthanide chelate labeled probes. J Med Virol 1999; 59: 378-384.

MacDonald NE, Hall CB, Suffin SC, Alexson C, Harris PJ, Manning JA. Respiratory syncytial viral infection in infants with congenital heart disease. N Engl J Med 1982; 307: 397-400.

Madhi SA, Venter M, Madhi A, Petersen K, Klugman KP. Differing manifestations of respiratory syncytial virus-associated severe lower respiratory tract infections in human immunodeficiency virus type 1-infected and uninfected children. Pediatr Infect Dis J 2001; 20: 164-170.

Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988; 319: 1112-1117.

Martinez FD, Taussig LM, Morgan WJ. Infants with upper respiratory illnesses have significant reductions in maximal expiratory flow. Pediatr Pulmonol 1990; 9: 91-95.

Martinez FD, Wright AL, Holberg CJ, Mogan WJ, Taussig LM. Maternal age as a risk factor for wheezing lower respiratory illnesses in the first year of life. Am J Epidemiol 1992; 136: 1258-1268.

Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ, and the Group Health Medical Associates. Asthma and wheezing in the first six years of life. N Engl J Med 1995; 332: 133-138.

Martinez FD, Stern DA, Wright AL, Taussig LM, Halonen M. Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. J Allergy Clin Immunol 1998; 102: 915-920.

Martinez FD. Recognizing early asthma. Allergy 1999; 54 Suppl 49: 24-28.

Martinez F, Godfrey S. Wheezing disorders in the preschool child. Pathophysiology and management. London and New York: Martin Dunitz, 2003.

McCarthy AJ, Kingman HM, Kelly C, Taylor GS, Caul EO, Grier D, Moppet J, Foot ABM, Cornish JM, Oakhill A, Steward CG, Pamphilon DH, Marks DI. The outcome of 26 patients with respiratory syncytial virus infection following allogeneic stem cell transplantation. Bone Marrow Transplant 1999; 24: 1315-1322.

McIntosh K, Halonen P, Ruuskanen O. Report of a workshop on respiratory viral infections: epidemiology, diagnosis, treatment and prevention. Clin Infect Dis 1993; 16: 151-164.

Meddens MJM, Herbrink P, Lindeman J, van Dijk WC. Serodiagnosis of respiratory syncytial virus (RSV) infection in children as measured by detection of RSV-specific immunoglobulins G, M and A with enzyme-linked immunosorbent assay. J Clin Microbiol 1990; 28: 152-155.

Meert K, Heidemann S, Abella B, Sarnaik A. Does prematurity alter the course of respiratory syncytial virus infection? Crit Care Med 1990; 18: 1357-1359.

Meurman O, Ruuskanen O, Sarkkinen H, Hänninen P, Halonen P. Immunoglobulin class-specific antibody response in respiratory syncytial virus infection measured by enzyme immunoassay. J Med Virol 1984; 14: 67-72.

Meurman O, Waris M, Hedman K. Immunoglobulin G antibody avidity in patients with respiratory syncytial virus infection. J Clin Microbiol 1992; 30: 1479-1484.

Morgan WJ, Martinez FD. Risk factors for developing wheezing and asthma in childhood. Pediatr Clin North Am 1992; 39: 1185-1203.

Mrazek DA, Klinnert M, Mrazek PJ, Brower A, McCormick D, Rubin B, Ikle D, Kastner W, Larsen G, Harbeck R, Jones J. Prediction of early-onset asthma in genetically at-risk children. Pediatr Pulmonol 1999; 27: 85-94.

Murray M, Webb MSC, O'Callaghan C, Swarbrick AS, Milner AD. Respiratory status and allergy after bronchiolitis. Arch Dis Child 1992; 67: 482-487.

Myint S, Johnston S, Sanderson G, Simpson H. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. Mol Cell Probes 1994; 8: 357-364.

Mäkelä MJ, Puhakka T, Ruuskanen O, Leinonen M, Saikku P, Kimpimäki M, Blomqvist S, Hyypiä T, Arstila P. Viruses and bacteria in the etiology of the common cold. J Clin Microbiol 1998; 36: 539-542.

Navas L, Wang E, de Carvalho V, Robinson J, the Pediatric Investigators Collaborative Network on Infections in Canada. Improved outcome of respiratory syncytial virus infection in a high-risk hospitalized population of Canadian children. J Pediatr 1992; 121: 348-354.

Noble V, Murray M, Webb MSC, Alexander J, Swarbrick AS, Milner AD. Respiratory status and allergy nine to 10 years after acute bronchiolitis. Arch Dis Child 1997; 76: 315-319.

Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkäranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. J Med Virol 2002; 66: 417-420.

Osundwa VM, Dawod ST, Ehlayel M. Recurrent wheezing in children with respiratory syncytial virus (RSV) bronchiolitis in Qatar. Eur J Pediatr 1993; 152: 1001-1003.

Paganelli R, Ansotegui IJ, Sastre J, Lange C-E, Roovers MHWM, de Groot H, Lindholm NB, Ewan PW. Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAP, in six European allergy clinics. Allergy 1998; 53: 763-768.

Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, LeSouëf PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. Am J Respir Crit Care Med 2001; 163: 37-42.

Papadopoulos NG, Sanderson G, Hunter J, Johnston SL. Rhinoviruses replicate effectively at lower airway temperatures. J Med Virol 1999; 58: 100-104.

Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, Meyer J, Lackie PM, Sanderson G, Holgate ST, Johnston SL. Rhinoviruses infect the lower airways. J Infect Dis 2000; 181: 1875-1884.

Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, Gourgiotis D, Kafetzis D. Association of rhinorivus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 2002; 165: 1285-1289.

Papadopoulos NG, Papi A, Psarras S, Johnston SL. Mechanisms of rhinovirus-induced asthma. Paediatr Respir Rev 2004; 5: 255-260.

Park J-H, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. Am J Respir Crit Care Med 2001; 163: 322-328.

Paton AW, Paton JC, Lawrence AJ, Goldwater PN, Harris RJ. Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by reverse transcription and polymerase chain reaction amplification. J Clin Microbiol 1992; 30: 901-904.

Peterson CGB, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover in vivo. Clin Exp Allergy 1991; 21: 561-567.

Petrie A, Sabin C. Assessing agreement. In: Medical statistics at a glance. Oxford: Blackwell Science, 2000, p. 93-95.

Phelan PD, Landau LI, Olinsky A. Clinical patterns of acute respiratory infections. Acute viral bronchiolitis. In: Respiratory illness in children. Oxford: Blackwell Scientific Publications, 1990, p. 66-73.

Pifferi M, Ragazzo V, Caramella D, Baldini G. Eosinophil cationic protein in infants with respiratory syncytial virus bronchiolitis: predictive value for subsequent development of persistent wheezing. Pediatr Pulmonol 2001; 31: 419-424.

Piippo-Savolainen E, Remes S, Kannisto S, Korhonen K, Korppi M. Asthma and lung function 20 years after wheezing in infancy. Results from a prospective follow-up study. Arch Pediatr Adolesc Med 2004; 158: 1070-1076.

The PREVENT Study Group. Reduction of respiratory syncytial virus hospitalization among premature infants and infants with bronchopulmonary dysplasia using respiratory syncytial virus immune globulin prophylaxis. Pediatrics 1997; 99: 93-99.

Price GW, Hogan AD, Farris AH, Burks AW, Platts-Mills TAE, Heymann PW. Sensitization (IgE antibody) to food allergens in wheezing infants and children. J Allergy Clin Immunol 1995; 96: 266-270.

Pullan CR, Hey EN. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. BMJ 1982; 284: 1665-1669.

Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TAE, Heymann PW. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. Am J Respir Crit Care Med 1999; 159: 785-790.

Rawlinson WD, Waliuzzaman Z, Carter IW, Belessis YC, Gilbert KM, Morton JR. Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. J Infect Dis 2003; 187: 1314-1318.

Reijonen T, Korppi M, Pitkäkangas S, Tenhola S, Remes K. The clinical efficacy of nebulized racemic epinephrine and albuterol in acute bronchiolitis. Arch Pediatr Adolesc Med 1995; 149: 686-692.

Reijonen T, Korppi M, Kuikka L, Remes K. Anti-inflammatory therapy reduces wheezing after bronchiolitis. Arch Pediatr Adolesc Med 1996; 150: 512-517.

Reijonen TM. Early childhood wheezing. A study on therapy and risk factors for recurrent bronchial obstructions (Doctoral dissertation). Kuopio: Kuopio University Printing Office, 1997a, p. 52.

Reijonen TM, Korppi M, Kuikka L, Savolainen K, Kleemola M, Mononen I, Remes K. Serum eosinophil cationic protein as a predictor of wheezing after bronchiolitis. Pediatr Pulmonol 1997b; 23: 397-403.

Reijonen TM, Korppi M, Kleemola M, Savolainen K, Kuikka L, Mononen I, Remes K. Nasopharyngeal eosinophil cationic protein in bronchiolitis: relation to viral findings and subsequent wheezing. Pediatr Pulmonol 1997c; 24: 35-41.

Reijonen TM, Kotaniemi-Syrjänen A, Korhonen K, Korppi M. Predictors of asthma three years after hospital admission for wheezing in infancy. Pediatrics 2000; 106: 1406-1412.

Remes ST, Korppi M, Remes K, Pekkanen J. Prevalence of asthma at school age: a clinical population-based study in eastern Finland. Acta Pædiatr 1996; 85: 59-63.

Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. J Allergy Clin Immunol 2001; 108: 509-515.

Rona RJ, Gulliford MC, Chinn S. Effects of prematurity and intrauterine growth on respiratory health and lung function in childhood. BMJ 1993; 306: 817-820.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Peñaranda S, Bankamp B, Maher K, Chen M, Tong S, Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman DD, Peret TCT, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Günther S, Pallansch MA, Anderson LJ, Bellini WJ. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003; 300; 1394-1399.

Ruohola A, Heikkinen T, Waris M, Puhakka T, Ruuskanen O. Intranasal fluticasone propionate does not prevent acute otitis media during viral upper respiratory infection in children. J Allergy Clin Immunol 2000; 106: 467-471.

Rusconi F, Galassi C, Corbo GM, Forastiere F, Biggeri A, Ciccone G, Renzoni E, the SIDRIA Collaborative Group. Risk factors for early, persistent and late-onset wheezing in young children. Am J Respir Crit Care Med 1999; 160: 1617-1622.

Ruuskanen O, Ogra PL. Respiratory syncytial virus. Curr Probl Pediatr 1993; 23: 50-79.

Saarinen UM, Juntunen K, Kajosaari M, Björksten F. Serum immunoglobulin E in atopic and non-atopic children aged 6 months to 5 years. Acta Pædiatr Scand 1982; 71: 489-494.

Sackett DL, Richardson WS, Rosenberg W, Haynes RB. Is this evidence about a diagnostic test important? In: Evidence-based medicine. How to practice and teach EBM. London: Churchill Livingstone, 1998, p. 118-128.

Samet JM, Wiesch DG, Ahmed IH. Pediatric asthma: epidemiology and natural history. In: Naspitz CK, Szefler SJ, Tinkelman DG, Warner JO, eds. Textbook of pediatric asthma. An international perspective. London: Martin Dunitz, 2001, p. 35-66.

Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997; 100: 444-451.

Santti J, Hyypiä T, Halonen P. Comparison of PCR primer pairs in the detection of human rhinoviruses in nasopharyngeal aspirates. J Virol Methods 1997; 66: 139-147.

van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC 2nd, Welliver RC. Increased production of IFN-γ and cysteinyl leukotriens in virus-induced wheezing. J Allergy Clin Immunol 1999; 103: 630-636.

Sherriff A, Peters TJ, Henderson J, Strachan D, the ALSPAC Study Team. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3½ years. Int J Epidemiol 2001; 30: 1473-1484.

Sigurs N, Hattevig G, Kjellman B, Kjellmann N-IM, Nilsson L, Björkstén B. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. J Allergy Clin Immunol 1994; 94: 757-763.

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Björkstén B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 1995; 95: 500-505.

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000; 161: 1501-1507.

Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005; 171: 137-141.

Silvestri M, Oddera S, Rossi GA, Crimi P. Sensitization to airborne allergens in children with respiratory symptoms. Ann Allergy Asthma Immunol 1996; 76: 239-244.

Sly PD, Hibbert ME. Childhood asthma following hospitalization with acute viral bronchiolitis in infancy. Pediatr Pulmonol 1989; 7: 153-158.

Smyth AR, Smyth RL, Tong CYW, Hart CA, Heaf DP. Effect of respiratory virus infection including rhinovirus on clinical status in cystic fibrosis. Arch Dis Child 1995; 73: 117-120.

Staunton DE, Merluzzi VJ, Rothlein R, Barton R, Marlin SD, Springer TA. A cell adhesion molecule, ICAM-1, is the major surface reseptor for rhinoviruses. Cell 1989; 56: 849-853.

Stein RT, Holberg CJ, Sherrill D, Wright AL, Morgan WJ, Taussig L, Martinez FD. Influence of parental smoking on respiratory symptoms during the first decade of life: the Tucson Children's Respiratory Study. Am J Epidemiol 1999a; 149: 1030-1037.

Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999b; 354: 541-545.

Stockton J, Stephenson I, Fleming D, Zambon M. Human metapneumovirus as a cause of community-acquired respiratory illness. Emerg Infect Dis 2002; 8: 897-901.

Strachan DP, Cook DG. Health effects of passive smoking. 1. Parental smoking and lower respiratory illness in infancy and early childhood. Thorax 1997; 52: 905-914.

Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995; 96: 549-557.

Thumerelle C, Deschildre A, Bouquillon C, Santos C, Sardet A, Scalbert M, Delbecque L, Debray P, Dewilde A, Turck D, Leclerc F. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: a prospective study in the Nord-Pas de Calais region (France). Pediatr Pulmonol 2003; 35: 75-82.

Timonen KL, Pekkanen J, Korppi M, Vahteristo M, Salonen RO. Prevalence and characteristics of children with chronic respiratory symptoms in eastern Finland. Eur Respir J 1995; 8: 1155-1160.

Tridon A, Fauquert JL, Dibet P, Betail G, Labbe A. Atopy parameters in asthmatic infants. J Investig Allergol Clin Immunol 1999; 9: 222-228.

Vignola AM, Campbell AM, Chanez P, Bousquet J, Paul-Lacoste P, Michel F-B, Godard P. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. Am Rev Respir Dis 1993; 148: 689-694.

Waris M. Pattern of respiratory syncytial virus epidemics in Finland: two-year cycles with alternating prevalence of groups A and B. J Infect Dis 1991; 163: 464-469.

Webb MSC, Henry RL, Milner AD, Stokes GM, Swarbrick AS. Continuing respiratory problems three and a half years after acute viral bronchiolitis. Arch Dis Child 1985; 60: 1064-1067.

Welliver RC, Kaul TN, Putnam TI, Sun M, Riddlesberger K, Ogra PL. The antibody response to primary and secondary infection with respiratory syncytial virus: Kinetics of class-specific responses. J Pediatr 1980; 96: 808-813.

Welliver RC, Sun M, Rinaldo D, Ogra PL. Predictive value of respiratory syncytial virus-specific IgE responses for recurrent wheezing following bronchiolitis. J Pediatr 1986; 109: 776-780.

Wendt CH, Hertz MI. Respiratory syncytial virus and parainfluenza virus infections in the immunocompromised host. Semin Respir Infect 1995; 10: 224-231.

Wennergren G, Hansson S, Engström I, Jodal U, Åmark M, Brolin I, Juto P. Characteristics and prognosis of hospital-treated obstructive bronchitis in children aged less than two years. Acta Pædiatr 1992; 81: 40-45.

Wennergren G, Åmark M, Åmark K, Óskarsdóttir S, Sten G, Redfors S. Wheezing bronchitis reinvestigated at the age of 10 years. Acta Pædiatr 1997; 86: 351-355.

Wever-Hess J, Kouwenberg JM, Duiverman EJ, Hermans J, Wever AMJ. Prognostic characteristics of asthma diagnosis in early childhood in clinical practice. Acta Pædiatr 1999; 88: 827-834.

Wright AL, Holberg CJ, Martinez FD, Morgan WJ, Taussig LM, Group Health Medical Associates. Breast feeding and lower respiratory tract illness in the first year of life. BMJ 1989; 299: 946-949.

Yunginger JW, Ahlstedt S, Eggleston PA, Homburger HA, Nelson HS, Ownby DR, Platts-Mills TAE, Sampson HA, Sicherer SH, Weinstein AM, Williams PB, Wood RA, Zeiger RS. Quantitative IgE antibody assays in allergic diseases. J Allergy Clin Immunol 2000; 105: 1077-1084.

Øymar K, Havnen J, Halvorsen T, Bjerknes R. Eosinophil counts and urinary eosinophil protein X in children hospitalized for wheezing during the first year of life: prediction of recurrent wheezing. Acta Pædiatr 2001; 90: 843-849.

ORIGINAL PUBLICATIONS (I-V)

Kuopio University Publications D. Medical Sciences

D 339. Pellikainen, Johanna. Activator protein -2 in breast cancer: relation to cell growth, differentiation and cell-matrix interactions.
2004. 81 p. Acad. Diss.

D 340. Nunes, Sílvia Ramos. Longitudinal clinical characterization of the acute respiratory distress syndrome (ARDS): with special reference to monitoring of respiratory mechanics.
2004. 108 p. Acad. Diss.

D 341. Punakallio, Anne. Balance abilities of workers in physically demanding jobs: with special reference to firefighters of different ages.
2004. 87 p. Acad. Diss.

D 342. Lupsakko, Taina. Functional visual and hearing impairment in a population aged 75 years and older in the City of Kuopio in Finland: associations with mood and activities of daily living.
2004. 72 p. Acad. Diss.

D 343. Sivenius, Katariina. Genetic studies on type 2 diabetes mellitus with special emphasis on obesity and microvascular complications.
2004. 140 p. Acad. Diss.

D 344. Ylinen, Jari. Treatment of chronic non-specific neck pain with emphasis on strength training.
2004. 99 p. Acad. Diss.

D 345. Sironen, Reijo. Gene expression profiles of chondrocytic cells exposed to hydrostatic pressure.
2004. 77 p. Acad. Diss.

D 346. Lapveteläinen, Tuomo. Effects of aging, training, and collagen gene defects on murine osteoarthritis.
2004. 76 p. Acad. Diss.

D 347. Purhonen, Maija. Event-related potential (ERP) studies of mother-infant interaction.
2004. 90 p. Acad. Diss.

D 348. Inkinen, Ritva. Proteoglycans and hyaluronan in the intervertebral disc and cartilage endplate: effects of aging and degeneration.
2004. 97 p. Acad. Diss.

D 349. Salonvaara, Marjut. Coagulation physiology and abnormalities in newborn infants: association with intraventricular haemorrhage and central venous catheter-related thrombosis.
2004. 97 p. Acad. Diss.

D 350. Randell, Kaisa. Effects of hormonal and other factors on fractures in peri- and early postmenopausal women.
2004. 89 p. Acad. Diss.

D 351. Laasonen-Balk, Teijamari. Neuroimaging of depression using single-photon emission computerized tomography.
2005. 102 p. Acad. Diss.

D 352. Tolmunen, Tommi. Depression, B vitamins and homocysteine.
2005. 130 p. Acad. Diss.