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VIRAL INFECTION INDUCED
RESPIRATORY DISTRESS IN
CHILDHOOD

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU
FACULTY OF MEDICINE;
MEDICAL RESEARCH CENTER OULU;
OULU UNIVERSITY HOSPITAL



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**VIRAL INFECTION INDUCED
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CHILDHOOD**

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Abstract

Dyspnoea associated with respiratory infection is a common symptom in infancy and early childhood. Inspiratory stridor is the main symptom in cases of croup and expiratory wheezing in cases of bronchiolitis, obstructive bronchitis and acute asthma exacerbations. Dyspnoea associated with respiratory infection is a common cause of emergency department visits and unplanned hospital admissions among infants and preschool children. The assessment of dyspnea associated with acute childhood respiratory infection is largely subjective, and evidence regarding the severity of acute dyspnoea is needed in order to target hospital admissions more accurately. Wheezing associated with respiratory infection in infancy has been recognized as an important predictor of recurrent wheezing and asthma at school age.

The aims of this study were to determine the risk factors for croup, to evaluate factors that reliably predict the need for hospitalizing children with acute wheezing and to find out whether respiratory infection with wheezing during infancy has a positive association with the development of asthma during childhood. The work included two register-based surveys and one prospective cohort study.

It is concluded that a family history of croup is an exceptionally strong risk factor for croup and its recurrence in childhood. The early phase of bronchiolitis is unstable in infants below 6 months of age. These infants are most likely to need medical interventions in the first 5 days after onset of the disease. A positive respiratory syncytial -virus test result, a fever of more than 38°C and low initial oxygen saturation are predictors of the need for hospitalization and medical interventions. An initial oxygen saturation >93% effectively identifies children aged more than 6 months with mild wheezing, and this limit can be used to avoid unplanned hospital admissions. There is an association between early respiratory syncytial -virus infections and subsequent wheezing and asthma, in that such infections select children who are prone to wheezing and asthma before school age, but the symptoms tend to decrease with time and an early respiratory syncytial -virus infection will not permanently alter bronchial reactivity.

Keywords: asthma exacerbation, bronchiolitis, croup, dyspnoea, infection-induced wheezing, obstructive bronchitis

Pruikkonen, Hannele, Virusinfektion aiheuttama hengitysvaikeus lapsilla.

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Tiivistelmä

Hengitysvaikeus on yleinen oire lapsilla virusten aiheuttamien hengitystieinfektioiden yhteydessä. Kurkunpää tulehdukseen liittyy sisäänhengitysvaikeus. Ilmatiehyttulehdukseen, ahtauttavaan keuhkoputkentulehdukseen ja akuuttiin astma-kohtaukseen liittyy uloshengitysvaikeus. Hengitystieinfektioihin liittyvä hengitysvaikeus on yksi yleisimmistä syistä päivystyspoliklinikkakäynteihin ja äkillisiin sairaalahoitojaksoihin lapsipotilailla. Hengitystieinfektioiden taudinkulun tuntemisella ja hengitysvaikeuden vaikeusasteen arvioinnilla on tärkeä merkitys näiden potilaiden hoidon toteuttamisessa. Hengitystieinfektioon liittyvää hengitysvaikeutta on pidetty riskitekijänä astman kehittymiselle.

Tämän tutkimuksen tarkoituksena oli selvittää kurkunpää tulehduksen riskitekijöitä ja sairaalahoitoon vaikuttavia tekijöitä hengitystieinfektioon liittyvän uloshengitysvaikeuden hoidossa sekä varhaislapsuudessa sairastetun hengitystieinfektion yhteyttä myöhempään astma- ja allergiasairastavuuteen. Tutkimukseen sisältyi kaksi rekisteriaineistoa ja yksi seuranta tutkimusaineisto.

Tutkimuksessa todettiin, että kurkunpää tulehduksen uusiutuminen on erittäin tavallista ja sisarusten ja vanhempien sairastama kurkunpää tulehdus on merkittävin riskitekijä kurkunpää tulehdukselle ja sen uusiutumiselle. Alle 6 kuukauden ikäisillä lapsilla ilmatiehyttulehduksen taudinkuva on epävakaata ensimmäisen 5 oirepäivän aikana. Kuume, matala happisaturaatioarvo ja respiratory syncytial -virusinfektio ennustavat osastohoidon ja invasiivisten toimenpiteiden tarvetta ilmatiehyttulehduksen yhteydessä. Yli 6 kuukauden ikäisillä lapsilla happisaturaatioarvo > 93 % ennustaa lievää taudinkuvaa hengitystieinfektioon liittyvän uloshengitysvaikeuden hoidossa. Käyttämällä tätä happisaturaatioarvoa raja-arvona, kun arvioidaan sairaalahoidon tarvetta, voidaan merkittävästi ja turvallisesti vähentää sairaalahoidon tarvetta lasten hengitystieinfektioon liittyvän uloshengitysvaikeuden hoidossa. Alle 6 kuukauden iässä sairastettu respiratory syncytial -virusinfektio on riskitekijä varhaislapsuudessa ilmeneville astmaoireille, mutta tämä riski vähenee iän myötä ja 8 vuoden iässä ei ole havaittavissa eroja astma- ja allergiasairastavuudessa, kun verrataan näitä potilaita muun hengitystieinfektion sairastaneisiin potilaisiin ja terveisiin kontrollipotilaisiin.

Asiasanat: ahtauttava keuhkoputkentulehdus, astma, bronkioliitti, hengitystieinfektioon liittyvä hengenahdistus, ilmatiehyttulehdus, kurkunpää tulehdus, laryngiitti, obstruktiivinen bronkiitti

To lifelong learning

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Hannele Pruikkonen

Abbreviations

CI	confidence interval
CF	cystic fibrosis
CPAP	continuous positive airway pressure
GINA	Global Initiative for Asthma
HR	hazard ratio
hMPV	human metapneumovirus
ICD-10	International Classification of Diseases, version 10
IgE	immunoglobulin E
IgG	immunoglobulin G
ISAAC	International study of Asthma and Allergies in Childhood
MMI	major medical intervention
NIC	neonatal intensive care
OR	odds ratio
PaO ₂	partial pressure of oxygen in arterial blood
PCR	polymerase chain reaction
PPB	parts per billion
ROC-curve	receiver operating characteristic -curve
RR	relative risk
RSV	respiratory syncytial virus
SaO ₂	oxygen saturation
SCORAD	severity scoring of atopic dermatitis
SD	standard deviation
SND-test	standard normal deviation -test
SP-A	surfactant protein A
SP-D	surfactant protein D
TLR4	toll-like receptor 4

List of original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:

- I Pruikkonen H, Dunder T, Renko M, Pokka T & Uhari M (2009) Risk factors for croup in children with recurrent respiratory infections: a case-control study. *Paediatr Perinat Epidemiol* 23(2): 153–9.
- II Pruikkonen H, Uhari M, Dunder T, Pokka T & Renko M (2014) Infants under 6 months with bronchiolitis are most likely to need major medical interventions in the 5 days after onset. *Acta Paediatr* 103(10): 1089–93.
- III Pruikkonen H, Uhari M, Dunder T, Pokka T & Renko M (2014) Initial oxygen saturation values can predict the need to hospitalise children with mild wheezing. *Acta Paediatr* 103(9): 951–6.
- IV Pruikkonen H, Juntti H, Uhari M, Dunder T, Pokka T & Renko M (2014) Early RSV infection does not permanently alter bronchial reactivity. Manuscript.

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1 Introduction

Croup is a common illness responsible for up to 15% of emergency department visits due to respiratory disease in children (Cherry 2008). It is characterized by a barking cough, hoarseness and inspiratory stridor as a result of viral infection (Bjornson & Johnson 2008). Even though croup is a common paediatric infection, little is known of its predisposing factors or recurrence rate.

Approximately 50% of children experience at least one episode of infection-induced wheezing before the age of 6 years (Martinez *et al.* 1995). The initial episode is most often diagnosed as bronchiolitis in a child <1–2 years old (Beigelman & Bacharier 2014). The terminology for subsequent episodes or episodes occurring at >1 years of age varies greatly (Beigelman & Bacharier 2014, Gotz *et al.* 2006, Mertsola *et al.* 1991, Wilson 1989).

Severe bronchiolitis in infancy and recurrent wheezing in cases of viral infections have been considered important predictors of asthma, which is often accompanied by infection-induced exacerbations (Beigelman & Bacharier 2014, Sigurs *et al.* 2000, Stein *et al.* 1999). Viral respiratory infections are associated with almost 85% of asthma exacerbations in childhood (Johnston *et al.* 1995). Infection-induced wheezing is one of the most common causes of emergency department visits and unplanned hospital admissions among preschool children (Kuehni *et al.* 2001). Hospital admission rates among children <4 years of age with infection-induced wheezing range from 15 to 39 per 1000 per year (Langley *et al.* 2003, Martinez *et al.* 1995). Although the admission rates for asthma among schoolchildren have decreased considerably during recent decades, admission rates for wheezing illness in young children have remained high (Wennergren & Strannegard 2002).

The evaluation of acute childhood wheezing is largely subjective, and it is difficult to predict the severity of the disease and the need for hospitalization (Gorelick *et al.* 2008). Evidence regarding the clinical course and severity of acute childhood wheezing disease is needed in order to relieve the burden on parents, hospitals and society at large.

2 Review of the literature

2.1 Infection-induced stridor in children

2.1.1 Croup

The word “croup” is used to refer to a number of respiratory illnesses that are characterized by varying degrees of inspiratory stridor, barking cough and hoarseness due to obstruction in the laryngeal region. The terminology for croup illnesses has evolved over time, and prior to the 20th century all croup-like illnesses were thought to be diphtheria (Cherry 2008).

Today, the vast majority of cases of croup are either laryngotracheitis or spasmodic croup (Bjornson & Johnson 2005). Acute laryngotracheitis and spasmodic croup entail a sudden onset of inspiratory stridor including mild upper respiratory infection. Acute laryngotracheitis involves inflammation of the larynx and trachea, whereas this inflammation is absent in spasmodic croup (Cherry 2008).

Occurrence

Croup is a common illness responsible for up to 15% of emergency department visits due to respiratory disease in children (Cherry 2008). About 5% of children have croup during the second year of life, the peak incidence being between 7 and 36 months of age. Croup rarely occurs in children younger than 3 months of age. The incidence in boys is about 1.5 times that in girls (Denny *et al.* 1983). Croup also has seasonal pattern, as the peak incidence typically appears in the autumn (Denny *et al.* 1983). Annual patterns are also found, with about 50% more cases occurring in odd-numbered years (Marx *et al.* 1997). Reported hospital admission rates for children with croup have varied from 1% to 8%, and less than 3% of those admitted receive intubation (Rosychuk *et al.* 2010, Sofer *et al.* 1991). A marked decrease in hospitalization rates was observed in the early 1990s (Segal *et al.* 2005).

Clinical course

Croup patients frequently present with an abrupt onset of barking cough, hoarse voice and often inspiratory stridor during the night, sometimes preceded by 12–24 hours of non-specific coughing, rhinorrhoea, coryza and fever (Bjornson &

Johnson 2008). Croup is a clinical diagnosis, and less than 1% of children with acute onset stridor have any another diagnosis (Bjornson & Johnson 2008, Bjornson & Johnson 2013). Croup symptoms can fluctuate rapidly and are typically short-lived, with about 60% of children having resolution of the barking cough by 48 hours and less than 2% having symptoms persisting longer than 5 nights (Bjornson & Johnson 2013). At least two-thirds of children with croup have mild symptoms (Bjornson & Johnson 2013). Death appears to be rare, as it has been estimated that it occurs in no more than 1 in 30 000 cases (Bjornson & Johnson 2013).

Pathogenesis

The symptoms of croup result from upper airway obstruction caused by an acute viral infection, most typically parainfluenza virus types 1 or 3 (Marx *et al.* 1997). Other viruses that cause croup include enterovirus, human bocavirus, influenza A and B viruses, respiratory syncytial virus (RSV), rhinovirus, coronavirus and adenovirus (Rihkanen *et al.* 2008). A common respiratory viral genome, as determined by PCR assays, was found in the nasopharynx of 80% of 144 children with croup in a recent study (Rihkanen *et al.* 2008). Influenza viruses cause more severe croup than parainfluenza viruses, and children with croup caused by influenza require longer hospitalization and more medication and have a greater risk of readmission than children whose croup is attributable to parainfluenza (Peltola *et al.* 2002). Measles, diphtheria and *Mycoplasma pneumoniae* are other occasional causes of croup (Zoorob *et al.* 2011).

Croup symptoms nearly always become worse during the night and a physiologically plausible explanation may be circadian fluctuations in endogenous serum cortisol and epinephrine cycling (Bjornson & Johnson 2008, Weitzman *et al.* 1971).

Acute croup involves erythema and swelling of the lateral walls of the trachea just below the vocal cords, resulting in narrowing of the subglottic region of the trachea (Davison 1959). The affected area becomes edematous histologically, with cellular infiltration in the lamina propria, submucosa and adventitia, and the infiltrate contains histiocytes, lymphocytes, plasma cells and neutrophils (Richards 1938). The parainfluenza virus contributes to airway swelling, as it activates chloride secretion and inhibits sodium absorption across the tracheal epithelium (Kunzelmann *et al.* 2004). Generalized airway swelling and inflammation of the larynx and trachea lead to epithelial necrosis and shedding (Bjornson & Johnson

2008). In spasmodic croup there is non-inflammatory swelling in the subglottic region of the trachea (Davison 1959).

Recurrent croup

Croup episodes occurring frequently (two or more episodes per year) have been defined as recurrent croup (Kwong *et al.* 2007). This is characterized by repeated bouts of barking cough that occur with the onset of upper respiratory tract infections (Kwong *et al.* 2007). Recurrent croup was identified in 6.4% of a population of 486 infants born during a 12-month period and followed up for the first 4 years of life (Hide & Guyer 1985). More than one episode of croup requiring hospitalization was reported in 23% of 204 children admitted for croup when hospitalizations during both the preceding and following 3 years were evaluated (Wall *et al.* 2009). More than two episodes of croup were observed in 52% of 110 children who were evaluated for 9 years after hospitalization for croup (Zach *et al.* 1981).

Recurrent croup can be a manifestation of an underlying intrinsic and/or extrinsic airway narrowing process (Joshi *et al.* 2014). A relapsing course and long duration of episodes should alert the clinician to consider the possibilities of congenital or traumatic airway abnormalities, a foreign body in the airways, gastro-oesophageal reflux, asthma, allergy, bacterial infections and tumors (Joshi *et al.* 2014).

Risk factors for croup

Croup symptoms vary from child to child depending on host factors such as immunity and the anatomy of the subglottic space (Zoorob *et al.* 2011). The peak incidence of croup at the age of 2 years could be attributable to increased exposure to viral pathogens combined with the smaller subglottic space found in toddlers, leaving them at a greater risk of airway narrowing (Bjornson & Johnson 2008). Parainfluenza virus infections are common in children, as 80% of children are seropositive for parainfluenza virus types 1, 2 and 3 by the age of 5 years, but croup develops in only a small percentage of those exposed (Schomacker *et al.* 2012). It is possible that primary infection with parainfluenza virus type 3 may lead to sensitization to the parainfluenza virus group rather than to type 3 alone, setting the stage for spasmodic croup due to parainfluenza virus types 1 and 2 (Cherry 2008). Sensitization to viral antigens may also play a role in recurrent croup (Ottolini *et al.* 2002).

Two-thirds of children with croup in the first 3 years of life have wheezing during the episode or during an episode of some other lower respiratory infection, while one-third show no wheezing during respiratory infections (Castro-Rodriguez *et al.* 2001). Children with croup accompanied by wheezing have significantly lower mean levels of small airway function shortly after birth, i.e. before any respiratory infection, than do infants who have croup without wheezing later in the first 3 years of life (Castro-Rodriguez *et al.* 2001). Children with croup without wheezing have higher inspiratory resistance before any respiratory infection than infants who do not have lower respiratory infections later in the first 3 years of life (Castro-Rodriguez *et al.* 2001).

A family history of asthma has been found to be a risk factor for croup during the first 2 years of life (Kuiper *et al.* 2007). A family history of asthma and allergy has been found to be a risk factor in children hospitalized for croup, but not in outpatient children, suggesting that it indicates a tendency to develop a more severe disease (Konig 1978, Van Bever *et al.* 1999, Zach *et al.* 1981). The effect of a positive family history of asthma on respiratory morbidity during childhood is increased by environmental factors such as parental smoking and exposure to inhaled house dust (Kuiper *et al.* 2007). Day care attendance increases the risk of croup among children with a familial history of atopy in the first year of life (Celedon *et al.* 1999). A family history of hay fever, eczema and chronic bronchitis is associated with croup and recurrent croup (Van Bever *et al.* 1999). The risk factors for recurrent croup include a patient history of asthma and wheezy bronchitis (Cohen & Dunt 1988).

A family history of croup has been found to be a risk factor for recurrent croup (Cohen & Dunt 1988, Zach *et al.* 1981).

2.2 Infection-induced wheezing in children

2.2.1 Terminology and occurrence

Wheezing, defined as a continuous high-pitched sound emitted from the chest during expiration, is the end result of narrowing of the intrathoracic airways and expiratory flow limitation (Brand *et al.* 2008).

In the Tucson Children's Respiratory Study 1246 newborns were followed up for lower respiratory tract infections and wheezing symptoms during the first 3 years and again at age 6 years (Martinez *et al.* 1995). Four phenotypes were

identified at the latter age: (1) early transient wheezers, the 20% of the children, who had wheezing during first 3 years of life but no longer at 6 years of age, (2) persistent wheezers, the 14% in whom wheezing had occurred before the age of 3 years and was still present at the age of 6 years, (3) late-onset wheezers, the 15% who had wheezing between 3 and 6 years of age, and (4) non-wheezers, the 52% who had never wheezed (Fig.1) (Martinez *et al.* 1995, Stein *et al.* 1997).

The initial episode of infection-induced wheezing is most often diagnosed as bronchiolitis in a child <1–2 years old (Beigelman & Bacharier 2014). The word “bronchiolitis” is a pathological description which has come to be used as a clinical diagnosis since the 1940s (Smyth & Openshaw 2006). Its definitions have varied between countries, and even between hospitals within the same country (Mecklin *et al.* 2014, Smyth & Openshaw 2006). According to current US guideline, the diagnosis of bronchiolitis includes wheezing in children <24 months of age whereas the current UK guideline includes wheezing children <12 months age (AAP 2006, SIGN 2006). In some studies, the definition of bronchiolitis has been restricted to respiratory syncytial virus (RSV) cases only, or to children with their first episode of wheezing (Jartti *et al.* 2009a, Zorc & Hall 2010).

The terminology for subsequent infection-induced wheezing episodes after bronchiolitis, or for episodes occurring at an age of >1 year of age varies greatly. The terms that have been used are: acute virus-associated wheezing illness, infection-induced wheezing, wheezy bronchitis, obstructive bronchitis, acute bronchial obstruction, asthmatic bronchitis and preschool wheeze (Beigelman & Bacharier 2014, Ducharme *et al.* 2014, Gotz *et al.* 2006, Mecklin *et al.* 2014, Mertsola *et al.* 1991, Paasilta *et al.* 2011, Wilson 1989).

Recurrent wheezing in cases of viral infection can progress to asthma, which is often accompanied by infection-induced exacerbations (Fig. 1) (Beigelman & Bacharier 2014, Sigurs *et al.* 2000, Stein *et al.* 1999). Viral respiratory infections are associated with nearly 85% of asthma exacerbations in childhood (Johnston *et al.* 1995).

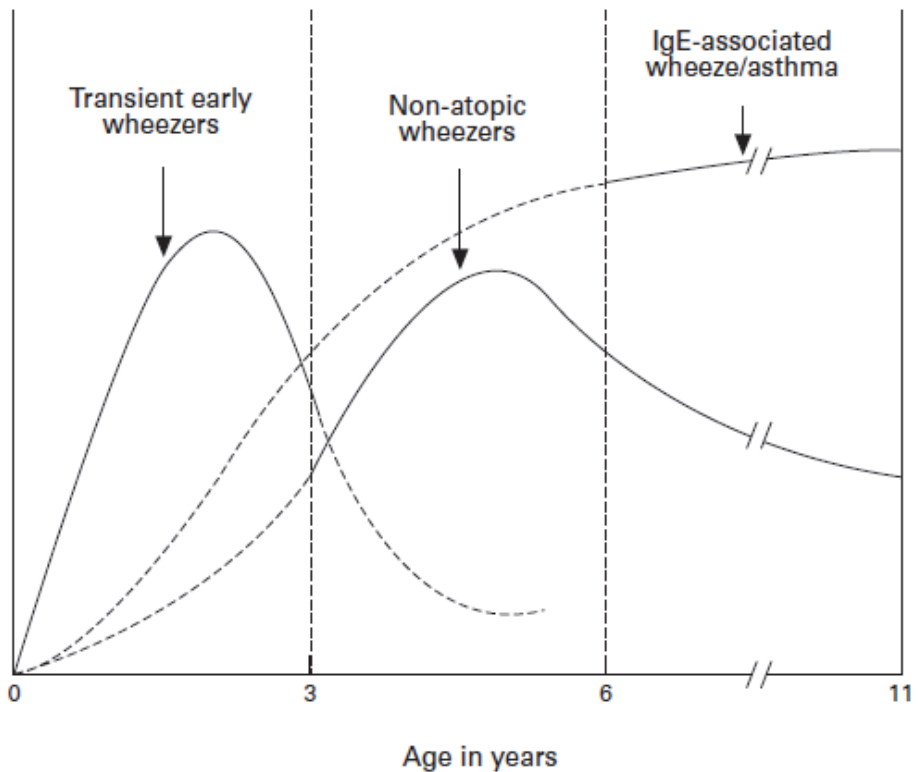


Fig. 1. Hypothetical yearly occurrence of wheezing for the three childhood wheezing phenotypes. The dashed lines suggest that wheezing curves can adopt different shapes due to the many factors involved, including overlapping between the groups (Stein *et al.* 1997). Reprinted with permission from BMJ Publishing Group Ltd.

The presentation of children with infection-induced wheezing is influenced by age, previous respiratory infections, genetic background, sensitization to aeroallergens, environmental influences and interactions between these factors (Beigelman & Bacharier 2014). Maternal asthma, maternal smoking, rhinitis apart from colds, eczema during the first year of life and male sex are risk factors for persistent wheezing during first 6 years of life (Martinez *et al.* 1995). One cohort study including 285 children with a high risk of allergic disease showed sensitization to an aeroallergen to be a risk factor for wheezing in the first 6 years of life (OR 1.9 95% CI 1.2 to 3.1), with this allergic sensitization preceding wheezing due to a

rhinovirus infection (Jackson *et al.* 2012). Genetic variants in an asthma-associated gene locus on chromosome 17 (17q21) were associated with future asthma diagnoses among the children in this same cohort who had wheezing due to rhinovirus infection in the first 3 years of life (Caliskan *et al.* 2013).

2.2.2 Aetiology

Evidence of viral infection is found in 80% to 90% of childhood wheezing episodes (Jackson *et al.* 2008). The major viral species causing wheezing in children include RSV, rhinovirus, human metapneumovirus (hMPV) and influenza viruses (Inoue & Shimojo 2013).

Respiratory syncytial virus (RSV)

Since its identification and isolation from children with pulmonary disease in 1956, RSV has been documented as the single most important virus causing acute respiratory infections in children (Simoes 1999). RSV has been the causative agent in 27–77% of bronchiolitis cases requiring hospitalization (Jartti *et al.* 2004, Stempel *et al.* 2009). RSV affects about 90% of infants and young children by the age of 2 years, with peak rates of infection in infants aged between 6 weeks and 6 months (Simoes 1999). Repeated infections are common in all age-groups, as previous infection does not prevent subsequent infections (Simoes 1999). RSV is a seasonal virus, with winter and spring epidemics occurring annually in temperate climates (Kim *et al.* 1973). In Finland RSV epidemics have typically occurred in two-year cycles, with a minor epidemic in spring preceding a major epidemic in winter (Waris 1991). RSV has two subtypes, A and B, according to variations in the large envelope glycoproteins (Papadopoulos *et al.* 2004).

Rhinoviruses

Rhinoviruses have been identified in 8–82% of children requiring hospitalization for lower respiratory tract infection with wheezing (Hasegawa *et al.* 2014a, Jartti *et al.* 2004). Children hospitalized with rhinovirus infection have been older than children hospitalized on account of an RSV infection (Jartti *et al.* 2009b, Korppi *et al.* 2004a). Infants with rhinovirus bronchiolitis have been reported to be more likely to have atopic dermatitis and eosinophilia than infants with RSV bronchiolitis, and to have higher levels of exhaled nitric oxide and more allergic

sensitization to foods (Jartti *et al.* 2009b, Korppi *et al.* 2004a). The prevalence of rhinovirus-associated wheezing increases with age, and it is significantly more common in children with recurrent wheezing episodes than in first-time wheezers in the age categories <24 and <36 months (Jartti *et al.* 2009a). There are over 100 types of human rhinovirus, which are classified into three species (A, B and C) on phylogenetic sequence criteria. The recently identified human rhinovirus group C has been shown to be associated with severe wheezing episodes in young children more frequently than the other rhinovirus groups (Cox *et al.* 2013).

Human metapneumovirus (hMPV)

The hMPV is a single-stranded RNA virus and a member of the newly discovered *Metapneumovirus* genus of the *Paramyxoviridae* family that infects humans (van den Hoogen *et al.* 2001). The clinical course of hMPV infection resembles that of RSV infection (Jartti *et al.* 2002). The proportion of hMPV detected in nasopharyngeal aspirate samples of children with acute wheezing has varied from 5% to 9% (Garcia-Garcia *et al.* 2010, Jartti *et al.* 2002, Smuts *et al.* 2008, Williams *et al.* 2005).

A recent prospective population-based surveillance project including over 10 000 children less than 5 years of age with acute respiratory illness detected hMPV in 6% of children admitted to hospital and 7% of outpatients (Edwards *et al.* 2013). The children hospitalized on account of an hMPV infection were older and were more likely to require supplementary oxygen and to have a longer stay in the intensive care unit than those hospitalized without an hMPV infection (Edwards *et al.* 2013).

Other viruses

Influenza viruses, which belong to the *Orthomyxoviridae* family, cause severe lower respiratory tract complications such as bronchitis and pneumonia (Inoue & Shimojo 2013). In addition, influenza is significantly associated with wheezing during the winter among children younger than 3 years of age and has been detected in 6–10% of such patients (Mansbach *et al.* 2008b, Miller *et al.* 2013).

The parainfluenza viruses, belonging to the *Pneumoviridae* genus of the *Paramyxoviridae* family, are classified into types 1–4 and cause respiratory illnesses (Weinberg *et al.* 2009). Parainfluenza virus 3 in particular may cause bronchiolitis and other lower respiratory tract infections in infants (Weinberg *et al.* 2009).

The human bocavirus, which belongs to the *Bocavirus* genus of the family *Parvoviridae*, has been associated with upper and lower respiratory infections and is detected all the year around with rates of 0.5–25% in patients with lower respiratory tract infection (Allander 2008). The pathogenicity of human bocavirus has been questioned, as it is mainly detected as a co-infection (Allander 2008). However, more solid data for the case that primary human bocavirus infection is an important cause of respiratory tract infections in young children have been provided by use of PCR in serum, by high viral copy numbers in nasopharyngeal samples and by serology (Peltola *et al.* 2013).

2.2.3 Bronchiolitis (wheezing in infancy)

Occurrence

More than a third of all children develop bronchiolitis during the first year of life, and approximately 3% are hospitalized (Smyth & Openshaw 2006, Zorc & Hall 2010). The annual incidence of emergency department visits on account of bronchiolitis was 36.3 per 1000 person-years in the United States in 2010 within children aged <2 years, implying total costs of \$389 million (Hasegawa *et al.* 2014b). Hospitalization rates for bronchiolitis doubled from 1980s to 2000s in United States and Europe (Bjor & Braback 2003, Langley *et al.* 2003, Shay *et al.* 1999). The annual incidence of bronchiolitis hospitalization has decreased from 17.9 to 14.9 per 1000 person-years between 2000 and 2009 in the United States, while the use of mechanical ventilation and also hospital charges increased significantly over the same period. The total hospital charges for bronchiolitis in the United States in 2009 were \$1.7 billion (Hasegawa *et al.* 2013). The risk of hospitalization is high in children aged <6 months with bronchiolitis, and these children face a high risk of needing mechanical respiratory support with this condition (Deshpande & Northern 2003, Mansbach *et al.* 2012). The annual rates of hospitalization for RSV bronchiolitis are 16.9 per 1000 for infants at 0–5 months of age in the United States and 5.1 per 1000 for those aged 6–11 months (Langley & Anderson 2011).

Clinical course

After a brief 1 to 3 day prodrome of upper respiratory symptoms including nasal obstruction and/or rhinorrhoea with low-grade fever, typical signs of bronchiolitis occur, characterized by coughing, wheezing, retractions, dyspnoea, respiratory distress, poor feeding and tachypnoea (>50/min) (AAP 2006, Horst 1994). On physical examination, diffuse wheezing, prolonged expiration and crackles are heard in the lungs (Horst 1994). The diagnosis of bronchiolitis is a clinical one (AAP 2006). The severity of bronchiolitis can vary from mild respiratory symptoms treated at home to severe respiratory distress and hypoxaemia requiring mechanical ventilation (Smyth & Openshaw 2006). The symptoms can progress rapidly at the beginning of the disease, and the first 48 to 72 hours of coughing and dyspnoea are the most critical in terms of respiratory compromise (Horst 1994). The duration of the disease varies, with the symptoms resolving within 13 days in 50% of patients, and within 21 days in 90% (Thompson *et al.* 2013). Bronchiolitis-associated deaths are rare, the annual rate reported among children under the age of 1 year in the United States being 2.0 per 100 000 live births (Holman *et al.* 2003).

Central apnoea is a serious and well-known complication in infants with bronchiolitis (Church *et al.* 1984). Previous studies among hospitalized infants found rates of apnoea ranging from 16% to 24%, with prematurity, young age and RSV infection identified as risk factors for its development (Ralston & Hill 2009, Willwerth *et al.* 2006). Recent studies have found a <1% incidence of apnoea in previously healthy term infants, the risk of apnoea being similar for all the major viral pathogens (Ralston & Hill 2009, Schroeder *et al.* 2013).

Pathogenesis

The word “bronchiolitis” refers to inflammation of bronchioles due to virus infection. The bronchioles are filled with necrotic debris and purulent exudative material and the mucosa and surrounding bronchiolar wall contain a neutrophilic infiltrate (Allen 2010). Plugs of sloughed necrotic epithelium and fibrin in the airways cause partial or total obstruction of the airflow, the degree of which may vary as these areas are cleared, resulting in rapidly changing clinical signs that confound any accurate assessment of the severity of illness (Zorc & Hall 2010). A “ball-valve” mechanism can result in the trapping of air in places distal to the obstructed areas, with subsequent absorption, atelectasis, and a mismatch of pulmonary ventilation and perfusion that may lead to hypoxaemia (Zorc & Hall

2010). Atelectasis may be accelerated by the lack of collateral channels in young children, and potentially by the administration of a high concentration of supplementary oxygen, which is absorbed more rapidly than room air. Smooth-muscle constriction seems to have a minor role in the pathological process (Zorc & Hall 2010).

The number of viruses recognized as causing bronchiolitis has markedly expanded with the use of molecular amplification techniques. The most common cause of bronchiolitis is RSV, while other causes include parainfluenza viruses, influenza, adenovirus, human metapneumovirus (hMPV), human bocavirus, coronaviruses, rhinovirus and *Bordetella pertussis* (Miller *et al.* 2013, Nuolivirta *et al.* 2010). Multiple viruses have been found in 10-30% of bronchiolitis cases (Zorc & Hall 2010).

Risk factors for severe bronchiolitis

Host factors

RSV is recognized by the transmembrane Toll-like receptor 4 (TLR4) and adapter proteins in the distal respiratory tract, leading to the production of proinflammatory cytokines and subsequent activation of the adaptive immune response (Rämet *et al.* 2011). The surfactant proteins A (SP-A) and D (SP-D) are able to bind both RSV and TLR4, modulating the inflammatory response. Genetic variations in TLR4, SP-A and SP-D have been associated with a risk of severe RSV bronchiolitis, but the results have varied between studies (Rämet *et al.* 2011).

The majority of severe bronchiolitis cases occur in infants <2 months old and at that age nearly all cases of bronchiolitis are caused by RSV (Mansbach *et al.* 2012). These young infants have more than a 4-fold risk of requiring intubation or mechanical respiratory support during the course of bronchiolitis compared to infants >1 year of age (OR 4.29 95% CI 1.7 to 11.5) (Mansbach *et al.* 2012). The risk factors for severe RSV infection are premature birth, especially with chronic lung disease of prematurity but also without, congenital heart disease and T-cell immunodeficiency (Boyce *et al.* 2000, Hall *et al.* 1986, MacDonald *et al.* 1982). In observational studies 7% to 13% of children born at or before 32 weeks of gestation were hospitalized for RSV as compared with 2% to 7% of children born after 32 weeks (Langley & Anderson 2011). In addition to a greater likelihood of hospitalization, premature infants have a longer stay in hospital (6–7 vs. 4 days)

and in the intensive care unit (6 vs. 4 days) than healthy infants (Langley & Anderson 2011). In a recent population-based birth cohort of nearly 300 000 children 7.5% were born prematurely, and prematurity was associated with hospitalization for RSV infection during the first year of life with a relative risk (RR) of 2.1 (95% CI 1.8 to 2.0) compared with infants born at term (Murray *et al.* 2014).

Infants having a low birth weight for their gestational age are more prone to be admitted to hospital for lower respiratory tract infections in the first year of life than normal birth weight infants (Barker *et al.* 1991). On the other hand, in a birth cohort of 298 healthy term newborns, birth weight >4000g proved to be a risk factor for lower respiratory infection caused by RSV in the first year of life (OR 2.2 95% CI 1.1 to 4.6) (Houben *et al.* 2011).

Children with neuromuscular impairment in all age groups are more likely to require intensive care and mechanical ventilation for RSV infection than children without neuromuscular impairment, despite their higher median age at diagnosis (Wilkesmann *et al.* 2007). Cystic fibrosis (CF) is a risk factor for severe viral respiratory infections, and RSV is an important cause of early acute respiratory tract morbidity in young infants with CF (Abman *et al.* 1988). Down's syndrome is an independent risk factor for severe RSV infections. In a prospective cohort study of 215 children with Down's syndrome, where other high-risk conditions were excluded, 8% were hospitalized for an RSV infection during the first 2 years of life (Bloemers *et al.* 2007).

The month of birth affects an infant's risk of RSV-related hospitalization during the entire first year of life, children born between October and February having the highest risk in the United States (Lloyd *et al.* 2014). One-month-old infants born in January have a 10-fold risk of RSV-related hospitalization than those born in October (RR 9.8 95% CI 7.8 to 12.4) (Lloyd *et al.* 2014). Male sex is a risk factor for severe RSV infection, the risk ratio of boys to girls being 1.4:1 (Simoes 2003). Breastfeeding has been reported to reduce the risk of hospitalization for bronchiolitis during the first year of life (Lanari *et al.* 2013). In a prospective cohort study including 1814 newborns with ≥ 33 gestational weeks, in which 23% had never been breastfed, in the breastfed group 65% had been exclusively breastfed and 35% were breastfed but received additional formula, the risk of hospitalization by 12 months age was significantly higher in those who had never been breastfed (HR 1.6 95% CI 1.0 to 2.5) (Lanari *et al.* 2013).

Aetiology

The severity of bronchiolitis, defined in terms of the length of hospitalization and need for supplementary oxygen, intensive care and mechanical ventilation, is significantly worse in children with an RSV infection than those with a non-RSV infection (Garcia *et al.* 2010, Hervas *et al.* 2012, Marguet *et al.* 2009) .

RSV subtype A has been reported to cause more severe bronchiolitis than subtype B (Papadopoulos *et al.* 2004). RSV shedding durations have been closely associated with age and the severity of infection, as young infants with severe bronchiolitis symptoms take longer to shed the virus than those with a milder infection (Munywoki *et al.* 2014). Symptomatic RSV infections have an average virus shedding time of 13.5 days as compared with 7.8 days in asymptomatic episodes (Munywoki *et al.* 2014). The level of RSV load has been observed to correlate positively with the duration of hospitalization and the severity of bronchiolitis (Scagnolari *et al.* 2012).

Clinical predictors

Findings at physical examination have been less consistently associated with the outcomes of bronchiolitis than either individual risk factors or viral findings (AAP 2006).

Tachypnoea, defined as a respiratory rate of 60 or more breaths per minute, is associated with an increased risk of severe bronchiolitis (Brooks *et al.* 1999, Corneli *et al.* 2012, Parker *et al.* 2009, Shaw *et al.* 1991). Severe retractions on arrival and a history of poor fluid intake have been observed to predict the need for medical interventions in infants brought to an emergency department with bronchiolitis (Damore *et al.* 2008, Mansbach *et al.* 2012, Parker *et al.* 2009). Tachycardia, defined as a heart rate of 150 or more per minute or greater than the 97th percentile for age, has been identified as a risk factor for admission and a longer stay in hospital (Walsh *et al.* 2004). On the other hand, it has been reported that the clinical assessment of respiratory rate, heart rate and the presence of retractions fails to differentiate between patients with bronchiolitis who are discharged from the emergency department but return requiring admission and those who do not return (Roback & Baskin 1997). A fever (>38°C) at the time of admission to hospital for bronchiolitis is reported to predict a severe course of the disease (Ricart *et al.* 2013).

Environmental factors

Crowded living conditions, day care attendance and living with siblings are significant risk factors for severe bronchiolitis (Simoes 2003). Exposure to environmental tobacco smoke is a universally established risk factor for susceptibility to bronchiolitis, its severity and a detrimental outcome (Simoes 2003). In a recent prospective cohort of 378 children admitted to hospital for bronchiolitis, household tobacco smoking predicted the need for oxygen supplementation on admission (OR 2.5 95% CI 1.6 to 3.7) and was a significant predictor of a need for mechanical ventilation during the course of the disease (OR 5.5 95% CI 2.8 to 10.8) (Semple *et al.* 2011).

Outcomes after bronchiolitis in infancy

RSV infection in infancy, particularly if severe enough to lead to hospitalization, has been recognized as an important predictor of recurrent wheezing, airway hyperresponsiveness, asthma and allergic sensitization in later childhood (Regnier & Huels 2013, Sigurs *et al.* 2000, Stein *et al.* 1999). It is not known whether RSV infection contributes to the inception of asthma or simply identifies infants who run an increased risk of subsequent wheezing due to an asthmatic predisposition, an atopic predisposition or pre-existing abnormal lung function (Martinez *et al.* 1995, Singh *et al.* 2007). The relationships between viral respiratory infections in early life, clinical bronchiolitis and the development of asthma are complex and it is likely that both host and environmental factors modify the risk of asthma (Beigelman & Bacharier 2013).

In the double-blind, placebo-controlled trial 429 otherwise healthy preterm infants born at gestational age of 33 to 35 weeks were randomly assigned to receive either monthly palivizumab injections or placebo (Blanken *et al.* 2013). Palivizumab treatment resulted in a significant reduction of 61% (95% CI 56 to 65) in the total number of wheezing days during the first year of life (Blanken *et al.* 2013). These findings implicate RSV infection as an important mechanism of wheeze during the first year of life in these infants.

Wheezing and asthma

Up to 40% of children with a history of RSV bronchiolitis have wheezing during the first 5 years of life, compared with 11% of patients without bronchiolitis, but this difference diminishes after the age of 5 years (Kneyber *et al.* 2000).

Asthma is a chronic inflammatory disorder of the airways, the typical symptoms of which are coughing, wheezing and shortness of breath (Lemanske & Busse 2003). The development of airflow limitation is the result of bronchoconstriction, airway oedema, mucus secretion and airway remodelling (Kudo *et al.* 2013). Generally most asthma starts from childhood in connection with sensitization to common inhaled allergens (Kudo *et al.* 2013). Asthma is diagnosed based on the symptoms observed and measurements of pulmonary function, revealing variable or reversible airways obstruction (Lemanske & Busse 2003).

According to the International Study of Asthma and Allergies in Childhood (ISAAC), the global prevalence of frequent or severe asthma symptoms is 4.9%, and wheezing occurs in 11.5% of children at the age of 6 to 7 years (Lai *et al.* 2009). The lifetime prevalence of asthma was estimated to be 4.0% in a non-selected population-based study of children aged from 7 to 12 years in Finland (Remes *et al.* 1996).

Existing clinical studies on the association of RSV infection during early childhood with asthma or wheezing in relation to the situation in healthy children are summarized in Table 1. The definition of wheezing as an outcome has mainly been based on parental reports and has varied from any wheezing during the follow-up to frequent or recurrent wheezing, i.e. at least three episodes of wheezing either during the whole follow-up or in the past year. Asthma has usually been defined in terms of either parentally reported and doctor-diagnosed asthma (with ongoing maintenance medication in some studies) or recurrent wheezing verified by a doctor. A positive association between RSV infection and asthma or wheezing has been found in most studies, but in several series either no association has been found or the positive association has been lost during follow-up. Two recent reviews and one meta-analysis concluded that the association between early RSV infection and subsequent asthma/wheezing was strong but tended to decrease with age at follow-up (Perez-Yarza *et al.* 2007, Regnier & Huels 2013). In the Tucson Children's Respiratory Study it was found that children with previous RSV infection were no more likely to have infrequent or frequent wheezing at the age of 13 years than the controls (Stein *et al.* 1999).

Hospitalization for wheezing caused by non-RSV viruses, especially rhinovirus, has been found to have an even closer association with the later development of asthma and wheezing than hospitalization for RSV-induced wheezing (Jackson *et al.* 2008). In series of 82 children who had been hospitalized for wheezing in infancy the risk of asthma at 7 years of age was 4-fold after rhinovirus wheezing relative to rhinovirus-negative wheezing (Kotaniemi-Syrjanen *et al.* 2003). Among children with a non-RSV infection, a history of wheezing, atopic dermatitis, skin prick test positivity and age more than 12 months were reported to be significant predictors of asthma 3 years after hospitalization (Reijonen *et al.* 2000). Children requiring hospitalization for wheezing during rhinovirus infection have atopic dermatitis and blood eosinophilia more often than those with RSV infection and they are older (Jartti *et al.* 2004, Korppi *et al.* 2004a).

Table 1. Clinical studies of the association of RSV infection with wheezing/asthma employing healthy controls.

References	Number of RSV patients/controls followed up	Age at the time of RSV infection	Hospitalized/ outpatients	Age at the end of follow-up	Outcome	Odds Ratio (OR) and 95% confidence intervals (CI) or Relative Risk (RR) and 95% confidence intervals (CI) or differences in proportions
Sims <i>et al.</i> 1978	35/35	Infancy	Hospitalized	8 years	Any wheezing	OR 10.1 (1.2 to 85.6)
Pullan & Hey 1982	130/111	<1 year	Hospitalized	10 years	Wheezing in last year	OR 1.4 (0.4 to 4.4)
Mok & Simpson 1982	100/200	<1 year	Hospitalized	7 years	Asthma	OR 2.5 (0.8 to 8.4)
Murray <i>et al.</i> 1992	42/73	<1 year	Hospitalized	5.5 years	Wheezing in last year	OR 3.8 (1.6 to 9.3)
Sigurs <i>et al.</i> 1995	47/93	<1 year	Hospitalized	3 years	Recurrent wheezing	RR 1.8 (0.8 to 3.9)
					Asthma	RR 21.8 (2.9 to 163.6)
					Any wheezing	RR 1.9 (1.3 to 2.7)
Sigurs <i>et al.</i> 2000	47/93	<1 year		7.5 years	Asthma	RR 12.7 (3.4 to 47.1)
					Any wheezing	RR 5.3 (2.2 to 12.5)
Sigurs <i>et al.</i> 2005	46/92	<1 year		13 years	Current asthma	OR 10.1 (3.4 to 29.8)
					Current asthma or recurrent wheezing	OR 9.3 (3.6 to 24.5)
					Current asthma	32.6% vs. 6.5%, diff. 0.26 (0.13 to 0.41)
					Current asthma or recurrent wheezing	39.1% vs. 8.7%, diff. 0.30 (0.16 to 0.46)
					Current asthma	
					Current asthma or recurrent wheezing	
Stein <i>et al.</i> 1999	207/369	<3 years	Outpatients	6 years	Frequent wheezing	OR 4.3 (2.2 to 8.7)
				8 years	Infrequent wheezing	OR 2.5 (1.5 to 4.3)
					Frequent wheezing	OR 1.9 (0.9 to 4.2)
				11 years	Infrequent wheezing	OR 1.7 (1.0 to 2.9)
					Frequent wheezing	OR 2.4 (1.3 to 4.6)
				13 years	Infrequent wheezing	OR 1.4 (0.7 to 2.7)
					Frequent wheezing	OR 1.4 (0.7 to 2.6)

References	Number of RSV patients/controls followed up	Age at the time of RSV infection	Hospitalized/ outpatients	Age at the end of follow-up	Outcome	Odds Ratio (OR) and 95% confidence intervals (CI) or Relative Risk (RR) and 95% confidence intervals (CI) or differences in proportions
Schauer <i>et al.</i> 2002	42/84	<1 year	Hospitalized	1 year	Recurrent wheezing	OR 8.9 (1.4 to 55.9)
Singleton <i>et al.</i> 2003	95/113	<2 years	Hospitalized	5-8 years	Asthma	RR 3.1 (1.6 to 6.1)
Juntti <i>et al.</i> 2003	76/76	<1 year	Hospitalized	8 years	Asthma	OR 1.9 (0.9 to 4.2)
Korppi <i>et al.</i> 2004b	36/45	<2 years	Hospitalized	18-20 years	Previous asthma	OR 2.1 (0.6 to 7.6)
Backman <i>et al.</i> 2014	27/86	<2 years		28-31 years	Current asthma	OR 1.4 (0.4 to 5.4)
					Asthma	33.3% vs. 12.8%, diff. 0.21 (0.03 to 0.41)
Henderson <i>et al.</i> 2005	96/9826	<1 year	Hospitalized	30-42 months	Reported wheezing	OR 2.3 (1.3 to 3.9)
	62/7216	<1 year		69-81 months	Reported wheezing	OR 3.5 (1.8 to 6.6)
	73/8039	<1 year		91 months	Asthma	OR 2.5 (1.4 to 4.3)
Fjaerli <i>et al.</i> 2005	35/64	<1 year	Hospitalized	7 years	Asthma	OR 17.7 (5.7 to 55.1)
Garcia-Garcia <i>et al.</i> 2007	32/38	<2 years	Hospitalized	4 years	Asthma	OR 10.1 (2.5 to 40.1)
					Recurrent wheezing	75.0% vs. 40.0%, diff. 0.35 (0.10 to 0.56)
Mikalsen <i>et al.</i> 2012	90/141	<1 year	Hospitalized	11 years	Asthma	OR 1.8 (0.8 to 4.1)
Zomer-Kooijker <i>et al.</i> 2014	159/549	<13 months	Hospitalized	6 years	Current wheezing	OR 3.2 (1.2 to 8.1)

Atopic allergy

The link between RSV infection in infancy and atopy in later childhood is uncertain, with some studies supporting an association and others refuting it (Table 2). In many studies the term atopy has been used solely to indicate positive findings of sensitization, e.g. in skin-prick tests.

Atopic manifestations after RSV infection other than asthma or atopic sensitization have seldom been assessed in clinical studies. No difference has been found in the occurrence of atopic dermatitis after RSV infection in infancy relative to healthy controls (Garcia-Garcia *et al.* 2007, Juntti *et al.* 2003, Schauer *et al.* 2002, Sigurs *et al.* 2000, Sigurs *et al.* 2005, Sigurs *et al.* 2010).

Table 2. Clinical studies of the association of RSV infection with atopic sensitization employing healthy controls

References	Number of RSV patients/controls followed up	Age at the time of RSV infection	Hospitalized/ outpatients	Age at the end of follow-up	Sensitization defined by	Association of RSV infection with sensitization; OR/RR (95% CI) or difference in proportions
Sims <i>et al.</i> 1978	32/26	Infancy	Hospitalized	8 years	Skin prick tests	20.0% vs. 17.1%, diff. 0.03 (-0.16 to 0.22)
Pullan & Hey 1982	130/111	<1 year	Hospitalized	10 years	Skin prick tests	15.0% vs. 27.5%, diff. -0.12 (-0.24 to -0.01)
Sigurs <i>et al.</i> 1995	47/93	<1 year	Hospitalized	3 years	Skin prick tests or serum IgE antibodies	RR 3.6 (1.6 to 8.0)
Sigurs <i>et al.</i> 2000	47/93	<1 year		7.5 years		RR 2.4 (1.1 to 5.5)
Sigurs <i>et al.</i> 2005	46/92	<1 year		13 years	Animal dander	OR 5.6 (2.2 to 14.4)
Sigurs <i>et al.</i> 2010	46/92	<1 year		18 years	Animal dander	32.5% vs. 10.6%, diff. 0.22 (0.07 to 0.39)
Stein <i>et al.</i> 1999	207/369	<3 years	Outpatients	6 years	Perennial allergens Skin prick tests	40.0% vs. 14.1%, diff. 0.26 (0.10 to 0.43) 37.4% vs. 39.7%, diff. -0.02 (-0.12 to 0.07)
Schauer <i>et al.</i> 2002	42/84	<1 year	Hospitalized	1 year	Serum IgE antibodies	OR 20.7 (3.5 to 120.8)
Juntti <i>et al.</i> 2003	76/76	<1 year	Hospitalized	8 years	Skin prick tests	7.8% vs. 43.1%, diff. -0.35 (-0.50 to -0.19)
Korppi <i>et al.</i> 2004b	36/45	<2 years	Hospitalized	18-20 years	Skin prick tests	60.0% vs. 47.7% diff. 0.12 (-0.10 to 0.33)

References	Number of RSV patients/controls followed up	Age at the time of RSV infection	Hospitalized/ outpatients	Age at the end of follow-up	Sensitization defined by	Association of RSV infection with sensitization; OR/RR (95% CI) or difference in proportions
Henderson <i>et al.</i> 2005	48/6377	<1 year	Hospitalized	7 years	Skin prick tests	OR 0.7 (0.2 to 1.7)
García-García <i>et al.</i> 2007	32/38	<2 years	Hospitalized	4 years	Skin prick tests	25.8% vs. 37.5% diff. -0.12 (-0.40 to 0.15)

2.2.4 Obstructive bronchitis (preschool wheeze)

Occurrence

Obstructive bronchitis is highly prevalent among children aged between 1 and 6 years. It has been shown in a population-based survey that about a third of all children in Europe and the United States had suffered from recurrent wheezing and coughing during the recent 6 winter months (Bisgaard & Szeffler 2007). A life-time prevalence of doctor-diagnosed obstructive bronchitis in a population of over 21 000 schoolchildren aged between 7 and 11 years was 12.3% in a cross-sectional study conducted in Central and Eastern Europe (Leonardi *et al.* 2002). The prevalence of obstructive bronchitis episodes among children aged between 1 and 5 years increased in England in the 1990s from 9% to 19% in repeated population surveys (Kuehni *et al.* 2001) .

Obstructive bronchitis is one of the most common causes of emergency department visits and unplanned hospital admissions among preschool children (Kuehni *et al.* 2001). Hospital admission rates among children <4 years of age with infection-induced wheezing, including obstructive bronchitis, ranged from 15 to 39 admissions per 1000 per year (Langley *et al.* 2003, Martinez *et al.* 1995). Hospital admission rates for asthma among schoolchildren have decreased considerably during recent decades, but admission rates for infection-induced wheezing illness in young children have remained high (Wennergren & Strannegard 2002).

Clinical course

Obstructive bronchitis is an acute respiratory illness characterised by cough, rhonchi and expiratory distress (Court 1973). Upper respiratory symptoms such as nasal discharge are frequent (Court 1973). The expiratory distress experienced during obstructive bronchitis is characterized by wheezing, shortness of breath, tachypnoea and a need for and response to inhaled short-acting beta-agonists (Konstantinou *et al.* 2013). Obstructive bronchitis is associated with viral respiratory tract infections, and repeated episodes tend to occur seasonally (Ducharme *et al.* 2014). The most common causative agents are rhinovirus, RSV, coronavirus, hMPV, parainfluenza virus and adenovirus (Brand *et al.* 2008).

Wheezing was reported to start a mean of 43 hours (SD 7 hours) after the first symptoms of respiratory infection and to persist for 3.8 days (SD 4.2 days) among

54 patients aged from 1 to 6 years with a history of recurrent attacks of obstructive bronchitis, who were prospectively followed up for 3 months (Mertsola *et al.* 1991).

Mechanisms of bronchoconstriction

The epithelial cells are a focal point in the pathogenesis of viral respiratory infections, serving as host cells for viral replication and initiators of innate immune responses. Damage to the epithelial cells can disturb the airway physiology through various pathways. Epithelial oedema and shedding, together with mucus production, can cause airway obstruction and wheezing (Gern 2003). It has been hypothesized that smooth muscle cells in the airways may become infected during viral invasion, although this has not been verified *in vivo* (Grunstein *et al.* 2001).

Information derived from animals and humans indicates that viruses can enhance the susceptibility of airways to virus-induced bronchoconstriction (Gern 2003). The mechanisms of virus-induced bronchoconstriction appear to be multifactorial, and contributing factors are likely to include impairment in the inactivation of tachykinins, virus effects on nitric oxide production and virus-induced changes in neural control over the airways (Smith *et al.* 1987). The tachykinins, which are synthesized by sensory nerves, are potent bronchoconstrictors and the airway epithelial cells help to regulate their levels through production of the enzyme neutral endopeptidase, which can be damaged by viral infection (Gern 2003). Nitric oxide synthesis, which can regulate both vascular and bronchial tone, is enhanced by viral infections (Gern 2003). Viruses can also affect airway tone by enhancing vagally mediated reflex bronchoconstriction, as demonstrated in both human and animal models (Gern 2003). One possible mechanism for this effect is that the M2 muscarinic receptor is damaged by a virus or virus-induced interferon, leading to enhanced bronchoconstriction (Bowerfind *et al.* 2002).

Risk factors for obstructive bronchitis

Maternal smoking has been associated with transient early wheezing, i.e. wheezing by the age of 3 years but no longer at the age of 6 years (Martinez *et al.* 1995). By contrast, late-onset wheezing, i.e. no wheezing at the age of 3 years but wheezing at age of 6 years, has been associated with maternal asthma, male sex and early-onset rhinitis (Martinez *et al.* 1995). Persistent wheezing, i.e. wheezing at the age of 3 years and at the age of 6 years has been associated with maternal asthma,

frequent wheezing and the diagnosis of asthma and atopy in childhood (Martinez *et al.* 1995).

It was found in the Tucson Children's Respiratory Study that infants exposed to other children at home or in day care experienced more frequent wheezing at an age of 2 years (OR 1.6 95% CI 1.1 to 2.1) (Taussig *et al.* 2003). In addition, a population-based prospective cohort study of 5125 children concluded that accelerated weight gain from birth to 3 months following normal fetal growth was associated with an increased risk of wheezing until the age of 4 years (OR 1.4 95% CI 1.2 to 1.7) (Sonnenschein-van der Voort *et al.* 2012).

Genome-wide association studies and candidate-gene analyses have identified several common genetic variants associated with asthma (Ducharme *et al.* 2014). The 17q21 locus is the most consistently replicated risk factor for childhood asthma and recent studies have documented a more pronounced effect of this locus among children exposed to environmental tobacco smoke in early life and children with early life respiratory infections, suggesting gene-environment interactions (Caliskan *et al.* 2013, Smit *et al.* 2010).

Outcomes after obstructive bronchitis

Wheezing symptoms and recurrent episodes of obstructive bronchitis during viral infections are common among preschool children, but the symptoms tend to decrease with time (Goksor *et al.* 2006, Wennergren *et al.* 1992). Thus 50% of children hospitalized for acute wheezing before the age of 2 years were symptom-free by the age of 5 years and 70% by the age of 10 years (Goksor *et al.* 2006, Wennergren *et al.* 1992). On the other hand, 25% of the children with persistent asthma in the Tucson Children's Respiratory Study had started to wheeze by the age of 6 months and 75% by the age of 3 years (Martinez *et al.* 1995).

Despite a seemingly benign prognosis regarding symptoms, findings from birth cohorts have consistently shown that, as a group, preschool children with wheezing have permanent deficits in lung function at 6 years of age (Grad & Morgan 2012). Population-based lung function studies have shown adolescents with a history of transient or persistent wheezing before the age of 3 years to have significantly lower forced expiratory flows than those without any history of early wheezing (Morgan *et al.* 2005). However, in some studies it has been lung function at birth rather than wheezing symptoms at preschool age that has explained later deficits in lung function (Haland *et al.* 2009).

2.3 Evaluation of the severity of childhood dyspnoea

2.3.1 Auscultation

The aim of auscultation of the chest is to assess the ratio of expiration to inspiration and the degree of dyspnoea, whereas the degree of airway narrowing can only be estimated crudely and indirectly by this means (Brand *et al.* 2008). One important aim of auscultation is to identify unusual or atypical wheezing features, that might suggest some other underlying condition such as a foreign body or an anatomical abnormality (Brand *et al.* 2008).

Croup

Inspiratory stridor is the main auscultation finding in the case of croup (Bjornson & Johnson 2005). Impeding respiratory failure in severe croup includes decreasing stridor and decreasing breath sounds (Bjornson & Johnson 2005).

Bronchiolitis

Crackles and/or wheezing are the typical auscultation findings in the case of bronchiolitis and these findings have variability according to the state of the disease (AAP 2006, Horst 1994).

Acute asthma exacerbations

Wheezing and prolonged expiration are the main auscultation findings in the case of asthma exacerbations and impending respiratory failure in severe exacerbations includes decreasing breath sounds (GINA 2014).

2.3.2 Clinical scores

Individual clinical signs such as auscultation findings, respiratory rate or accessory muscle use do not correlate well with the degree of airway narrowing in acute dyspnoea (Commey & Levison 1976, Kerem *et al.* 1991). Many clinical trials have used clinical scores to describe the degree of acute dyspnoea (Powell *et al.* 2013, Wu *et al.* 2014). These scores have included measurements of respiratory rate, heart

rate, respiratory effort and oxygenation in addition to auscultation findings (Bekhof *et al.* 2014).

The substantial temporal variability in physical findings and the fact that clinical scores have not been validated for their clinical predictive value in cases of croup, bronchiolitis, obstructive bronchitis and acute asthma exacerbations have not allowed these scores to be used in a clinically meaningful manner, and comparison between studies is difficult (AAP 2006, Bekhof *et al.* 2014, Bjornson & Johnson 2008, Bjornson & Johnson 2013).

2.3.3 Pulse oximetry

Measurement of the partial pressure of oxygen in arterial blood (PaO₂) has been the standard method for evaluating oxygenation in a clinical setting. Pulse oximetry is a widely available technology that provides an easy, non-invasive and reliable method of monitoring oxygenation (Schnapp & Cohen 1990). Clinical assessment of hypoxaemia is unreliable, and arterial blood oxygen saturation ~75% is required before central cyanosis becomes clinically detectable even under optimal conditions (Martin & Khalil 1990).

The estimation of arterial haemoglobin oxygen saturation by pulse oximetry is based on the specific characteristics of oxygenated and deoxygenated haemoglobin with regard to light absorption in the red and infrared spectra (Fouzas *et al.* 2011). Deoxygenated haemoglobin is characterized by greater red light absorption than oxygenated haemoglobin, whereas oxygenated haemoglobin exhibits higher absorption in the infrared spectrum (Fouzas *et al.* 2011). By obtaining the ratio of light absorption in the red and infrared spectra and then calculating the ratio of these 2 ratios, the percentage of oxygenated haemoglobin can be calculated (Fouzas *et al.* 2011).

The estimation of arterial haemoglobin oxygen saturation by pulse oximetry and PaO₂ are related through the oxygenated haemoglobin dissociation curve, and their relation is not linear. A series of factors can influence the shape of the curve (Fig.2) (Fouzas *et al.* 2011).

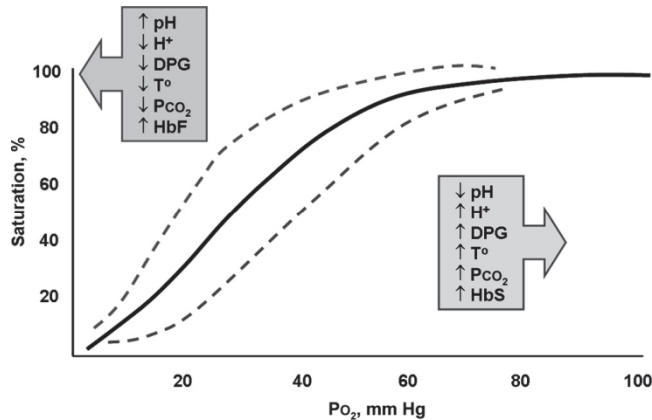


Fig. 2. The oxygenated haemoglobin dissociation curve and factors that influence its shape. The curve is shifted to the right by increases in the hydrogen ion (H⁺) concentration (acidosis), 2,3diphosphoglycerate (DPG), temperature (T°), and the partial pressure of carbon dioxide (Pco₂) and by the presence of haemoglobin S (HbS) (sickle cell disease). Decreases in H⁺ (alkalosis), DPG, T°, and Pco₂ and the presence of fetal haemoglobin (HbF) shift the curve to the left (Fouzas *et al.* 2011). Reprinted with permission from The American Association of Pediatrics.

Pulse oximetry readings vary with age and altitude, with mean oxygen saturation values of 97% to 99% (–2SDs: 95%–96%) reported in healthy infants and children at sea level (Balasubramanian *et al.* 2006, Mau *et al.* 2005).

In paediatric practise, pulse oximetry should be available in any situation associated with hypoxaemia (Fouzas *et al.* 2011). Oxygen saturation is a sensitive indicator of disease severity in children with wheezing (Rosen *et al.* 1989, Sole *et al.* 1999). Oxygen saturation is not reliable indicator of disease severity in proximal airway obstruction such as acute laryngotracheitis, foreign body aspiration or vocal chord dysfunction (Fouzas *et al.* 2011).

Croup

Continuous pulse oximetry is indicated in children with severe croup, as they can have low oxygen saturation as a result of intrapulmonary involvement of their viral infection, but is not necessary in mild croup cases (Bjornson & Johnson 2008). It was concluded in a study of 199 patients with a mean age of 2 years requiring hospitalization for croup that there was no correlation between initial oxygen saturation and the length of stay (Stoney & Chakrabarti 1991).

Bronchiolitis

Pulse oximetry is among the measures that are most closely correlated with the outcomes of bronchiolitis (Table 3) (Zorc & Hall 2010). However, there is no consensus on the oxygen saturation thresholds that should be used to admit, treat and discharge infants with acute bronchiolitis (Fouzas *et al.* 2011).

The American Academy of Pediatrics guideline recommends the administration of supplementary oxygen if oxygen saturation values fall to <90% (AAP 2006). The Scottish Intercollegiate Guidelines Network recommends admission to hospital for all symptomatic infants with oxygen saturation values of $\leq 92\%$, whereas the decision to admit and/or treat patients with an oxygen saturation of 93% to 94% should be made on an individual basis (SIGN 2006).

In a multicentre prospective study including 1456 bronchiolitis patients aged <2 years, initial oxygen saturation values $\geq 94\%$ predicted safe discharge from the emergency department (Mansbach *et al.* 2008a). Furthermore, an initial oxygen saturation of <94% proved to be the best predictor of hospital admission in a recent multicentre study of 598 infants aged 2 to 12 months with moderate to severe bronchiolitis (OR 5.5 95% CI 2.9 to 10.2) and served better for this purpose than did a respiratory rate of 60 or more breaths per minute (Corneli *et al.* 2012).

Table 3. Studies evaluating the threshold for initial oxygen saturation values in relation to the outcomes of bronchiolitis.

References	Number of patients	Age	Outcome	Oxygen saturation value predicting the outcome
Mulholland <i>et al.</i> 1990	60	≤15 months	Severe disease	<90%
Shaw <i>et al.</i> 1991	213	<13 months	Severe disease	<95%
Brooks <i>et al.</i> 1999	542	<12 months	Deterioration during admission	<85%
Voets <i>et al.</i> 2006	378	2 weeks-24 months	Admission	<95%
Mansbach <i>et al.</i> 2008a	1456	<24 months	Safe discharge	≥94%
Parker <i>et al.</i> 2009	312	2-23 months	Major medical intervention	≤92%
Marlais <i>et al.</i> 2011	449	<12 months	Admission	<97%
Corneli <i>et al.</i> 2012	598	2-12 months	Length of stay >1 night	<94%
Evans <i>et al.</i> 2012	169	<12 months	Nasal CPAP	<95%
Mansbach <i>et al.</i> 2012	2207	<24 months	CPAP and/or intubation	<85%

Acute asthma exacerbations

Current guidelines as accepted by the Global Initiative for Asthma (GINA) state that oxygen saturation should be monitored by pulse oximetry during asthma exacerbations to assess the severity of the disease and the response to treatment (GINA 2014). Mild asthma exacerbations are associated with oxygen saturation values of >95% and severe exacerbations with values of <92% in children ≤5 years age (GINA 2014). Children aged ≤5 years should be admitted if their acute exacerbation fails to resolve within 1-2 hours despite repeated doses of short-acting beta-agonist and their oxygen saturation is <92% when breathing room air (GINA 2014). Mild to moderate asthma exacerbations in children ≥6 years age are associated with oxygen saturation values of 90% to 95% and severe exacerbations with values of <90% (GINA 2014). Although it has been suggested that initial oxygen saturation values predict the outcome of acute asthma exacerbations in children, some studies have focused on post-treatment values as being predictive of the need for hospitalization (Table 4). According to the current guidelines, children with an acute asthma exacerbation can be discharged after initial treatment if oxygen saturation values of 94% to 98% are maintained while breathing room air (GINA 2014).

Table 4. Studies evaluating the threshold for initial oxygen saturation values in relation to the outcomes of acute asthma exacerbations.

References	Number of patients	Age	Outcome	Oxygen saturation value predicting the outcome
Bishop & Nolan 1991	100	6 months-17 years	Admission	<91%
Geelhoed <i>et al.</i> 1994	280	1-15 years	Admission	≤91%
Wright <i>et al.</i> 1997	85	1-17 years	Admission	≤91% (post-treatment value)
Sole <i>et al.</i> 1999	174	4 months-15 years	Increased severity of acute asthma	<94%
Keogh <i>et al.</i> 2001	278	≥1 year	Long duration (>12 hours) of bronchodilator therapy	≤92%
Keahey <i>et al.</i> 2002	1040	2-17 years	Admission rate >55%	≤92%
Mehta <i>et al.</i> 2004	273	1-17 years	Prolonged (>4 hours) frequent bronchodilator therapy	≤91%
Boychuk <i>et al.</i> 2006	1219	1-18 years	Admission rate >45%	<92%

3 Aims of the research

The specific aims of the research were:

1. To determine the risk factors for croup and recurrent croup (I).
2. To examine the need for and timing of major medical interventions in infants under 6 months of age with bronchiolitis (II).
3. To identify factors that reliably predict the need to hospitalize children with mild acute wheezing disease (III).
4. To evaluate whether RSV infection during infancy has a positive association with the development of asthma and atopy during childhood (IV).

4 Risk factors for croup and recurrent croup (I)

4.1 Subjects

All the children who visited the emergency outpatient department at the Paediatric Department of Oulu University Hospital during 1996-2000 because of acute croup were included in this survey. At that time the catchment area for the emergency department at night (10 pm to 8 am) consisted of the city of Oulu, with about 120 000 inhabitants, for which it was the only primary care unit. There were 1752 visits because of acute croup, bronchiolitis, bronchitis, tonsillitis or upper respiratory infection, and almost half of these, i.e. 817 visits, were attributable to croup. A total of 597 patients actually had croup, 67% of whom were boys.

We selected control patients from among those who had had other airway infections and matched them for age, (± 3 months for children ≤ 2 years, and ± 6 months for children > 2 years) and sex. Altogether we were able to identify 492 pairs and after excluding eight pairs for which the data for either the croup patient or the control patient were inadequate, we sent a questionnaire to the remaining 484 pairs in 2003.

We received 308 replies from the parents of the croup patients, giving a response rate of 64%. After excluding from the set of 283 control patients (58%) who replied the 85 who were reported to have had croup on another occasion and those whose questionnaires were incompletely filled in, we had a series of 194 control patients with adequate data, 58% of whom were boys. For the final analysis we rematched the controls and cases for age (± 6 months) and sex and eventually arrived at 182 pairs of patients representing croup versus other airway infections (Fig.3) (Table 5). The age of the croup and the control patients was 8.9 years (SD 2.4) at the time when the questionnaire was sent (Table 5).

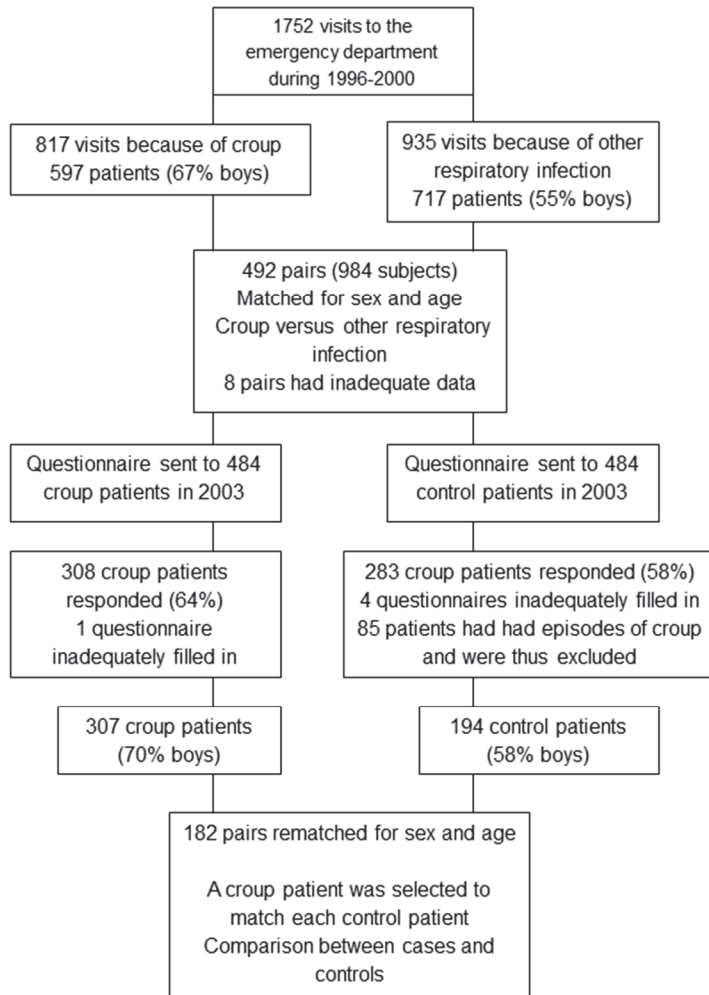


Fig. 3. Study profile (I).

Table 5. Demographic data of the 182 croup cases and their age- and sex-matched controls (I).

Characteristic	Cases	Controls
Age at the time of the questionnaire, years, mean (SD)	8.9 (2.4)	8.9 (2.4)
Number of boys (%)	111 (61%)	111 (61%)
Episodes of croup, mean (SD)	6.4 (7.4)	
Visits to a physician because of croup, mean (SD)	4.3 (5.5)	
Hospitalizations because of croup, mean (SD)	2.1 (3.5)	
Episodes of otitis media, mean (SD)	9.1 (8.9)	9.3 (7.7)
Episodes of bronchitis diagnosed by a physician, mean (SD)	3.1 (4.8)	2.9 (4.1)
Adenoidectomy performed, n (%)	75 (42%)	79 (44%)
Tympanostomy performed, n (%)	35 (20%)	48 (27%)
Mother's smoking, n (%)	35 (20%)	56 (32%)

4.2 Methods

The questionnaire asked about the number of croup episodes in the patient's history, the number of hospitalizations owing to croup episodes and whether the child's parents or siblings had suffered from croup episodes and, if so, in how many episodes. The number of episodes of bronchitis and otitis media were also asked for, as well as the use of anti-asthmatic medication during respiratory infections. Physician-diagnosed diseases and medical procedures such as anaesthesia, adenoidectomy, tympanostomy and intensive neonatal care were recorded. In addition, there were questions on a tendency to snore or suck their thumb, duration of breast feeding, use of a dummy and feeding bottle, form of day care, parental smoking, level of parental education and family pets.

Some of the variables evaluated are known risk factors for respiratory infections. As we took the controls from children visiting the outpatient clinic because of respiratory infections, these factors could occur more commonly among the controls than in a general paediatric population. To exclude this kind of selection bias we took a second unselected control group from our previous survey. This unselected population was used to compare the frequency of parental smoking and having pets among the croup cases and their matched controls.

We used conditional logistic regression analysis for pairs matched for sex and age to estimate the risk factors for croup and recurrent croup (patients who had three or more episodes). We performed two case-control analyses, the first comparing all the patients who had ever suffered from croup with the control patients who had had some other airway infection, and the second comparing the

patients who had suffered from recurrent croup with their controls. The results are presented in terms of odds ratios (OR) and their 95% confidence intervals (CI). The level of education of the mother was taken as a confounding factor in the multivariate risk factor analysis.

4.3 Results

The mean number of croup episodes among croup population was 6.4, the mean number of episodes requiring a visit to a physician was 4.3 and the mean number of hospitalizations for croup was 2.1 (Table 5). Boys were over-represented among the croup patients (67%).

The most significant risk factor for croup appeared to be a history of croup among the patient's siblings, with an OR of 3.9 (95% CI 2.2 to 6.9, $P<0.01$) (Table 6). Another risk factor was a history of croup in the parents (Table 6). Parental smoking appeared to be more common among the control patients with respiratory infections than the croup patients or unselected controls (Table 6). A cat as a pet was more common among both the respiratory controls and the unselected controls, so that this factor was looked on as reducing the risk of croup (Table 6). Multivariable analysis adjusted for maternal education gave an OR of 3.2 (95% CI 1.5 to 7.1, $P<0.01$) for a history of croup in the parents and 4.4 (95% CI 2.1 to 9.2, $P<0.01$) for a history of croup in the siblings (Table 6).

Altogether 111 (61%) of the patients with croup had had recurrent episodes. The risk factors for recurrent croup were similar to those for croup, except that snoring increased the risk of recurrent croup with an OR of 1.7 (95% CI 1.1 to 2.9, $P=0.03$) and a pet cat was equally common among the cases and controls (Table 7). Multivariate analysis adjusted for the mother's level of education, gave an OR of 4.1 (95% CI 1.4 to 11.7, $P<0.01$) for a history of croup in the parents and an OR of 5.5 (95% CI 1.9 to 15.6, $P<0.01$) for a history of croup in the siblings (Table 7). No other factors such as asthma, hay fever or atopic dermatitis were associated with the risk of croup or recurrent croup.

Table 6. Risk factors for croup among 182 patients and their age- and sex-matched controls (I).

Risk factor	Crude OR				Multivariate analysis					
	N	n ₁	n ₂	OR	(95%CI)	P-value	N	OR	95%CI	P-value
History of croup										
Parents	159			2.8	(1.6, 5.1)	<0.01	111	3.2	(1.5, 7.1)	<0.01
Siblings	146	59	15	3.9	(2.2, 6.9)	<0.01	111	4.4	(2.1, 9.2)	<0.01
Snoring	167			1.4	(1.0, 2.0)	0.08				
Parental smoking	178			0.8	(0.6, 0.9)	0.01				
Number of siblings	166			1.0	(0.8, 1.1)	0.76				
Day care	148	4	7	0.6	(0.2, 2.0)	0.37				
Breast feeding	174	5	4	1.3	(0.3, 4.7)	0.74				
Asthma	176	35	36	1.0	(0.6, 1.5)	0.91				
Hay fever	179	32	37	0.9	(0.5, 1.4)	0.55				
Atopic dermatitis	177	24	25	1.0	(0.5, 1.7)	0.89				
Animal allergy	179	20	26	0.8	(0.4, 1.4)	0.38				
Pet dog	172	31	32	1.0	(0.6, 1.6)	0.90				
Pet cat	172	10	22	0.5	(0.2, 1.0)	0.04				
Pet rodent	172	18	18	1.0	(0.5, 1.9)	1.00				
NIC	172	21	15	1.4	(0.7, 2.7)	0.32				
Otitis media episodes	166			1.0	(1.0, 1.0)	0.55				
Bronchitis episodes	95			1.0	(0.9, 1.1)	0.98				

N is the total number of pairs in the analysis, and varies because of missing data, n₁ is the number of pairs in which the cases were exposed and the controls unexposed, and n₂ is the number of pairs in which the cases were unexposed and the controls exposed.

The following variables were included in the multivariate stepwise conditional logistic regression analysis: history of croup in siblings, parents' smoking, history of croup in a parent, pet cat, tympanostomy tubes, child's snoring and maternal education as a confounding factor, P>0.05 for all variables excluded.

NIC, neonatal intensive care, yes/no

Table 7. Risk factors for recurrent croup (three or more episodes) among 111 patients and their age- and sex-matched controls (I).

Risk factor	Crude OR					Multivariate analysis				
	N	n ₁	n ₂	OR	(95%CI)	P-value	N	OR	95%CI	P-value
History of croup										
Parents	101			3.4	(1.6, 7.2)	<0.01	74	4.1	(1.4, 11.7)	<0.01
Siblings	89	40	7	5.7	(2.6, 12.8)	<0.01	74	5.5	(1.9, 15.6)	<0.01
Snoring	103			1.7	(1.1, 2.9)	0.03				
Parental smoking	110			0.7	(0.6, 0.9)	0.01				
Number of siblings	102			1.0	(0.8, 1.2)	0.84				
Day care	87	3	6	0.5	(0.1, 2.0)	0.33				
Breast feeding	108	4	3	1.3	(0.3, 6.0)	0.71				
Asthma	108	27	24	1.1	(0.6, 1.9)	0.68				
Hay fever	109	23	24	1.0	(0.5, 1.7)	0.88				
Atopic dermatitis	107	17	15	1.1	(0.6, 2.3)	0.72				
Animal allergy	109	16	16	1.0	(0.5, 2.0)	1.00				
Pet dog	106	22	15	1.5	(0.8, 2.8)	0.25				
Pet cat	106	8	10	0.8	(0.3, 2.0)	0.64				
Pet rodent	106	8	9	0.9	(0.3, 2.3)	0.81				
NIC	106	13	8	1.6	(0.7, 3.9)	0.28				
Otitis media episodes	107			1.0	(1.0, 1.0)	0.51				

N is the total number of pairs in the analysis, and varies because of missing data, n₁ is the number of pairs in which the cases were exposed and the controls unexposed, and n₂ is the number of pairs in which the cases were unexposed and the controls exposed.

The following variables were included in the multivariate stepwise conditional logistic regression analysis: history of croup in siblings, parents' smoking, history of croup in a parent, tympanostomy tubes, child's snoring and maternal education as a confounding factor, P>0.05 for all variables excluded.

NIC, neonatal intensive care, yes/no

5 Evaluation of childhood wheezing (II and III)

5.1 Subjects

This survey included all the children aged ≤ 16 years who visited the emergency outpatient department at the Paediatric Department of Oulu University Hospital between 1 January 2007 and 31 December 2008 because of wheezing during a respiratory infection. Oulu University Hospital has the only paediatric department in the Northern Ostrobothnia Hospital District and served a total population of 384 280 people when the study started in 2007, including 5 650 infants younger than 6 months of age and 91 511 children aged 6 months to 16 years.

We carried out an electronic search of the hospital records for all visits during the period concerned, using version 10 of the International Classification of Diseases (ICD-10) codes and accepting all those that showed a primary diagnosis of 'bronchitis' (J20), 'bronchiolitis' (J21), 'asthma' (J45 and J46) or 'obstructive breathing' (R06.0 and R06.2). Data were collected on each patient's history, status and treatment. Initial oxygen saturation (SaO_2) values had been measured using the Nellcor OxiMax N-560-pulse oximetry device in the emergency room without supplementary oxygen. The professional experience of the physicians in charge of the emergency room and the ward was assessed, based on how long they had been working for the Paediatric Department. A fluoroimmunoassay was used to detect viral antigens in the patient's nasopharyngeal aspirates based on monoclonal antibodies for RSV, influenza A and B viruses, parainfluenza viruses of types 1, 2 and 3, adenovirus and metapneumovirus.

We focused on each patient's first visit during the period and excluded patients who did not have any symptoms of respiratory infection despite wheezing or had a confirmed diagnosis of pneumonia or sepsis in addition to wheezing.

We identified 353 patients younger than 6 months of age with the specified ICD-10 codes and 539 patients aged between 6 months and 16 years (Figs. 4 and 5).

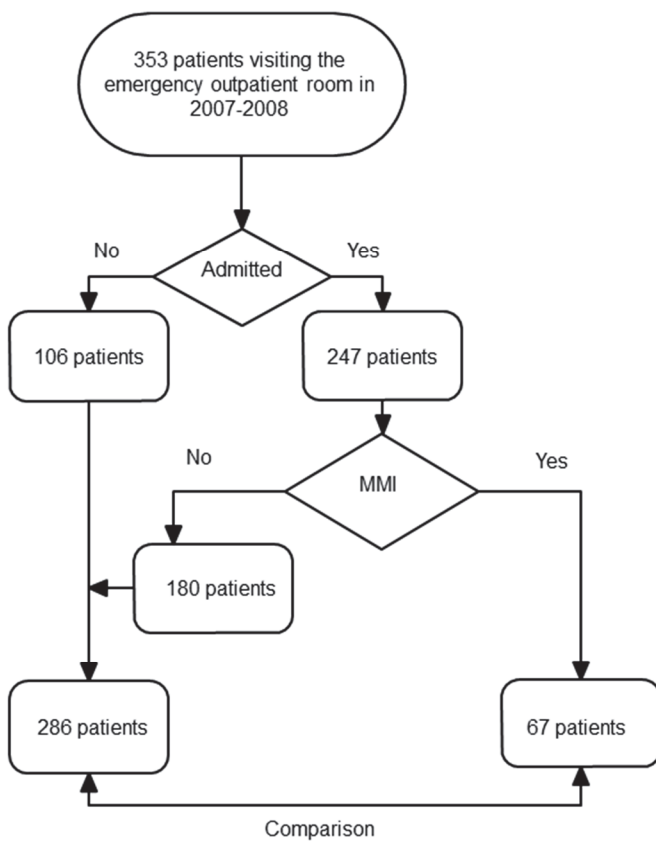


Fig. 4. Study profile (II), 353 patients, age <6 months (MMI; major medical intervention).

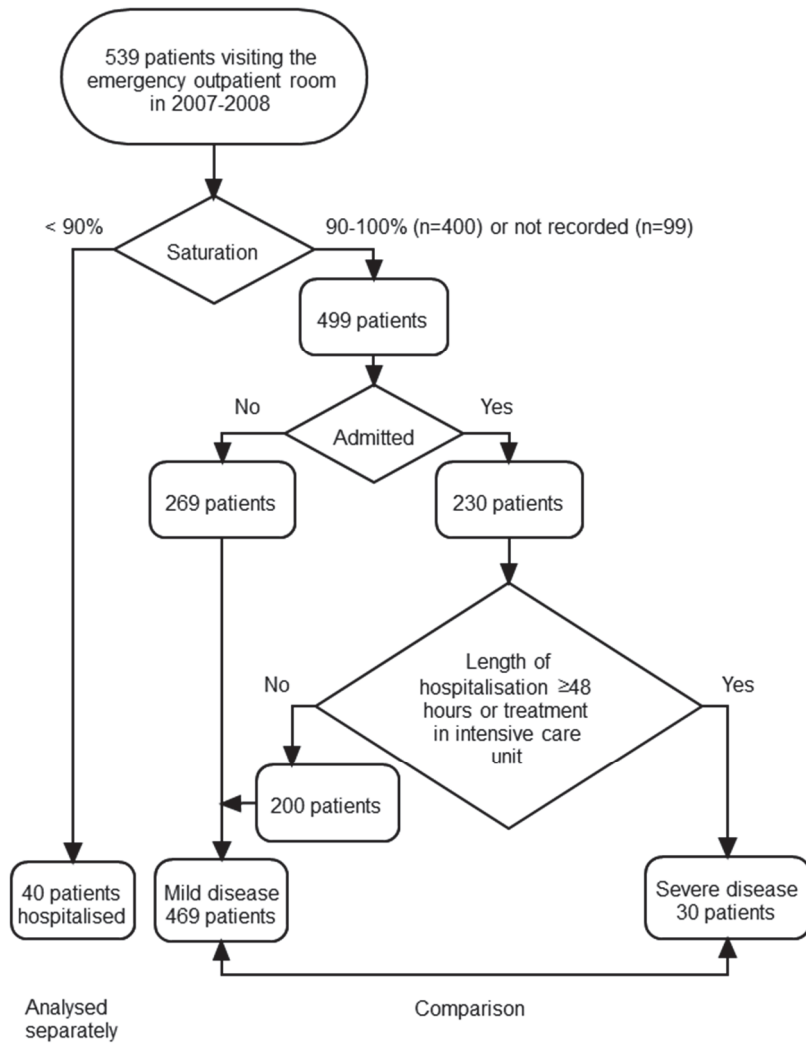


Fig. 5. Study profile (III), 539 patients, age from 6 months to 16 years.

5.2 Methods

5.2.1 Infants younger than 6 months of age

We set out to evaluate the progression of bronchiolitis in young infants by analysing the time course of the disease in order to identify the need for, and timing of, medical interventions (Fig. 4).

We recorded the need for any major medical intervention (MMI), with MMI defined as the need for any of the following interventions during admission: supplementary oxygen, intravenous fluids, intravenous antibiotics or admission to the intensive care unit. We also recorded all apnoeas occurring in the emergency room or the ward. Intravenous fluids were routinely used in our clinic at that time rather than nasogastric tubes if the patient had feeding problems during bronchiolitis.

We calculated that a sample size of 330 patients would provide 80% power with a 5% type I error when detecting a factor with a risk ratio of 2.0 or greater for MMI. The Kaplan-Meier method was used to analyse the timing of the MMIs. Comparisons between the two groups, MMI versus no MMI, were performed using the standard normal deviate -test (SND-test) for dichotomous variables and Student's t-test for continuous variables. Variables associated with a MMI with a P value of <0.10 were selected as candidate explanatory variables and further tested by multivariate forward stepwise logistic regression analysis with adjustment for age (Table 8).

The local ethical committee said that it did not need to approve the study design, as it was observational, with no interventions.

5.2.2 Children aged from 6 months to 16 years

In the children who were older than 6 months we explored the parameters that reliably predict the severity of acute wheezing disease. To analyse the factors that represent the severity of the disease, we classified the patients into two groups according to their length of stay in hospital. Those remaining there for less than 48 hours were deemed to have a mild disease and those remaining for at least 48 hours or taken to the intensive care unit were said to have a severe disease. As it was clear that most patients with initial SaO₂ values below 90% should be hospitalized, we analysed these cases separately (Fig. 5).

We calculated that a sample size of 460 patients would provide 80% power with a 5% type I error when detecting a factor with a risk ratio of 1.7 or greater for severe disease. The comparisons between the mild and severe disease groups were performed using the standard normal deviate -test (SND-test) for dichotomous variables and Student's t-test for continuous variables. A receiver operating characteristic (ROC) curve for the initial SaO₂ values between 90-100% was constructed to predict the length of hospitalization, and an optimum cut-off value at which both sensitivity and specificity were at their best was calculated. Variables affecting the severity of the disease with a p-value <0.10 were selected as explanatory variables to be tested by multivariate forward stepwise logistic regression analysis (Table 9).

Table 8. Characteristics of patients (age <6 months) with and without MMI (major medical intervention) (II).

Characteristic	No MMI N ₁ =286 N ₁ (%)	MMI N ₂ =67 N ₂ (%)	P value for the difference
Background			
Age in days, mean (SD)	82 (45)	63 (41)	0.002
Male	181 (63)	37 (55)	0.213
Prematurity	18 (6)	5 (7)	0.595
Underlying comorbidity	8 (3)	3 (4)	0.230
Distance of hospital from home			
0-50 km	215 (75)	49 (73)	0.644
50-100 km	16 (6)	3 (4)	0.999
Over 100 km	55 (19)	15 (22)	0.502
Earlier visits on account of wheezing	81 (28)	9 (13)	0.009
Home*			
Fever	92 (33)	27 (43)	0.112
Fever over 38°C	49 (18)	17 (28)	0.056
Duration of symptoms in days, mean (SD)			
Fever	2.0 (1.4)	2.0 (1.3)	0.804
Dyspnoea	1.8 (1.6)	1.6 (1.1)	0.579
Respiratory infection symptoms	4.6 (4.2)	3.9 (2.9)	0.177
Respiratory infection symptoms <5 days	187 (68)	54 (82)	0.018
Emergency room			
Initial oxygen saturation %, mean (SD)**	97.1 (2.4)	93.3 (5.5)	<0.001
Abnormal auscultation finding	220 (77)	53 (79)	0.635
Hospital			
Treatment in ward	180 (63)	67 (100)	
Duration of hospitalization in days, mean (SD)	1.5 (1.0)	3.9 (2.9)	<0.001
Viral tests			
RSV-positive***	178 (71)	58 (92)	<0.001

*Up to 10 patients with missing values.

** Initial oxygen saturation values available from 241 patients without MMI and 61 with MMI.

*** RSV test performed on 251 patients without MMI and 63 with MMI.

Table 9. Characteristics of the patients (age from 6 months to 16 years) according to the severity of the disease (III).

Characteristic	Mild disease N ₁ =469 N ₁ (%)	Severe disease N ₂ =30 N ₂ (%)	P value for the difference
Background			
Age in days, mean (SD)	2.2 (2.0)	2.4 (2.9)	0.673
Male	300 (64)	18 (60)	0.567
Prematurity	25 (5)	5 (17)	0.017
Underlying comorbidity			
Disease affecting respiratory tract	8 (2)	1 (3)	0.264
Disease not affecting respiratory tract	14 (3)	1 (3)	0.418
Distance of hospital from home			
0-50 km	327 (70)	19 (63)	0.423
50-100 km	31 (7)	4 (13)	0.100
Over 100 km	111 (24)	7 (23)	0.999
Earlier visits on account of wheezing	203 (43)	13 (43)	0.999
Earlier visits to the asthma outpatient clinic	35 (7)	4 (13)	0.177
Home*			
Fever	268 (59)	18 (60)	0.999
Fever over 38°C	165 (38)	13 (43)	0.451
Duration of symptoms in days, mean (SD)			
Fever	2.1 (1.4)	1.8 (1.3)	0.412
Wheezing	1.7 (1.7)	1.5 (1.0)	0.472
Respiratory infection symptoms	4.3 (4.3)	3.0 (2.7)	0.109
Short-acting inhaled beta-receptor agonist use	266 (57)	17 (57)	0.999
Anti-asthmatic medication use	83 (18)	6 (20)	0.632
Emergency room			
Initial oxygen saturation %, mean (SD)**	95.3 (2.6)	92.9 (2.8)	<0.001
Initial oxygen saturation ≤93%	81 (22)	19 (63)	<0.001
Abnormal auscultation finding	412 (88)	30 (100)	0.035
Short-acting inhaled beta-receptor agonist use	380 (81)	29 (97)	0.016
Systemic corticosteroid use	41 (9)	3 (10)	0.532
Hospital			
Treatment in ward	200 (43)	30 (100)	

*Up to 25 patients with mild disease had missing values. ** Initial oxygen saturation values were available for 370 patients with mild disease and 30 with severe disease.

5.3 Results

5.3.1 Infants younger than 6 months of age

The incidence of visits to the emergency room by children younger than 6 months with bronchiolitis was 37 per 1 000 per year and the admission rate was high, as 247 (70%) of the 353 patients were hospitalized. The incidence of recorded apnoea among the study population was 3%, and out of the 314 children (89%) who underwent viral testing 236 (75%) tested positive for RSV. Just under three-quarters (74%) of the patients who were admitted had suffered from respiratory infection symptoms for less than 5 days before admission and the mean duration of hospitalization was 2.2 days (SD 2.0).

Out of the total of 353 patients, 67 (19%) needed an MMI, while 286 (81%) did not (Table 8). The risk of a patient needing an MMI continued for up to 5 days after the onset of the respiratory infection symptoms (Fig. 6). All the recorded apnoeas occurred within 4 days of the onset of symptoms (Fig. 6). Most of the MMI cases resolved within 8 days of the onset of the symptoms (Fig. 6). The need for MMI occurred within the first 2 days after admission, and the apnoeas occurred within 1 day of admission. Almost all the MMIs resolved within 4 days of admission.

A positive RSV test predicted an MMI with an OR of 11.5 (95% CI 2.6 to 50.5, $P=0.001$) in a logistic regression model adjusted for age, while a fever of over 38°C predicted an MMI with an OR of 3.5 (95% CI 1.4 to 8.8, $P=0.007$). There was an inverse association between the initial SaO₂ value and MMI, with each 1% increase in the initial SaO₂ value lowering the OR for an MMI by 0.7 (95% CI 0.6 to 0.8, $P<0.001$).

Of the 106 discharged patients, 31 (29%) revisited the emergency room within two weeks of the first visit, including 21 who had experienced respiratory infection symptoms for less than 5 days when they paid their first visit. As a result of their subsequent visit, 17 patients were admitted and 7 needed an MMI.

Physicians with at least 5 years' professional experience in the Paediatric Department were more likely to discharge patients from the emergency room than those with less experience, with a discharge rate 50% versus 28% ($P=0.007$).

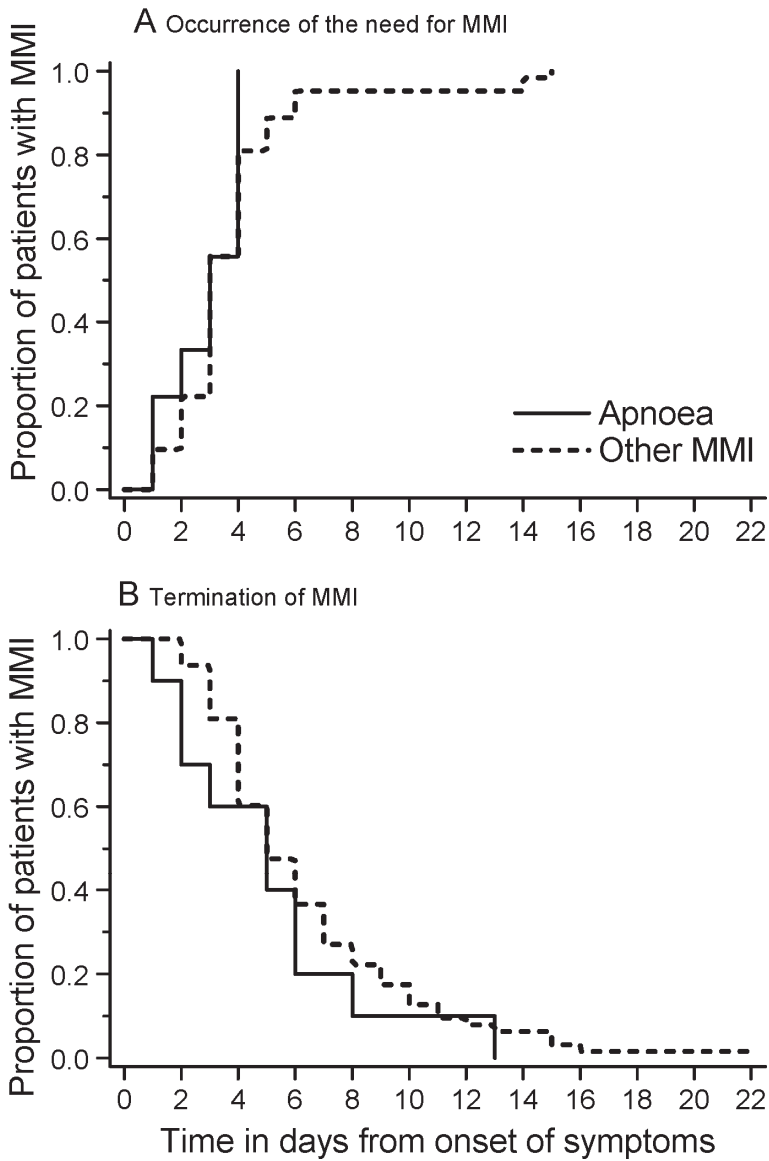


Fig. 6. (A) Occurrence of a need for major medical interventions (MMIs) and (B) timing of their termination in relation to the duration of respiratory infection symptoms (II).

5.3.2 Children aged from 6 months to 16 years

The study population included 469 patients (87%) with mild disease, 30 (6%) with severe disease and 40 (7%) with an initial SaO₂ below 90% (Table 9). The area under the ROC curve for an initial SaO₂ of 90–100%, predicting mild disease was 0.75 (95% CI 0.53 to 0.97) (Fig. 7). The cut-off value for optimum sensitivity and specificity was 93%. An initial SaO₂ >93% had a negative predictive value of 93% and a positive predictive value of 21%. An initial SaO₂ of >93% was the only variable that predicted a mild disease in the stepwise logistic regression analysis, with an OR of 6.3 (95% CI 2.8 to 14.3, P<0.001).

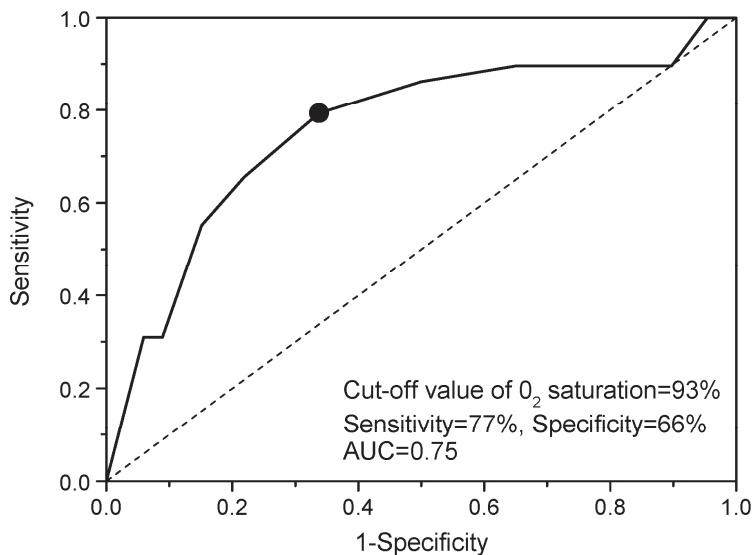


Fig. 7. Receiver operating characteristic (ROC) curve of initial oxygen saturation values between 90-100% as used to predict mild disease among 400 children visiting the emergency room on account of wheezing during respiratory infection. The area under the ROC curve equals 0.75 (III).

The admission rate was high, as 270 (50%) of the patients were hospitalized. The incidence of emergency room visits by children aged from 6 months to 16 years because of wheezing during respiratory infection was 440 per 100 000 per year. The mean age of the study population was 2.3 years (SD 2.1) and 88% of the patients were aged between 6 months and 4 years. The mean initial SaO₂ was 93.3%

(SD 3.9) in the patients who were admitted and 95.8% (SD 2.5) in those who were discharged from the emergency room. If an initial limit of $\text{SaO}_2 > 93\%$ had been used when considering discharge, only 140 (26%) of the patients would have required admission and the admission rate could have been reduced by 48%.

Three-quarters (75%) of the patients who were admitted had been treated with short-acting inhaled beta-agonists at the referral outpatient clinic and 86% received this treatment in the emergency room. Just under two-thirds (65%) of the discharged patients had been treated with short-acting beta-agonists at the referral outpatient clinic and 78% received this treatment at the emergency room.

The patients who were admitted were hospitalized for a mean of 1.4 days (SD 1.4), and 16 patients of them (6%) were treated in the intensive care unit, although only one needed mechanical ventilation. Just under half of the admitted patients (130, 48%) had an initial SaO_2 of $>93\%$, but only 14 of these patients (11%) required oxygen in hospital.

A total of 269 patients were discharged directly from the emergency room, including 27 (10%) who had an initial $\text{SaO}_2 \leq 93\%$. Of these, 35 (13%) revisited the emergency room within 48 hours of their first visit, and 13 (5%) were then admitted, with a mean hospital stay of 1.5 days (SD 1.4).

All the 40 patients (7%) who had an initial SaO_2 below 90% were hospitalized and 6 of them (15%) had a chronic disease of some kind. The mean duration of hospitalization was 2.0 days (SD 1.8) and 13 of these patients (33%) remained in hospital for more than 48 hours.

6 Outcomes after RSV infection in infancy (IV)

6.1 Subjects

The study was carried out in the Department of Paediatrics at Oulu University Hospital, the only children's hospital in the area. From August to November 2001, before the RSV epidemic season, we collected a cord blood sample from each newborn of at least 37 gestational weeks (n=1084) whose parents provided their informed consent (Fig. 8). If the parents were interested in participating in the study we asked them to contact the authors immediately if their child presented with symptoms of respiratory tract infection before the age of 6 months. In cases of infection we examined and, if necessary, admitted the child to the hospital within 24 hours. We used an in-house viral antigen-specific enzyme immunoassay method and PCR (for detection of rhinovirus) to determine the cause of the viral infection from a nasopharyngeal aspiration sample, after which the samples that still contained enough nasopharyngeal secretion were stored at -20°C.

Infants with a clinical respiratory infection who tested positive for RSV formed the initial study group (n=48: 27 hospitalized [1 later excluded due to a chromosomal abnormality] and 21 treated as outpatients; mean age 3.0 months; range 3 weeks to 5.5 months) (Fig. 8). Those who tested negative for RSV (n=28, 3 hospitalized; mean age 3.1 months; range 3 weeks to 5.7 months) formed the control group, which comprised 10 patients with a rhinovirus infection and 18 patients with a negative viral test result (Fig. 8). Later in 2014, we used a multiple PCR assay (Seeplex RV12; Seegene; Seol, Korea) to analyse five nasopharyngeal samples, which had tested negative in the initial viral analysis and had then been stored. Two of these samples tested positive for coronaviruses and two for the parainfluenza 4-virus, while one sample tested positive for both influenza A-virus and rhinovirus. A control group of healthy subjects with no respiratory tract infections before the age of 6 months (based on parental reports, n=84) and matched with the above infants for date of birth and sex were selected from those whose cord blood specimens were available (Fig. 8). None of the patients or control subjects required respiratory assistance during the neonatal period or had any concomitant disease such as congenital heart disease or a severe neurological disease.

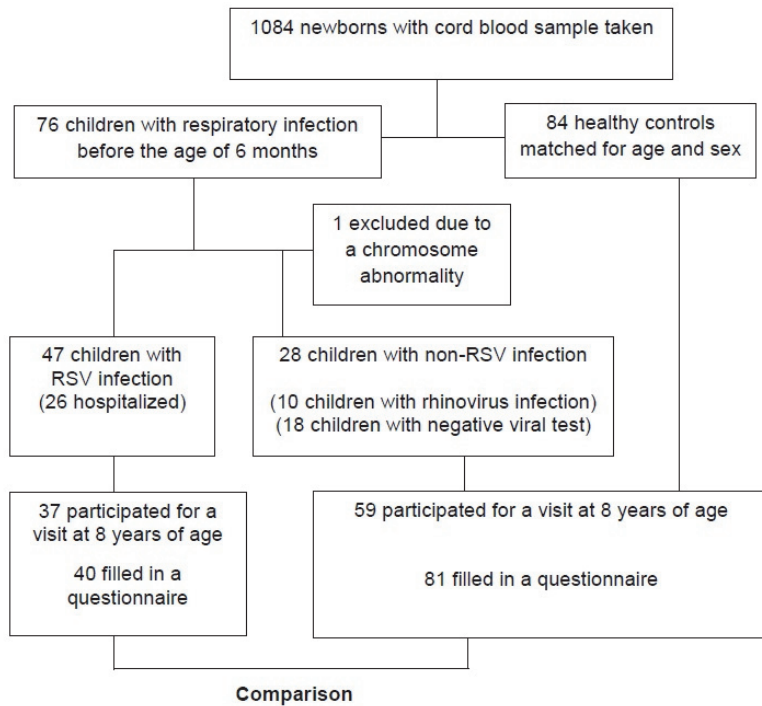


Fig. 8. Study profile (IV).

Altogether 93% of the subjects were contacted by telephone for a follow-up visit during the autumn of 2009, when they were 7 to 8 years old. We asked their parents to complete a questionnaire about their child's asthma and allergic symptoms. The children were invited for a clinical examination, lung function tests, skin-prick tests for 13 common allergens, and blood tests to determine their serum IgE concentrations, eosinophil count and mononuclear cells. A total of 127 (76%) families returned the questionnaire, and 96 (60%) subjects visited the outpatient department during the winter of 2010 (Fig. 8). The mean age of the 121 children either attending the control visit or returning the questionnaire was 8.3 years (SD 0.1), and 70 (58%) of them were male (Table 10).

6.2 Methods

We used the same questionnaire as in the ISAAC, along with some additional questions about the type of day-care, breastfeeding, number of siblings, occurrence of otitis media, food allergy, pets and smoking at home. The questionnaire was checked during the visit, and if the parents were unwilling to participate in the clinical examinations, they were interviewed by telephone to determine whether their child had had any visits or examinations on account of symptoms of asthma or allergy.

Current asthma was considered to be present if the child was on continuous maintenance medication for asthma at the time of the study. The term “asthma ever” was used if the child had either a history of doctor-diagnosed asthma or current asthma. Atopic eczema was clinically assessed with the SCORAD index and graded as mild (SCORAD index <25), moderate (SCORAD index 25-50) or severe (SCORAD index >50).

A trained nurse performed the lung function tests, spirometry (Medikro spirometry software 3.0.3, Medikro, Kuopio, Finland) with a bronchodilator test and exhaled nitric oxide (Niox Mino, Aerocrine AB, Solna, Sweden), with no knowledge of the group to which the child belonged. The results of the spirometry were expressed as percentages of the national reference values. The results of the exhaled nitric oxide measurements were expressed as ppb (parts per billion), with values above 10 ppb considered to be elevated.

Skin-prick testing was performed on the volar aspect of the forearm with allergens produced by ALK (Allergologisk Laboratorium A/S, Horsholm, Denmark). These included allergens of birch, mugwort, timothy, meadow grass, milk, egg, wheat, fish, dermatophagoides pteronyssinus, epithelia of dog, cat and horse and latex. We used a 1-mm, one-peak lancet with a shoulder to prevent deeper penetration. Histamine dihydrochloride (10mg/ml) served as a positive control. The reactions were read at 15 min, and considered positive if the mean diameter of the weal was over 3 mm and/or at least half the size of the histamine reaction. The reactions were analysed by a trained nurse with no knowledge of the group to which the child belonged.

We used a chemiluminescence method to determine the concentrations of IgE, and, according to our laboratory reference values, concentrations above 130 kU/l for children <8 years of age and concentrations above 320 kU/l for children >8 years of age were considered to be elevated.

Table 10. Demographic characteristics of patients with RSV infection and controls (IV).

Characteristic	RSV	Control subjects	RSV vs. Control subjects	
	N=40	N=81	Difference	P
	Mean/no (Range/%)	Mean/no (Range/%)	(95%CI)	
Age at the time of the study, years	8.3 (8.1-8.6)	8.3 (8.1-8.6)	0.01 (-0.03 to 0.06)	0.673
Number of children in the family	3.7 (1-12)	3.3 (1-14)	-0.43 (-1.32 to 0.47)	0.344
Number of siblings	2.8 (0-11)	2.4 (0-13)	-0.38 (-1.48 to 0.72)	0.494
Birth order	2.6 (1-9)	2.4 (1-11)	-0.19 (-1.48 to 0.72)	0.617
Gestational age, weeks	40.0 (37.4-42.3)	39.7 (37.7-41.7)	-0.03 (-0.49 to 0.42)	0.383
Number of acute otitis media episodes	7.5 (0-30)	5.6 (0-30)	-1.91 (-4.92 to 1.09)	0.209
Tympanostomy performed	7 (18)	15 (19)	-1 (-14 to 16)	1.0
Adenoidectomy performed	9 (23)	19 (23)	-1 (-16 to 16)	1.0
Duration of breastfeeding, months	7.7 (0.0-24.0)	8.1 (0.3-23.0)	0.95 (-1.47 to 3.37)	0.810
Type of day-care				
At age 3-5 years				
Home	17 (43)	29 (36)	7 (-11 to 25)	0.436
Family day-care	4 (10)	14 (17)	-7 (-19 to 7)	0.295
Day-care centre	19 (48)	38 (47)	1 (-18 to 19)	1.0
Mother smoked during pregnancy	7 (18)	9 (11)	6 (-6 to 22)	0.275
Smoking in the household during the past year	13 (33)	28 (35)	-2 (-18 to 16)	1.0
Pets at home at the time of the study	21 (53)	44 (54)	-2 (-20 to 17)	0.850

The routine method used in the laboratory served to determine the eosinophil count, and values above 0.4 ($\times 10^9/l$) were considered to be elevated.

The data were analysed with SPSS for Windows version 20.0. The patients with an RSV infection in infancy were compared with the control patients (Fig. 8) The patients who tested negative for RSV formed heterogenic group as 10 tested positive for rhinovirus (7 of these patients participated at control visit) and 18 had a negative viral test result. Due to this heterogeneity we formed a one combined control group including those who tested negative for RSV and healthy control subjects (Fig. 8). The Student's t-test, the Chi-squared test, Fisher's exact test, and the test for a difference in proportions served to calculate the statistical

significances of the differences between the groups. We also calculated odds ratios and 95% confidence intervals.

The Ethics Committee of the Northern Ostrobothnia Hospital District found the study protocol acceptable. All the patients provided their written informed consent during the control visit.

6.3 Results

Asthma ever was diagnosed among 10 (25%) patients with an RSV infection in infancy, whereas 7 (9%) children among the control subjects had ever had asthma ($P=0.014$) (Table 11). An RSV infection in infancy predicted asthma ever with an odds ratio (OR) of 3.5 (95% CI 1.2 to 10.5, $P=0.022$). The mean age at the time of asthma diagnosis between the two study groups did not differ, 2.6 years (SD 2.1) vs. 3.0 years (SD 1.9), ($P=0.693$).

Current asthma was present in 11 (7%) children: 5 (13%) among the former RSV patients (4 of whom were hospitalized for RSV infection in infancy) and 6 (7%) of the controls (Table 11). This difference was not statistically significant.

Wheezing symptoms were more common in the history of patients with an RSV infection in their infancy than in the history of the control patients (48% vs. 27%, $P=0.027$) (Table 11). An RSV infection in infancy predicted wheezing ever with an OR of 2.4 (95% CI 1.1 to 5.4, $P=0.031$). Wheezing symptoms within the last 12 months and the lung function tests, as measured by spirometry and exhaled nitric oxide showed no differences between the study groups (Table 11).

We observed atopic eczema, as measured by the Scrad index, more often in the patients with an RSV infection in infancy than in the controls, but this difference was not statistically significant (32% vs. 17%, $P=0.058$) (Table 11).

Symptoms of sneezing, a runny or blocked nose or allergic rhinitis were reported equally often in patients with an RSV infection in their infancy as in the control subjects (Table 11). Food intolerance or atopic symptoms reported by the parents did not differ between the groups (Table 11). Patients with an RSV infection in their infancy and control subjects showed no differences in family history of atopy and/or asthma.

The children who had either participated in the control visit or their parents had completed the questionnaire had had more visits and examinations on account of their history of allergy and atopy than had the children who did not participate, and this pattern occurred to an equal extent in both the RSV infection and control groups.

Table 11. Asthma and atopy among patients with RSV infection and controls; symptoms and diagnoses (IV).

Symptoms and diagnoses	RSV	Control subjects	RSV vs. Control subjects	
	N=40 N (%)	N=81 N (%)	Difference (95%CI)	P
Wheezing ever	19 (48)	22 (27)	20 (2 to 38)	0.027
Wheezing in the past 12 months	7 (18)	6 (7)	10 (-2 to 25)	0.075
Asthma ever	10 (25)	7 (9)	16 (3 to 33)	0.014
Anti-asthmatic medication in use at the time of study	5 (13)	6 (7)	6 (-6 to 20)	0.339
Symptoms of sneezing, or a runny or blocked nose	11 (28)	27 (33)	-6 (-22 to 12)	0.424
Allergic rhinitis ever	7 (18)	19 (23)	-6 (-20 to 11)	0.371
Atopic eczema ever	20 (50)	36 (44)	6 (-13 to 24)	0.569
Mild eczema (Scorad index<25) at the time of study*	12 (32)	10 (17)	15 (2 to 34)	0.058
Moderate eczema (Scorad index 25-50) at the time of study*	2 (5)	7 (12)	-6 (-18 to 7)	0.311
Food intolerance ever	9 (23)	24 (30)	-7 (-22 to 10)	0.398
Food intolerance at the time of study	8 (20)	12 (15)	5 (-8 to 21)	0.451
One positive skin-prick test*	10 (27)	5 (8)	19 (4 to 36)	0.012
Totally negative skin-prick tests*	21 (57)	42 (71)	-14 (-34 to 5)	0.132
Blood eosinophil count above reference values**	6 (17)	9 (16)	-1 (-14 to 19)	1.0
Serum IgE above reference values***	7 (21)	12 (21)	0 (-17 to 18)	1.0

* Follow-up visit and skin-prick tests were performed on 37 patients with RSV infection and 59 control subjects.

** Eosinophil counts were available for 35 patients with RSV infection and 56 control subjects.

*** Serum IgE values were available for 34 patients with RSV infection and 56 control subjects.

7 Discussion

7.1 Risk factors for croup and recurrent croup (I)

The recurrence rate for croup was significant, as 61% of the croup patients had had at least three croup episodes during the last 3–7 years after the initial visit to the emergency outpatient room. Recurrence rates reported in the literature have varied from 6% to 52%, wide variation that could be due to the fact that previous studies have employed different definitions of recurrent croup (Hide & Guyer 1985, Wall *et al.* 2009, Zach *et al.* 1981).

A history of croup among the siblings and parents of the index case appeared to be an exceptionally powerful risk factor for croup and its recurrence, a result which is in line with earlier observations (Cohen & Dunt 1988, Zach *et al.* 1981). One explanation for this could be that there are some families with inherited structural features in the area of the larynx that lead to an increased risk of croup during viral respiratory infection in childhood.

The fact that the patients with croup had a cat as a pet less often than the controls leads us to consider the controversial data that have emerged in earlier studies regarding the effect of cat exposure on airway diseases. Exposure to a cat at the age of 2–3 months has been found to reduce the risk of wheezing in case of that the mother did not have asthma, whereas it increased the risk of wheezing if the mother had asthma (Celedon *et al.* 2002). High exposure to a cat can induce high IgG antibody levels to cat in some children without any IgE-mediated sensitisation associated with a low risk of wheezing and bronchial hyper-reactivity (Lau *et al.* 2005). In families with a history of atopy, high exposure to a cat allergen in children has been associated with IgE-mediated sensitization, high IgG antibody levels to cat and an increased risk of asthma (Lau *et al.* 2005).

Parental smoking is known to be a risk factor for respiratory infections in childhood (DiFranza *et al.* 2004). The mothers are less likely to start smoking and more likely to quit if their child has an airway disease (Meinert *et al.* 1994). Nevertheless, the desirable outcomes of environmental tobacco smoke exposure can lead to situation in which parental smoking is more common in households with healthy children (DiFranza *et al.* 2004). This phenomenon can create the biased impression in case-control studies that environmental tobacco smoke exposure provides a protective effect (DiFranza *et al.* 2004). This possibility also

arose here, as we found that smoking by both parents was associated with a decreased occurrence of both croup and recurrent croup.

The strengths of our investigation lie in its design and the fact that the emergency outpatient room from which the patients were recruited was the only primary care unit available at night during at the time of the survey. This was a case-control study and each croup patient had control patient matched for sex and age. The information on the risk factors for croup and its recurrence can be of value to the families of affected children, as it enables the prediction of possible recurrent episodes. Both the controls and the croup patients in this survey had had multiple respiratory infections, and it is possible that more families in whom respiratory infections were common returned the questionnaire. The main limitation, however, is that the questionnaire was sent out 3–7 years after the child's initial visit to the emergency outpatient room, so that some parental reporting bias concerning especially on history of croup is possible.

7.2 Evaluation of childhood wheezing (II and III)

7.2.1 Infants younger than 6 months of age (II)

Altogether 19% of the infants below 6 months of age coming to our emergency room with bronchiolitis required an MMI and 3% had apnoea. These infants were most likely to need MMIs in the first 5 days after the disease onset. We identified three predictors of MMIs: a positive RSV test result, a fever of more than 38°C and a low initial oxygen saturation value. All the patients with apnoea were under 2 months of age and 40% were born prematurely. Underlying comorbidities did not predict the need for MMIs in our analysis.

To emphasize the course of bronchiolitis in young infants, we focused on patients below 6 months of age. These infants belong to the age group most likely to be hospitalized with bronchiolitis and face a high risk of needing mechanical respiratory support with this condition (Deshpande & Northern 2003, Mansbach *et al.* 2012). Age less than 2 months is the strongest predictor of apnoea during RSV infection (Kneyber *et al.* 1998). The definition of bronchiolitis and especially its upper age limit, have been challenged recently and there has been a call for clinical studies that only include patients younger than 12 months age (Korppi *et al.* 2012, Mecklin *et al.* 2014, Zorc & Hall 2010). Bronchiolitis is a clinical diagnosis and the most important characteristics are an unstable early phase of the disease,

increased respiratory effort and poor response to bronchodilators (Gadomski & Scribani 2014, Horst 1994, Mecklin *et al.* 2014).

RSV infection was the strongest predictor of MMIs in our infants with bronchiolitis. Although the high-risk groups for severe forms of RSV infection are traditionally infants younger than 6 weeks, premature infants and those with immunodeficiency or chronic lung, congenital heart or neurological diseases, prematurity and underlying chronic illness did not significantly predict the need for MMIs in our series (Boyce 2000, Hall 1986, Wilkesmann 2007). This is in line with recent claims that most children with RSV infections in developed countries have no underlying medical conditions, whereas non-RSV bronchiolitis has been associated with underlying comorbidities (Garcia *et al.* 2010, Hall *et al.* 2009). Prematurity is a risk factor for apnoea during RSV infection (Ralston & Hill 2009), this was also the case in our analysis. A fever higher than 38°C predicted the need for an MMI even when the patients with a confirmed diagnosis of pneumonia and sepsis were excluded, indicating that patients with severe bronchiolitis can have a bacterial co-infection, as also reported previously (Ricart *et al.* 2013). We found an inverse association between the initial SaO₂ value and the need for MMI. This result is in line with earlier reports in which initial SaO₂ values below 95% have been considered predictive of severe bronchiolitis (Mansbach *et al.* 2012, Parker *et al.* 2009, Shaw *et al.* 1991).

The strengths of our study lie in its focus on young infants who are at risk of serious deterioration during bronchiolitis and our use of the need for MMI as a measure of the severity of the disease. Our findings that the possibility of needing an MMI continued for up to 5 days after the onset of symptoms of bronchiolitis and that 19% of the patients eventually needed an MMI provide evidence-based information for use in developing and implementing guidelines concerning bronchiolitis. RSV infection, a fever higher than 38°C and a low initial SaO₂ value predict the need for MMIs and infants with these symptoms should be hospitalized. Discharge is safe when the respiratory symptoms have lasted for at least 5 days, as the risk of deterioration diminishes once the unstable phase of the disease is over. We found that length of professional experience has an effect on decision making, as those doctors who had longer professional experience were more likely to discharge patients from the emergency room than those with less experience. This is interesting as it is very common that the decisions to admit patients are often made by most junior and inexperienced clinical staff. The limitations of our study were that our data did not include systematic observations of respiratory rate and heart rate or clinical scoring of the severity of the disease and that our hospital did

not systematically check for the rhinovirus infection at the time when it was carried out.

7.2.2 Children aged from 6 months to 16 years (III)

We found that 87% of the children aged from 6 months to 16 years coming to the emergency room with wheezing during respiratory infection had a mild disease, defined as a stay in hospital of less than 48 hours. Altogether 6% of the patients had a severe disease and 7% had an initial SaO₂ value below 90%. An initial SaO₂ value >93% effectively identified the children with a mild wheezing disease, and the use of this figure as the limit for hospitalization would have almost halved the number of admissions.

There is poor agreement on the terminology and diagnosis of infection-induced wheezing in children after the initial episode, which is most commonly diagnosed as bronchiolitis (Ducharme *et al.* 2014). Bronchiolitis has typical features, whereas wheezing at preschool age has been defined according the duration of wheezing or according the triggers of wheezing (Brand *et al.* 2008). In addition, a diagnosis of asthma in young children is usually based on a series of clinical criteria, because the confirmatory lung-function testing has traditionally been available only at 6 years of age (Ducharme *et al.* 2014). Some task forces even hesitate to recommend to make a diagnosis of asthma in young children (Brand *et al.* 2008). Bronchiolitis has an unstable early phase and the response to bronchodilators is poor, whereas with obstructive bronchitis the symptoms are worst at the time of admission and there is a clear response to bronchodilators (Ducharme *et al.* 2014, Horst 1994, Reindal & Oymar 2006). There are clear pathophysiological differences between bronchiolitis and obstructive bronchitis. Virus-induced bronchoconstriction in obstructive bronchitis is obvious, whereas smooth muscle constriction seems to have only a minor role in bronchiolitis (Gern 2003, Zorc & Hall 2010).

The decision to admit children because of wheezing is partly determined by social and behavioural factors that are specific to the patient population, such as parental compliance, the availability of medication and the distance from the patient's home to the hospital (Brand *et al.* 2008). We believed that the length of hospitalization would be a more realistic measure of the severity of the wheezing episode rather than just hospitalization, as hospital stays of less than 48 hours indicate a relatively mild disease. We analysed patients with an initial SaO₂ below 90% separately, because it is evident that most of these patients have a severe disease and need hospitalization (Keahey *et al.* 2002).

We found that an initial SaO₂ value >93% effectively identified children with mild wheezing disease and the use of this value as a limit for hospitalization would have almost halved the number of admissions. Pulse oximetry is an accurate, simple and non-invasive method for measuring arterial oxygen saturation, and it can reliably detect desaturation in wheezing children (Rosen *et al.* 1989, Schnapp & Cohen 1990). Initial SaO₂ has been shown to be the dominant predictor of hospitalization in wheezing children (Boychuk *et al.* 2006). Although SaO₂ has been evaluated in several studies, there is no consensus on the initial thresholds for admission, treatment and discharge in case of children with acute wheezing (Fouzas *et al.* 2011). A two percentage point difference in initial SaO₂ may represent a very small difference in the partial pressure of arterial oxygen, but this difference can significantly influence the decision about whether to admit or discharge wheezing children (Mallory *et al.* 2003, Schuh *et al.* 2014). The routine use of pulse oximetry has led to changes in bronchiolitis management and may have lowered the hospitalization threshold for patients with bronchiolitis (Schuh *et al.* 2014). In our study just under half of the admitted patients (48%) had an initial SaO₂ of >93%, but only 11% of these patients required additional oxygen in hospital. Our study established a threshold that predicts mild wheezing disease, and we suggest that children with acute wheezing disease who are at least 6 months old do not need to be hospitalized if their initial SaO₂ is more than 93%. Our finding is in accordance with earlier studies (Corneli *et al.* 2012, Geelhoed *et al.* 1994, Parker *et al.* 2009). Most of our patients were male, but male gender as such did not have any effect on the severity of the disease. Although prematurity was one of the variables predicting the duration of hospitalization for children younger than 2 years of age with respiratory infections in earlier study (Weigl *et al.* 2004), it did not have any predictive power with respect to the severity of wheezing disease in the present series.

The strengths of our study are that we used a prolonged stay in hospital as an indicator of severe wheezing and focused on patients with an initial SaO₂ of at least 90%. One limitation was that we collected the data from the hospital's patient records and they did not include systematic recording of respiratory rates or clinical scoring of the severity of the wheezing episode.

7.3 Outcomes after RSV infection in infancy (IV)

We found that patients who had an RSV infection in early infancy more often had symptoms of asthma and wheezing before school age than did control patients.

However, these symptoms decreased with time, and so we found no differences in current asthma, wheezing symptoms and lung function measurements at the age of 8 years. We found no association between RSV infection in early infancy and subsequent atopic eczema or allergic sensitization.

In previous post-bronchiolitis studies the prevalence of asthma at the age of 6 to 8 years after an RSV infection in early infancy has varied from 8% to 48% (Castro *et al.* 2008, Koponen *et al.* 2012). We found that the prevalence of asthma was 13% after an RSV infection in infancy and 7% among the controls. Our study setting is comparable to that of earlier prospective studies in which the control subjects were recruited concomitantly with the index children at birth or during infancy (Korppi *et al.* 1994, Sigurs *et al.* 2000, Zomer-Kooijker *et al.* 2014). These prospective studies have reported the prevalence of asthma at the age of 6 to 8 years after hospitalization due to an RSV infection in infancy to be 15–23% and asthma diagnoses in 2–5% of healthy control subjects, as well as an association between hospitalization for RSV infection and current asthma during follow-up visits (Korppi *et al.* 1994, Sigurs *et al.* 2000, Zomer-Kooijker *et al.* 2014). The lack of this association and the lower prevalence of current asthma after an RSV infection in infancy in our study could be due to the fact that we recruited children with an RSV infection who required hospitalization as well as those who were treated as outpatients. The prevalence of asthma among the controls in our study is close to 4-6% of the asthma prevalence in Finnish children at school-age (Remes *et al.* 1996).

A severe early RSV infection requiring hospitalization has been considered a predictor for wheezing and asthma that persists into early adulthood, while in outpatient RSV population the association steadily subsides after the age of 3 years suggesting that a dose-response exists between infection severity and the higher odds of developing childhood asthma (Sigurs *et al.* 2010, Stein *et al.* 1999). A recent meta-analysis found an association between infant RSV hospitalization and subsequent asthma and wheezing that decreases with age (Regnier & Huels 2013). Our results are in line with this finding.

Rhinovirus wheezing illness in early life has been identified as a stronger predictor of the development of asthma than episodes due to RSV infection (Jackson *et al.* 2008, Koponen *et al.* 2012, Kotaniemi-Syrjanen *et al.* 2003). Children with a rhinovirus infection in early life are older and more likely to have a family history of asthma than those who have an RSV infection (Jackson & Lemanske 2010). Our study population included infants younger than 6 months of age with respiratory infection symptoms, most of whom had an RSV infection.

One recent epidemiological study and two recent birth cohort studies suggest that severe bronchiolitis in infancy most likely represents a genetic predisposition to asthma (Chawes *et al.* 2012, Jackson *et al.* 2012, Thomsen *et al.* 2009). In our previous analysis of this study population, infants who were later hospitalized on account of their RSV infection differed from their healthy control subjects in their higher capacity to produce stimulated proinflammatory cytokines at birth (Juntti *et al.* 2009). Thus, these results and those published in the recent literature support the interpretation that RSV infections pick up those children who are prone to wheezing and asthma before school age, but do not permanently alter their bronchial reactivity.

Our finding of no association between RSV infection in early infancy and subsequent atopic eczema is in line with earlier studies (Garcia-Garcia *et al.* 2007, Juntti *et al.* 2003, Schauer *et al.* 2002, Sigurs *et al.* 2010). The association between RSV infection in early infancy and subsequent atopic sensitization as measured by skin-prick tests remains controversial, and we did not find any such association, although both positive and negative ones has been found earlier (Juntti *et al.* 2003, Sigurs *et al.* 1995, Sigurs *et al.* 2000, Sigurs *et al.* 2005, Sigurs *et al.* 2010).

The strengths of our study lie in its prospective design and the fact that our control patients belonged to the same birth cohort as the patients with an RSV infection. The limitation of our study is that the number of patients with respiratory infection was low, so the subgroups formed were rather small and in this cohort study we could not assess the effect of rhinovirus infection in infancy considering wheezing and atopy at school age. Participation in the study visit was in parents' interest and the patients with a history of asthma and allergy symptoms were more likely to participate. Just over three-quarters (76%) of the study population returned the questionnaire and 60% participated in the study visit. The limitation of our study is that the number of patients with respiratory infection was low, so the subgroups formed were rather small.

8 Conclusions

1. A family history of croup is an exceptionally strong risk factor for croup and its recurrence in childhood.
2. The early phase of bronchiolitis is unstable in infants below 6 months of age. These infants are most likely to need medical interventions in the first 5 days after the disease onset. A positive RSV test result, a fever of more than 38°C and a low initial oxygen saturation value are predictors of a need for medical interventions.
3. Mild wheezing disease during respiratory infection is a common cause of paediatric hospitalization. An initial oxygen saturation >93% effectively identifies children aged more than 6 months with mild wheezing and this limit could be used in order to avoid unplanned hospital admissions.
4. There is an association between early RSV infections and subsequent wheezing and asthma. Early RSV infections select those children who are prone to wheezing and asthma before school age, but the symptoms tend to decrease with time and early RSV infection does not permanently alter bronchial reactivity.

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