Respiratory syncytial virus (RSV) infections in infancy
epidemiological and clinical aspects

Respiratoir Syncytieel Virus (RSV) infecties op jonge leeftijd
epidemiologische en klinische aspecten

Martin C.J. Kneyber

Cover illustration:
Erasmus Bridge (also known as "The Swan") which connects the northern part of Rotterdam with the southern part, crossing the river "Maas" (reproduced with kind permission by Paper Mill Cards, Amsterdam)

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Voor Inge en mijn moeder

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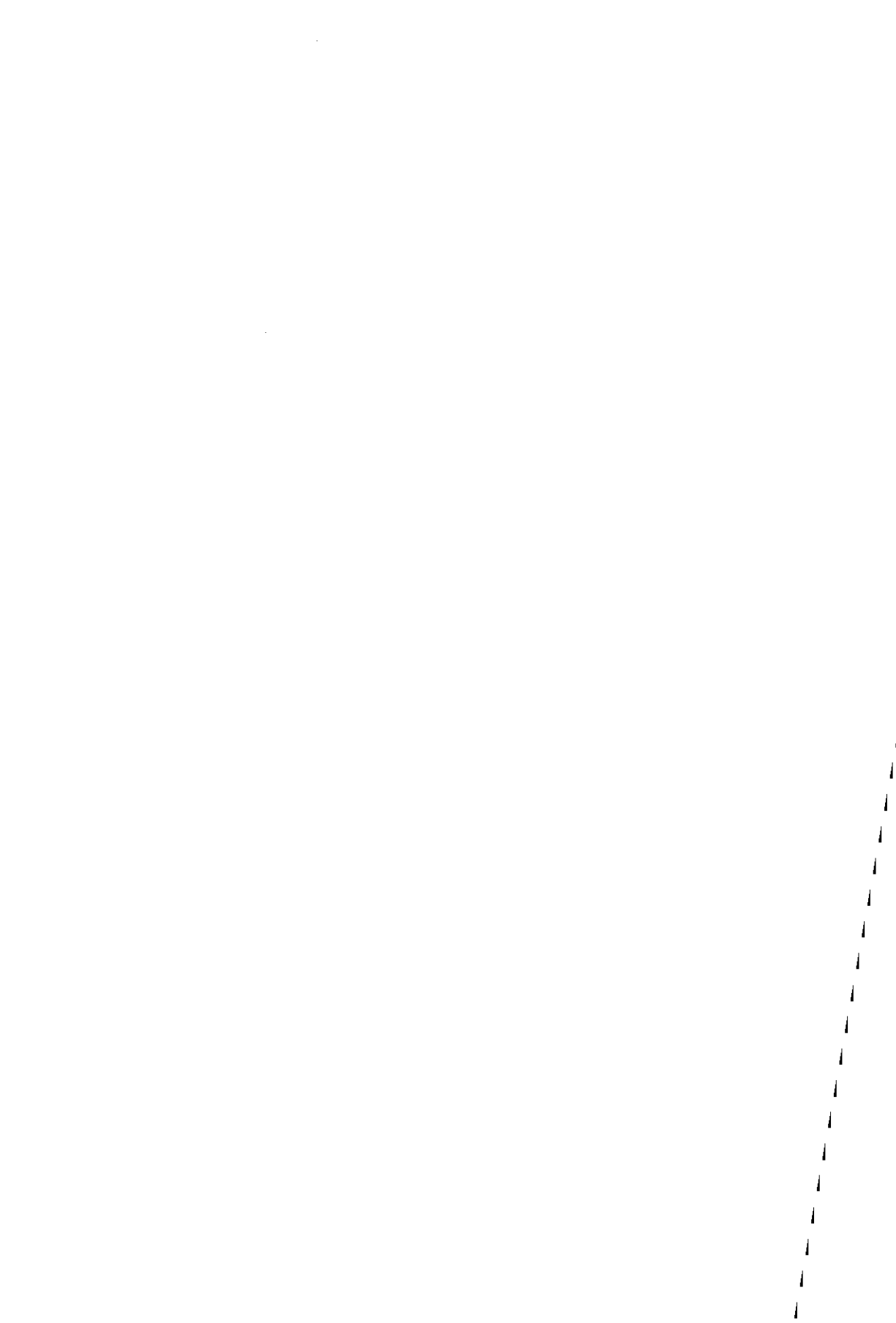


CHAPTER 1

GENERAL INTRODUCTION AND AIMS OF THE STUDIES

1.1 General introduction

Respiratory syncytial virus (RSV) is the most important cause of viral respiratory tract infections in infants and young children [1]. The number of infants which are annually hospitalised with acute bronchiolitis in The Netherlands is depicted in Figure 1.1. The virus causes yearly outbreaks with a seasonal distribution [2,3]. Nearly all children have been infected by the virus at least once by the age of two years [4].

1.1.1 Virological aspects

RSV is classified within the genus *pneumoviridae* of the family of *paramyxoviridae*. Two major subtypes of RSV (A and B) can be identified by the use of specific monoclonal antibodies [5,6]. Both subtypes can circulate simultaneously or independently during an epidemic (Figure 1.2). The proportion varies every season and per geographic location, although a predominance of subtype A is usually noted [7,8]. During an outbreak various antigenically distinct strains can be found. Multiple evolutionary lineages of RSV cocirculate globally during a single epidemic. For instance, an identical strain was

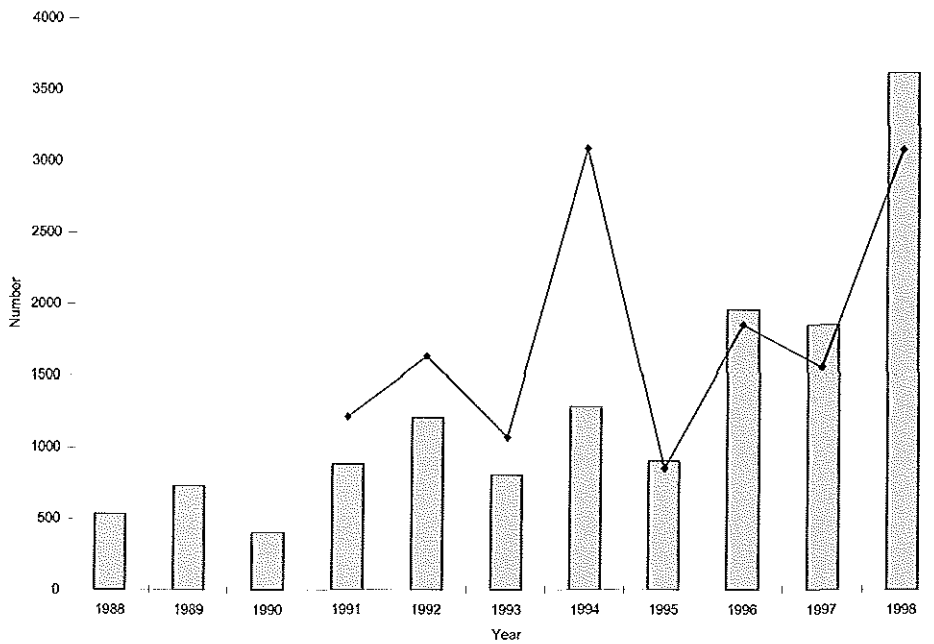


Figure 1.1 *Number of infants younger than 12 months of age hospitalised with acute bronchiolitis (ICD-9 diagnosis code 466.1) and number of positive RSV isolates between 1988 and 1998. The closed bars represent the number of hospitalised infants (data from SIG/Informatiecentrum voor de gezondheidszorg), the continued line represents the number of positive RSV isolates (data kindly supplied by Dr. M.L. Heijnen, National Institute of Public Health and the Environment (RIVM), The Netherlands)*

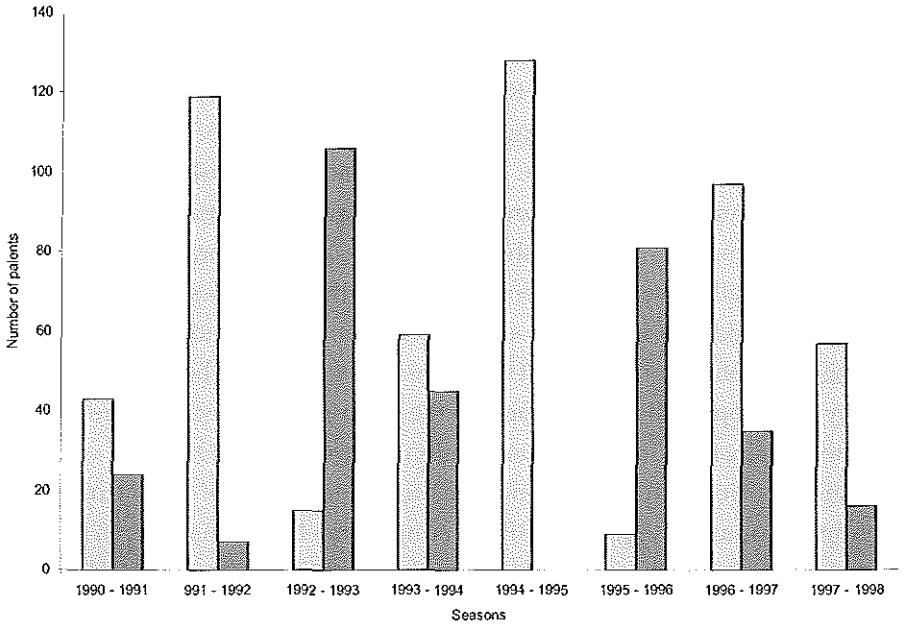


Figure 1.2 *Distribution of RSV subtypes A and B in all patients diagnosed between 1990 - 1998 at the Sophia Children's Hospital, Rotterdam (data kindly supplied by A.H. Brandenburg MD, Department of Virology, University Hospital Rotterdam, The Netherlands)*

found in Hannover, Germany and Montevideo, Uruguay during the same epidemic [9]. Several investigators previously reported that subgroup A infections lead to a more severe disease course [7, 10-12]. However, other groups could not confirm a relationship between subtype and severity of disease [13-16].

1.1.2 Epidemiology of infections by RSV

RSV infections are relatively uncommon during the first month of life [17]. The clinical presentation of RSV infection may vary geographically, even within one country or continent [18, 19]. The disease course of RSV infection is similar in tropical and developed countries [20]. Epidemics usually last five to six months with the highest incidence during the third or fourth month of the epidemic [21]. Not all infections by RSV result in lower respiratory tract disease [22]. The "Houston Family" study, carried out between 1975 and 1980, demonstrated RSV associated lower respiratory tract disease in 21.6% of a cohort of children followed during their first year of life [4]. In a hospitalised population RSV may be accountable for up to 13% of the upper respiratory tract infections and approximately 65% to 90% of the lower respiratory tract diseases (bronchiolitis and/or pneumonia) [23]. Reinfections with RSV occur frequently and tend to be mild [24-26]. The majority of RSV lower respiratory tract disease occurs in infants

younger than 12 months of age with a peak incidence between the age of two and six months [27].

Age is a predictor for severity of RSV infection (Figure 1.3) [28]. Severe LRT disease may occur especially in infants younger than six months of age [29]. Other risk factors include low socio-economic status, day-care attendance, smoking, low maternal antibody level, number of siblings, overcrowding, month of birth, air humidity and outdoor temperature [17,30-34]. Low birthweight may be a risk factor, because it has been proposed that small airways predispose to bronchiolitis [35]. Studies on the relationship between RSV infections in infancy and the development of atopy have generated conflicting data. Some groups suggest that there is no relationship between bronchiolitis in infancy and atopy; others have come to the opposite conclusion [35]. Breastfeeding may protect against RSV infection, but again controversy exists [36,37].

Underlying conditions that may result in severe RSV infection include bronchopulmonary dysplasia (BPD), congenital heart disease (CHD) with hemodynamic consequenses i.e. left to right shunting , T-cell immunodeficiency and prematurity [38-40]. Especially infants with congenital heart disease and pulmonary hypertension may be at an increased risk for severe infections by RSV [39, 41, 42]. Immunodeficiency, both acquired and congenital, results in increased viral shedding and increased morbidity [40,43]. RSV-associated pneumonia was found among patients

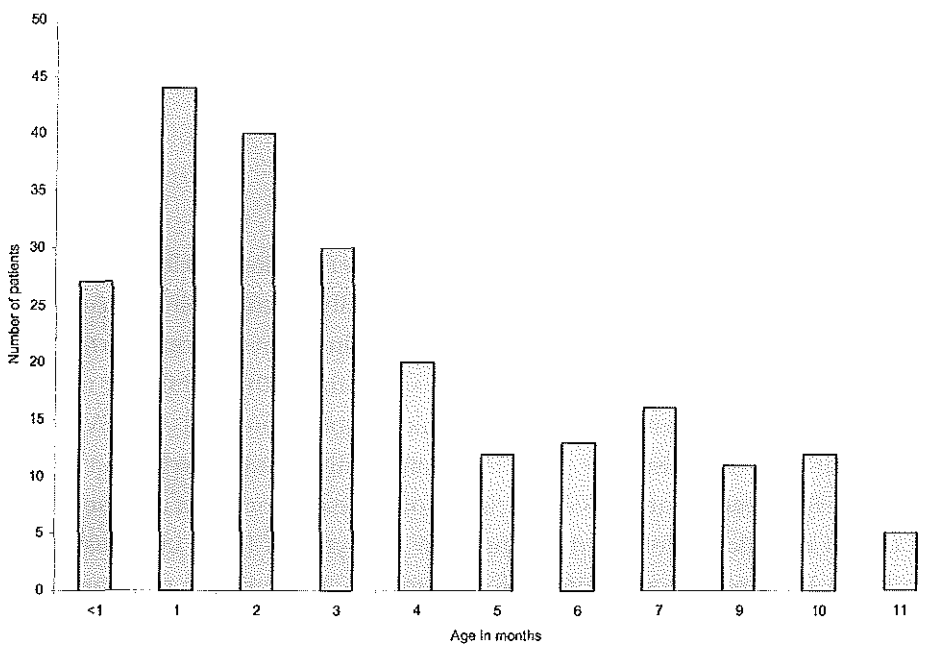


Figure 1.3 *Age distribution of infants diagnosed with RSV infection between 1992 - 1995 at the Sophia Children's Hospital, Rotterdam*

with HIV-infection [41]. Premature birth is associated with an increased necessity for supplemental oxygen, mechanical ventilation and admission to the intensive care unit [45].

1.1.3 Clinical manifestations of infections by RSV

The clinical spectrum of RSV-associated disease extends from mild upper respiratory tract involvement to severe lower respiratory tract disease necessitating mechanical ventilation, and apnoea. Symptoms by RSV infection usually begin in the nasopharynx with coryza and nasal congestion. A low grade fever may be noticed. Approximately two to five days later infection may progress to involve the lower respiratory tract as shown by the development of cough, dys- and/or tachypnoea and wheezing [46,47]. Physical examination may reveal tachypnoea, signs of rhinitis, cough, chest wall retractions, nasal flaring and seldomly fever. Wheezing may be noted even without the use of a stethoscope. On auscultation in- and/or exspiratory crackles may be noted. Hypoxaemia due to a ventilation/perfusion mismatch with or without hypercapnia is frequently observed [46-48].

Apnoea may be the first clinical sign of RSV infection. The incidence of apnoea in hospitalised patients varies between 10% and 20% [49-52]. RSV associated apnoea specifically occurs in young infants and premature infants [49,51,53]. The occurrence of apnoeic spells may necessitate mechanical ventilation [53].

The large majority of infections by RSV have a mild disease course in which neither hospitalisation nor the use of medication is indicated. In approximately 0.5% to 2% of all patients hospital admission is required [17,51]. The duration of hospitalisation for RSV infection varies extensively depending on the geographic area. For instance, in the United States and Australia, the mean duration of hospitalisation is four days, whereas children spend eight to 10 days in hospital in several European countries [55].

Of all hospitalised patients with infections by RSV, intensive care admission is necessary in 15% to 35% [56]. In approximately 7% to 21% of hospitalised patients mechanical ventilation is indicated because of respiratory distress, hyperapnia or recurrent apnoea [56,57]. Risk factors for mechanical ventilation include young age and low weight [53,58]. Neonatal RSV infection may follow an atypical course with nonspecific signs [19]. Bradycardia may the first presenting sign [59].

The mortality of infants with RSV infection is low and estimated to be between 1% and 3% in hospitalised children [23]. High-risk children have an increased mortality rate: 5% - 8% for children with CHD and up to 13% for children with chronic lung disease (CLD) [42,53,60].

RSV has also been identified as a nosocomial pathogen with infection rates varying

between 5% and 45% of the total number of (hospitalised) RSV infections and leading to a more severe disease course [61-64]. Infants at high risk for severe infections by RSV have a high hospitalisation rate and are at increased risk for severe nosocomial RSV infections [61,65]. The risk of acquisition of a nosocomial RSV infection increased with increasing stay in hospital for any underlying condition [61]. Hospital staff are thought to be a main vector for the transmission of RSV [66]. Various strains may be found among infants and children with a nosocomial infection.

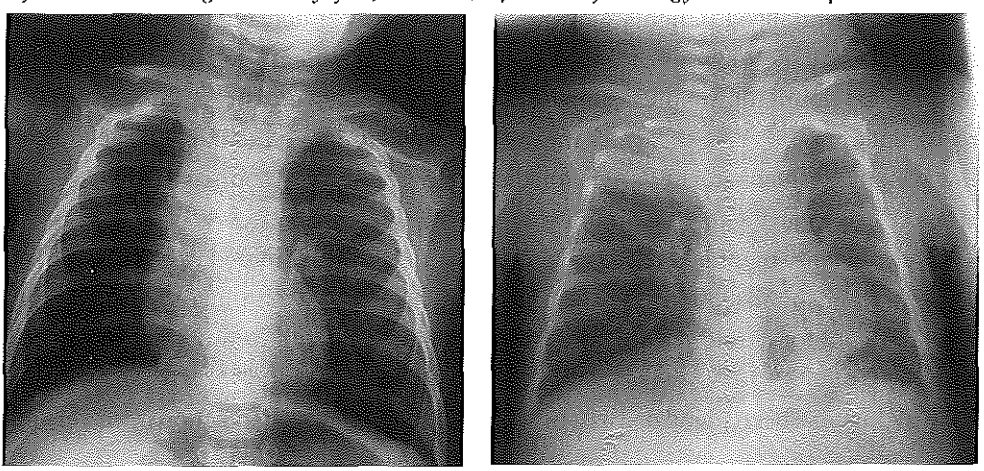
1.1.4 The value of chest radiography in infections by RSV

RSV infections are diagnosed mostly on clinical symptoms whereas virological confirmation is necessary in children admitted to the hospital in order to prevent nosocomial infections. The clinical diagnosis of infections by RSV is based upon symptoms such as wheezing, rales, tachypnoea and dyspnoea. In addition, chest radiography is often performed. The spectrum of findings on chest radiographs in RSV infection may vary from a normal chest radiograph to atelectasis, hyperinflation or pulmonary infiltrate (Figure 1.4) [67,68]. However, there seems to be no correlation between the severity of RSV infection and the findings on chest radiographs [69]. The routine use of chest radiographs in the initial management of infections by RSV has been questioned [70].

1.1.5 Treatment and prevention of infections by RSV

Treatment of RSV bronchiolitis has been mainly supportive. Oxygen administration and nasogastric tube feeding are usually indicated for hospitalised infants. The use of

Figure 1.4 *Chest radiographs of patients with respiratory syncytial virus infections showing hyperinflation (left) and atelectasis (right) (courtesy of F. Jansen MD, department of Radiology, Catharina Hospital Eindhoven).*



bronchodilators, corticosteroids and the antiviral drug ribavirin have been extensively investigated as will be discussed in Chapter 8 [71,72,73]. Preventive approaches include novel therapies such as passive immunisation with RSV-immunoglobulin (RSV-IVIG) or monoclonal antibodies such as palivizumab [74,75]. Despite dramatic effects in the '60s with a formalin inactived alum precipitated vaccin, vaccin development is currently progressing rapidly and some of the new vaccines such as subunit vaccines or live attenuated *cold passaged-temperature sensitive* vaccines hold great promise [76,77].

1.1.6 Long term effects of infections by RSV

Pulmonary function testing in infants hospitalised with bronchiolitis may either reveal increased airway resistance or restrictive, parenchymal lung disease as found in RSV pneumonia [78-81]. In contrast to these observations, a recent controlled study including patients with mild/moderate severe RSV bronchiolitis failed to identify significant differences in pulmonary function [82]. It has also been suggested that pre-existing abnormalities in lung function may be found among children with bronchiolitis when compared to controls and that this difference in lung function disappears between six and 12 months of age [83]. Others observed no increased incidence of airway hyperreactivity or pulmonary function eight to twelve years after mild bronchiolitis in infancy [84]. Bronchial hyperreactivity during RSV bronchiolitis may occur [85].

Bronchiolitis in infancy may result in obstructive pulmonary disease during (early) childhood [86]. Over the years, numerous studies have been published on the relationship between bronchiolitis in infancy and long term morbidity. Wheezing episodes can occur up to eight to ten years after RSV bronchiolitis in infancy [87]. It has been suggested that RSV bronchiolitis in infancy may lead to asthma in later life [88]. Two hypothesis have been proposed: *i)* a causal association - RSV induced lower respiratory tract infection directly damages the lung leading to long-term morbidity - and *ii)* an inherent predisposition: RSV infects the predisposed host resulting in severe infection and long term morbidity with subsequent asthma [89]. Controversy exists whether an atopic background is related to long-term morbidity by RSV [88,90]. Some clinical studies failed to identify a relationship between personal or family history of atopy and respiratory morbidity during childhood whereas others identified such a relationship [91-93].

1.2 Outline and aims of the studies

This thesis describes the results of epidemiological and clinical studies on infections by RSV in infancy. The studies were carried out in the Department of Paediatrics of the Sophia Children's Hospital, Rotterdam, a university children's hospital with both secondary and tertiary health care functions. **Chapter 2** provides an overview of clinical and pathophysiological aspects of RSV infections in young children.

Specific aims of the studies were:

1. Study of the relationship between the clinical severity of RSV infection and subtypes A or B. This issue is addressed in **chapter 3.**
2. Evaluation of characteristics of RSV-associated apnoea: identification of independent risk factors and assessment of the risk for recurrent apnoea and for mechanical ventilation once RSV-associated apnoea occurred. These issues are addressed in **chapter 4.**
3. To develop a prediction model for a normal chest radiograph based on clinical parameters in infants with infections by RSV (**chapter 5**).
4. In **chapter 6** a previously published model for the prediction of the duration of hospitalisation in RSV infections is evaluated and a new model is developed.
5. In **chapter 7** the number of nosocomial infections by RSV over the years and the association with morbidity at the Sophia Children's Hospital is studied. Furthermore, a review of preventive approaches which have to be be undertaken to reduce the number of nosocomial RSV infections is presented.
6. **Chapter 8** reviews the therapeutic and preventive approaches in the treatment of infections by RSV.
7. In a quantitative review the long term effects of RSV-bronchiolitis in infancy with a focus on the later development of recurrent wheezing/asthma during childhood is evaluated in **chapter 9.**

In **chapter 10** the results of the previous studies are summarised. Also, recommendations for further research are given. Finally, the results of this thesis are summarised in Dutch (**chapter 11**).

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CHAPTER 2

PATHOPHYSIOLOGICAL AND CLINICAL ASPECTS OF RESPIRATORY SYNCYTIAL VIRUS INFECTIONS

2.1 History

Morris and co-workers isolated in 1956 a cytopathogenic agent from chimpanzees with coryza [1]. Because of the purulent rhinorrhea, the virus was called Chimpanzee Coryza Agent (CCA). However, since animal handlers became ill and CCA was recovered from throat swabs of infants with lower respiratory tract illness, human origin of the virus was highly suspected [2]. Because of its clinical manifestations and the characteristic cytopathologic findings in tissue culture where it forms syncytia of epithelial cells, the virus was named *respiratory syncytial virus* (Figure 2.1). Epidemiological research carried out over the last four decades demonstrated the tremendous impact of RSV on lower respiratory tract infections in young children [3-7].

This chapter discusses the current perspectives on the pathophysiology of infections by RSV.

2.2 Virology

2.2.1 RSV

RSV is classified within the genus *pneumoviridae* which is a member of the family of *paramyxoviridae*. The virus is a pleomorphic (exhibiting both spherical and filamentous forms) enveloped cytoplasmic virus measuring 80 to 500 nm (Figure 2.2). The spherical particles measure 100 – 350 nm [8]. The genome of RSV is a single-stranded negative sense RNA which contains 15.222 nucleotides (RSV A2 strain) from which ten mRNA's are transcribed [9, 10]. These encode for the viral proteins. The G and F proteins are envelope associated proteins. Both are the major antigenic determinants of the virus inducing neutralising and protective antibodies [11]. The G protein, a large glycoprotein,

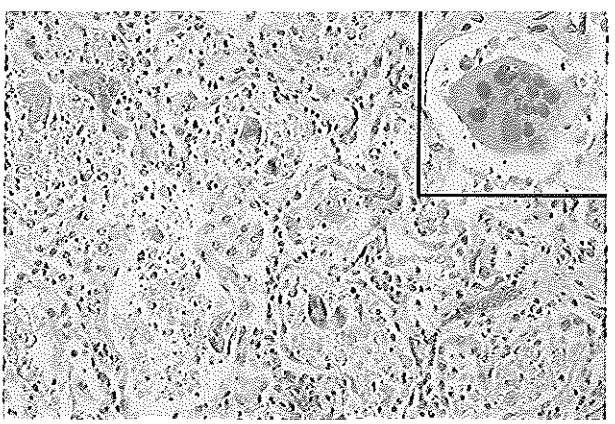


Figure 2.1
Histopathological example of post-mortal lung tissue infected with RSV (200x). Note the characteristic syncytia formation highlighted in the black box (400x). (source: www-medlib.med.utah.edu, the internet pathology laboratory for medical education. Courtesy by Prof. Dr. E.C. Klatt, MD)

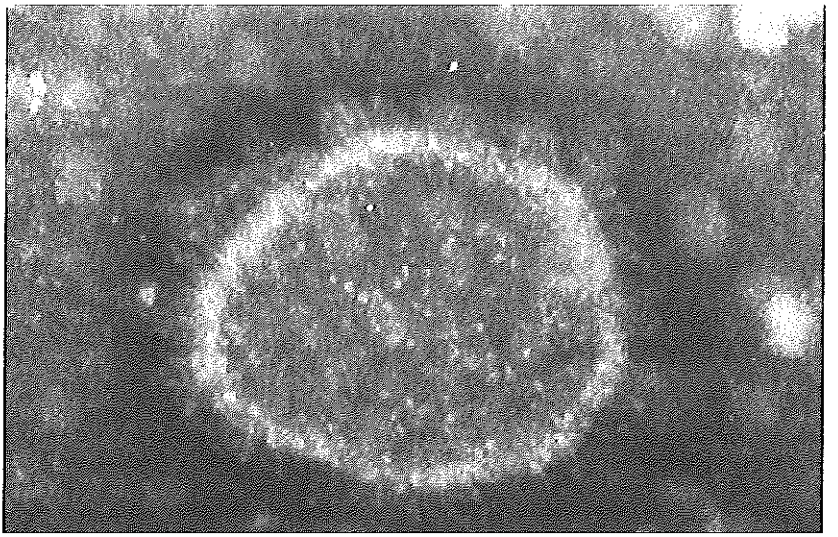


Figure 2.2 *Electronmicroscopy of respiratory syncytial virus (magnification 85.000 x, courtesy by Prof.Dr. A.D.M.E. Osterhaus)*

is responsible for attachment of the virus to host cells, whereas the F protein – a disulphide bonded glycoprotein - is responsible for the characteristic syncytium formation. Heparin-like structures on the G protein seem to play a role in the attachment of RSV to the host cell surface [12]. The L protein is believed to have polymerase activity. The functions of the small hydrophic protein (SH) and of the non-structural proteins NS1 and NS2 have not yet been elucidated.

The use of specific monoclonal antibodies allows for the identification of two major groups of RSV, designated A and B [13,14]. Differences between the two groups are mainly found in the G protein, which share only one of six epitopes and in the F protein, where differences in one epitope can be detected [13]. The G protein may have only 53% amino acid homology between subtype A and B. This indicates that the F protein is rather conserved and demonstrates a degree of both genetic and antigenic homology between both subtypes [11]. Genetic differences may be found among subtype A and B. Differences up to 20% have been described in the G protein sequences of subtype A and up to 9% for subtype B [15].

2.2.2 Transmission of the virus

RSV infects through the eye and the nose. Transmission is conferred by direct inoculation with large droplets or self-inoculation after contact with contaminated surfaces [16, 17]. Direct inoculation occurs only after close contact with an infected patient [17]. Contaminated surfaces are an important source of infection. RSV may survive

for one hour on cloth and tissues whereas surival on the skin may persist for 30 minutes. RSV can be recovered from countertops for up to seven hours [16]. Touching these countertops without handwashing results in self-inoculation.

The incubation period of RSV infection varies between two and eight days with a median of five days [18]. Viral shedding may persist for up to three weeks in the individual patient. In the majority of patients viral shedding persists for ten days to two weeks [19-21]. Young infants were found to shed more virus than older infants [20]. Viraemia may occur as has been demonstrated by the presence of RSV-RNA in polymorphonuclear cells, although no correlation between viraemia and severity of illness was found [22].

2.2.3 *Virological diagnosis of infections by RSV*

Techniques for diagnosis of RSV infection include viral culture, (in)direct immunofluorescent antibody assay, enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) on respiratory secretions. The immunofluorescent assay (IFA) can easily be carried out with results within one hour [23]. The sensitivity of the direct IFA is 95% to 97% with a specifity of 94% to 95% [24-27]. However, the interpretation of immunofluorescence assays requires the availability of skilled laboratory personnel. Other techniques include ELISA transformed into commercial kits such as the Abbott TestPack® with a sensitivity between 92% and 96% and a specifity of more than 95% [28-33]. Reverse transcriptase (RT)-PCR is highly specific with a sensitivity of approximately 95% and a specificity of 97% [34, 35]. With RT-PCR, RSV can be detected at much lower 50% tissue culture infectious dose ($TCID_{50}$) per milliliter [36].

Serological diagnosis of RSV infection is by far inferior to viral culture, DIFA and RT-PCR [37]. For the nonculture techniques, IFA remains the most cost effective and most rapid technique currently available [38].

2.3 Pathogenesis of infections by RSV

2.3.1 *Pathology*

RSV infects respiratory tract cells. Airways with a diameter of 75 to 300 µm are affected in children with bronchiolitis. After infection, necrosis of bronchiolar epithelium occurs followed by a peribronchiolar lymphocytic infiltration. The submucosa and adventitial tissues become oedematous and mucus secretion is enhanced resulting in the formation of thick plugs in the airways leading to obstruction

of air flow. This obstruction results in air trapping or consolidation of pulmonary areas. Elastic and muscular tissues remain unaffected [39]. RSV antigen can be detected in epithelial cells throughout the respiratory tract, with paranuclear eosinophilic inclusions in a great number of RSV positive cells [40].

RSV pneumonia is characterised by a interstitial infiltration of mononuclear cells with interalveolar wall thickening. The degree of pathological changes varies from relatively mild to severe epithelial necrosis and eosinophilic sloughing of the lumen [39]. The virus load is relatively high in bronchiolitis compared to pneumonia, [39,41].

Reduced levels of surfactant protein (SP) A, B and D are found in ventilated infants with RSV infection as measured by bronchoalveolar lavage. This may be due to an invasion of the type II pneumocytes by RSV. However, no clear relationship between SP level and arterial alveolar oxygen ratio was observed [42]. Adequate levels of SP-A seem to be important in RSV-infection. A recent study in mice demonstrated that SP-A played an important role in the host defense against RSV infection with lung inflammation and viral titers increased in SP-A negative mice [43]. Low levels of SP-A may contribute to the obstruction of smaller airways because of possible collapse of small airways by higher surface tension [44].

Bronchiolar obstruction may further be enhanced through the release of potent bronchoconstrictors such as IL-11, histamine and LTC_4 by mast cells and macrophages with subsequent bronchoconstriction [45-47]. Neural mechanisms governing bronchomotor tone and various neuropeptides further contribute to bronchoconstriction [48].

2.3.2 *Cellular immune response*

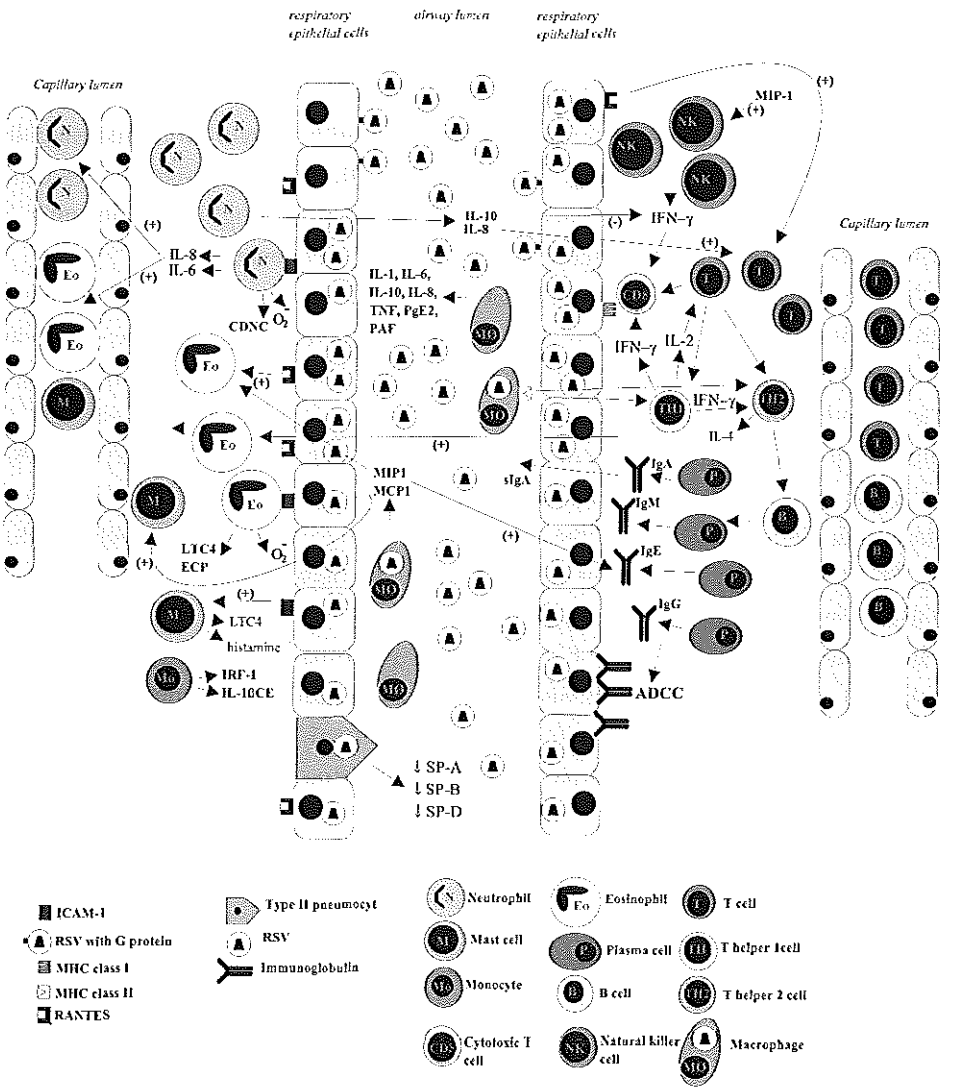
A graphic model of the pathogenesis of RSV infection is presented in Figure 2.3. The inflammatory proces caused by RSV takes place in pulmonary tissue. Immune cells leave the blood and redistribute to the lungs with the use of vascular cell adhesion molecule (VCAM)-1 [49,50,51].

2.3.2.1 *Initial response to RSV infection*

RSV activates both the classical and alternative pathways of complement [52]. Neutrophils, the predominant cell type in RSV lower respiratory tract disease, adhere to infected epithelial cells with subsequent release of oxygen radicals induced by RSV antigen-antibody complexes [53-56]. This results in complement-dependent neutrophil-mediated cytotoxicity and subsequent lysis of RSV infected cells [57].

RSV particles are then phagyocytosed by neutrophils with subsequent production

Figure 2.3 *Hypothethical graphic model of the pathophysiology of infections by RSV*



of cytokines such as IL-6, IL-8 and IL-10 [58]. IL-6 has two functions: it may act as an anti-inflammatory agent by promoting humoral and cellular defence mechanisms, but it may also act as a pro-inflammatory cytokine [59]. Neonatal blood cells have a diminished capacity to produce IL-6 [60]. IL-8 acts as a chemoattractant and activator for neutrophils, T-cells, basophils and primed eosinophils with functional IL-8 receptors [61]. IL-10 functions as a regulatory cytokine controlling IFN-γ expression during RSV-infection [62].

Respiratory epithelial cells express MHC class I molecules and are further destroyed by the cytolytic action of multiplying and budding virus. Natural killer cells are

activated and produce interferon (IFN)-γ. Subsequently, CD8+ cytotoxic T lymphocytes appear and destroy RSV infected cells which express MHC class I [63,64].

Macrophages release cytokines such as TNF-α, IL-1, IL-6, IL-8, IL-10, platelet activating factor (PAF) and prostaglandin E_2 [65-67]. The production of IL-6 and TNF-α further enhances the immune response [68]. IL-10 acts as an anti-inflammatory cytokine which may interfere with the establishment of an effective immune response [69].

Monocytes stimulate the synthesis of interferon regulatory factor (IRF) – 1 and interleukin-1ß converting enzyme (ICE) [70].

2.2.3.2 Activation of T-cells

Viral particles are presented by the macrophage in association with MHC class II to CD4+ T lymphocytes which respond by proliferation and cytokine production. Within the T-helper cells, two subsets can be identified: T_H-1-cells which produce IL-2 to promote further T-cell proliferation and IFN-γ. T_H-1 cells are involved in cellular immune responses. T_H-2 cells are involved in the production of immunoglobulins and the induction of eosinophilia. T_H-2 cells produce IL-4 and IL-5. IL-4, when expressed in abundance, decreases viral clearance and diminishes induction of CTL's [71]. Anti IL-4 treatment after immunisation with inactivated RSV can shift the immune response to a T_H-1 like response with augmented CTL activity [72]. IL-12 reduces the level of eosinophilia follow RSV challenge, which indicates that it directs towards a T_H-1 like response [62].

Both CD4+ and CD8+ T lymphocytes are involved in the termination of RSV replication after infection. The number of CD8+ T cells increases during the course of RSV infection [50]. They downregulate T_H–2 cytokine secretion and decrease pulmonary eosinophilia [73]. This may be done through IFN-γ [74]. The appearance of CD8+ cytotoxic T lymphocytes (CTL) is related to the clearance of the virus six to nine days after infection [75,76]. However, the clearance of virus by CD8 + lymphocytes also appears to mediate illness [77,78].

Live RSV has been found to induce a T_H-1 like reponse [79,80]. However, others reported evidence of a T_H-2 like response [81]. Hussel and co-workers showed both T_H-1 and T_H2-responses in mice [82]. The character of the RSV antigen initially presented to the immune system is important in inducing different T_H subsets. The F-protein elicits in vitro a T_H-1 response whereas the G protein elicits a T_H-2 response with extensive pulmonary eosinophilia [83,84]. Further evidence for this observation can be found in the presence of abundant IL-4 and Il-5 secretions despite high concentrations of IFN-γ indicating the activation of both T-helper subsets, thus suggesting that the initial exposure to either F or G antigen dictates the cytokine response [84]. Recently, Spender and co-workers

observed in mice eosinophilia despite an abundant IFN-γ production [62]. Thus, the *phenotype* of effector cells rather than the cytokine pattern alone is also important in determining disease [85].

Conflicting results have been reported on the production of IFN-γ in RSV infection. Hall and colleagues found that infants with RSV produced significantly less interferon when compared to infants with influenza and para-influenza virus infection [86]. Nakayama and co-workers did find IFN-α in nasopharyngeal secretions. The decline in mean titer was parallel to the quantity of RSV antigen [87].

2.3.2.3 Attraction of inflammatory cells

Eosinophils are attracted towards pulmonary tissue as a result of T_H-2 cell activation and release of IL-4. Superoxide and LTC_4 are released [88]. Activated eosinophils also release eosinophilic cationic protein (ECP) [89,90]. Viral induced eosinopenia may occur during RSV infection [91]. Infected epithelial cells express RANTES (Regulated on Activation, Normal T cell Expressed and presumably Secreted) and MIP-1 (macrophage inflammatory protein 1), and IL-8 is produced with resulting further attraction of neutrophils, basophils, monocytes, T-cells and primed eosinophils [54]. Production of monocyte chemotactic protein (MCP)-1 and MIP-1 occurs in epithelial cells of small airways and lungs. RANTES and MIP-1 enhance IgE and IgG4 production and induce degranulation of NK cells and CTL's. The production of RANTES is stimulated by IFN- γ, and eosinophils also can secrete RANTES [92].

The pro-inflammatory cells adhere to the respiratory epithelium through intercellular adhesion molecule-1 (ICAM-1) using CD18 receptors [93-95]. Noninfected cells may also express ICAM as a result of pro-inflammatory cytokine expression by neighbouring cells [96]. The expression of ICAM-1 is primarily mediated by IL-1α [97].

2.3.2.4 Antibody-dependent cell mediated cytotoxicity

As a result of T_H-2 cell stimulation, plasma cells produce immunoglobulins such as IgG and IgE. Antibody-dependent-cell mediated-cytotoxicity (ADCC) associated with RSV-IgG antibody, reaches a maximum between one and two weeks after the onset of illness [98,99]. In contrast, antibody-dependent enhancement of infection may also occur and form an explanation for the striking observation that young infants develop severe disease despite the presence of maternal antibodies [100].

2.3.3 ANTIBODY MEDIATED IMMUNITY

The humoral response of infants with RSV infection is influenced by their age, by the presence of maternal antibodies and by the viral antigen. Young age influences the response to both the G and F protein of RSV. The G protein is heavily glycosylated and may thus be less immunogenic in young infants. Also, the decreased response to the G protein is influenced by the presence of maternal antibodies [101]. Low levels of maternal antibodies are not related to a severe disease course [102].

Primary infection of infants with RSV results in production of serum antibodies that are subgroup specific and cross-reactive [103,104]. Especially antibodies against the F protein are cross-reactive given the conserved region in the F protein. Yamazaki *et al* reported that patients infected with subtype B did not develop an antibody response to the G protein of the A2 strain but did develop antibodies to the F protein of the A2 strain [104]. The presence of antibodies against both glycoproteins leads to some degree of resistance to reinfection [105]. Others observed that prior group A infection primed for a more extensive cross-reacting antibody response at the time of the second RSV infection than infections by group B [106].

RSV infection induces IgM, IgG, IgA and IgE antibodies. IgM serum levels can be detected after approximately three days of illness and may persist for two to ten weeks [107,108]. The quantity of IgG antibodies measured in the infant is influenced by the height of serum levels of maternal antibodies. Large quantities of maternal antibody may protect against severe disease [109]. In infants younger than three months of age greater geometric mean titers of IgG were observed than in infants aged 3 to 6 and 6 to 12 months of age. Within 10 to to 14 days after the onset of illness, an increase in geometric mean titer may be observed with a peak titer three to four weeks later followed by a decrease [107]. Convalescent phase titers were twofold greater in infants older than 6 months of age. Subclass specific antibodies are mainly in the IgG 1 and 3 subclass [110, 111]. Secondary infections lead to a greater, more persistent IgG response than primary infections [107].

RSV infected cells may be coated with immunoglobulins with IgA being the predominant immunoglobulin [112]. IgA coated RSV infected cells are detected in the first available nasal sample taken at day 1 or 2 of hospitalisation with an average of 3.5 days. Subsequently cell-free anti-RSV IgA appears with a gradual increase after 10 to 14 days after the onset of illness [108]. The appearance of IgA coincided with the termination of viral shedding, so that these cell-bound immunoglobulins neutralise virus after budding from the cell surface [108]. Cell-free IgA may contribute to recovery from primary infection since its increase in titers is stronger than IgG [113].

Cell-bound IgE may be detected within two days after the onset of illness [114]. IgE has

also been found in nasopharyngeal secretions, indicating that RSV-IgE production takes place in the mucosa [115]. This IgE response may be observed as early as six to eight days after the onset of illness [116]. Peak concentrations do not occur until some time after the acute illness [117]. The development of IgE correlated with increasing concentrations of histamine as a possible result of latent sensitisation to RSV [117,118]. It has been suggested that the titers of RSV-IgE in nasopharyngeal specimens may be predictive for recurrent wheezing [119]. However, others were unable to demonstrate specific RSV-IgE in nasopharyngeal specimens [120].

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CHAPTER 3

RELATIONSHIP BETWEEN CLINICAL SEVERITY OF RESPIRATORY SYNCYTIAL VIRUS INFECTION AND SUBTYPE

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Abstract

We investigated whether a relationship between clinical severity of RSV infection and distribution of subtype A or B could be demonstrated. The data of 232 children, who were admitted to or diagnosed with RSV infection in the outpatient department of the Sophia Children's Hospital, Rotterdam between 1992 and 1995, were studied. The diagnosis of RSV was confirmed by a direct immunofluorescence assay. Subtyping was performed by an indirect immunofluorescence assay using specific monoclonal antibodies. Gender, age at diagnosis, gestational age and birthweight, the presence of underlying diseases, impaired feeding, the presence of wheezing and retractions, respiratory rate, temperature, clinical diagnosis at presentation, SaO_2, pCO_2 and pH, characteristics of hospitalisation and the need for mechanical ventilation were observed. Analysis was performed on data from all patients diagnosed with RSV infection in the period between 1992 and 1995 spanning three RSV seasons, and seperately on the RSV season 1993 - 1994. The outcome of the three year analysis (150 (64.7%) subtype A vs 82 (35.3%) subtype B) was compared with the outcome of the season 1993 - 1994, a mixed epidemic with 37 (60.7%) subtype A and 24 (39.3%) subtype B isolates. None of the variables observed in the season 1993 - 1994 differed significantly between RSV-subtype A and B. Similar results were obtained from the analysis in the period 1992 until 1995, with the exception of pCO_2 (a more high pCO_2 was found in subtype A, $p < 0.001$) and retractions (more retractions were noted in patients with subtype A, $p = 0.03$). After correcting for possible confounders using regression analysis, these differences were not significant anymore.

Our data indicate that there is no relationship between clinical severity of RSV infection and subtype.

3.1 Introduction

Respiratory syncytial virus (RSV) is the most common cause of respiratory tract infections in infants and young children. The clinical presentation can vary from a mild upper respiratory tract infection to severe bronchiolitis. The incidence of RSV bronchiolitis reaches a maximum at the age of two months and declines within the first year of life [1]. The majority of infections have a mild clinical course. A minority of infants, 0.5 to 2%, has to be admitted to the hospital. Between 7% and 21% of these patients will need mechanical ventilation [2,3].

Severe infections may occur in premature infants or patients suffering from an underlying disease, such as congenital heart disease (CHD), bronchopulmonary dysplasia (BPD) or T-cell-immunodeficiency [4]. The mortality of infants hospitalised with RSV is approximately 0.5% to 1.5%. In infants with an underlying disease higher mortality rates are found [5,6].

Two major antigenic groups of RSV, subtype A and subtype B, can be identified by specific monoclonal antibodies [7]. The antigenic diversity between these two subtypes is determined mainly by G-proteins, which are responsible for attachment of the virus and to a lesser extent by the fusion proteins, the F-proteins. The two subtypes can circulate independently from each other, but they may also cocirculate during one epidemic [8]. The identification of the two subtypes has led to the speculation that there might be a relationship between clinical severity of infection and RSV subtype. A limited number of studies were carried out to establish such a relationship [9-12]. These studies provide conflicting results.

The objective of our study was to investigate whether a relationship between clinical severity of RSV infection and subtype could be demonstrated in children younger than 12 months.

3.2 Patients and methods

Patients younger than 12 months who either were admitted to the Sophia Children's Hospital or visited the outpatient department due to RSV infection in the period between 1992 and 1995, were included in this study. The Sophia Children's Hospital is a combined secondary-tertiary care university hospital.

RSV infection was confirmed by a positive direct immunofluorescent assay (IFA) using FITC labeled monoclonal antibodies against RSV (DAKO, Ely, UK) performed on cells of nasopharyngeal washing and/or a positive viral culture on HEp2 cells[13]. In 97.4% (n = 226) both DIFA and viral culture were positive, in 1.3% (n = 3) only the DIFA was positive and in 1.3% (n = 3) only the viral culture was positive. RSV

subtyping was performed using RSV subtype specific monoclonal antibodies (MAB) in an immunofluorescence assay on cells of nasopharyngeal washings as described by Taylor *et al* [14]. MAB's used were 92-11C for subtype A and 102-10B for subtype B (Chemicon, Temecula USA)[7]. Briefly, cells of nasopharyngeal washings were fixed on glass slides in acetone. RSV subtype specific MAB's were incubated for 30 minutes at 37° C. After washing three times in PBS the slides were incubated with the conjugate anti-mouse FITC (Dako, Ely,UK). When the IFA was negative and viral culture was positive, the subtype specific immunofluorescence was performed on RSV infected HEp2 cells.

Epidemiological and clinical data were retrospectively obtained from the medical charts. Patient characteristics include gender, age at diagnosis, birthweight, gestational age and the presence of underlying disease (congenital heart disease with hemodynamic consequences i.e. left/right shunt, bronchopulmonary disease and T-cell immunodeficiency). Clinical observations at presentation include impaired feeding (defined as normal feeding, slow feeding with normal volume, decreased volume and no oral feeding), the presence of wheezing and retractions, and temperature. The use of mechanical ventilation was registered during admission. The decision for ICU admission and mechanical ventilation was made before subtype was known. Other clinical parameters include characteristics of hospitalisation: number of admissions and length of stay in hospital and number of intensive care unit (ICU) admissions and length of stay in ICU.

Laboratory parameters measured at presentation included oxygen saturation (SaO_2), pCO_2 and pH. SaO_2 was measured transcutaneously with the use of a pulse oximeter, pCO_2 was measured on capillary blood samples. All parameters were measured irrespective of severity of disease.

Three diagnostic categories were defined: bronchiolitis/pneumonia (lower respiratory tract infection, LRTI), upper respiratory tract infection (URTI) and apnoea. The diagnosis bronchiolitis was based on clinical features and hypertranslucency, atelectasis or bronchial thickening on chest radiograph. URTI was defined as coughing and/or rhinitis, with no abnormalities on chest radiograph. Apnoea at presentation was defined as a cessation of respiration for a period over 20 seconds and/or bradycardia with accompanying cyanosis. The diagnosis pneumonia was based on clinical features and the presence of an infiltrate on chest radiograph.

Statistical analysis was performed using the χ^2 test, the students t-test and the Mann Whitney-U test. *P*-value ≤ 0.05 was considered significant. In order to examine the independent effect of virus subtype on the severity of disease we adjusted for gender, age in months, prematurity (gestational age $\leq$ 37 weeks), the presence of underlying disease and year of diagnosis with logistic and linear regression analysis.

Linear regression was used to investigate if there were differences between the two virus subtypes in SaO_2 or pCO_2. Logistic regression was performed with dichotomous outcome variables such as impaired feeding, the presence of wheezing and retractions, the clinical diagnosis (bronchiolitis/pneumonia or upper respiratory tract infection), need of mechanical ventilation and ICU admission.

3.3 Results

In the period between 1992 and 1995 covering three RSV seasons, 232 children with RSV infection visited our hospital. In 1992 - 1993 a predominance of subtype B was found. Ten out of 68 children were infected with subtype A (14.7 %) versus 58 children with subtype B (85.3 %). The season 1993 - 1994 showed a mixed epidemic: 37 children were infected with subtype A (60.7%) and 24 children were infected with subtype B (39.3%). The season 1994 - 1995 showed an epidemic in which all 103 children were infected with subtype A.

Based upon the subtype distribution, two analysis were perfomed: one on the entire period from 1992 until 1995, covering three RSV seasons and one on the mixed A/B season 1993 - 1994. The results of the outcome of the analysis of the period 1992 - 1995 were compared with the outcome of the analysis of the season 1993 - 1994.

Table 3.1 *Patient characteristics*

		Subtype A (n = 150)	Subtype B (n = 82)
Boys		63.3	67.1
Girls		36.7	32.9
Mean age (months)		3.5 ± 3.0	3.6 ± 2.9
Patients	< 3 months	48.0	47.6
	3 – 6 months	26.7	26.8
	> 6 months	25.3	25.6
Mean gestational age (weeks)		37.3 ± 3.8	37.8 ± 3.3
Gestational age	≤ 34 weeks	18.4	12.7
	35 – 37 weeks	10.3	12.7
	> 37 weeks	71.3	74.6
Mean birthweight (gr)		2936 ± 875	3023 ± 804
Underlying disease state		16.3	11.0
Prematurity and BPD (n)		10	4
CHD (n)		14	3
BPD (n)		0	2

No significant differecens between RSV subtype A and B were observed.
BPD: bronchopulmonary dysplasia, CHD: congenital heart disease
Numbers expressed as % unless stated otherwise

Table 3.2 *Clinical parameters at presentation*

	Subtype A (n = 150)	Subtype B (n = 82)
Impaired feeding	71.6	64.6
Wheezing	36.0	34.1
Retractions	58.0	41.8
Mean respiratory rate (/min)	52 ± 15	51 ± 13
Mean temperature (°C)	37.8 ± 1.0	38.0 ± 0.8
Mean SaO_2 (%)	90.3 ± 11.3	90.4 ± 9.2
Mean pCO_2 (kPa)	6.8 ± 2.2	5.8 ± 1.0
Mean pH	7.35 ± 0.1	7.37 ± 0.1

No significant differences between RSV subtype A and B were observed, except for the presence of retractions ($p = 0.03$) and pCO_2 ($p < 0.001$).
Numbers expressed as % unless stated otherwise.

3.3.1 Analysis of the data from the period 1992 until 1995

This period included 232 children: 150 (64.7%) were infected with subtype A, 82 (35.3%) with subtype B. The male/female ratio was 1.8 (150/82). Table 3.1 (page 43) shows the population characteristics.

Clinical parameters are shown in Table 3.2. No significant differences were found regarding impaired feeding, the presence of wheezing, respiratory rate, temperature, SaO_2 and pH. Significant differences were found regarding pCO_2 and the presence of retractions. The average pCO_2 for subtype A is 6.8 kPa against 5.8 kPa for subtype B ($p < 0.001$). Retractions were noted in 84 children infected with subtype A and in 28 children infected with subtype B ($p = 0.03$). No significant differences were found regarding underlying disease. Table 3.3 shows the clinical diagnosis at presentation. In Table 3.4 (page 46) the characteristics of hospitalisation are shown.

The pCO_2 was measured in 213 children: 136 (90.1%) samples were collected in patients with subtype A and 77 (94.0%) for subtype B. We noted in the season 1994 - 1995 more children with a high pCO_2 (above 10.0 kPa) than in the other two seasons. We also found in the season 1994 - 1995 a significant difference in the number of children < 2 months of age (in the first two seasons 19.4% against 34.0% of the children in the season 1994 - 1995, $p = 0.01$). Furthermore we found a significantly higher pCO_2 in children < 2 months of age (7.5 kPa vs. 6.0 kPa for children ≥ 2 months of age, $p < 0.001$). This also applies to all three seasons separately.

Twenty-eight (12.1%) children required mechanical ventilation. Twenty-one of these were infected with subtype A, 7 with subtype B ($p = 0.22$). Two children died. One of them was infected with subtype A, the other child was infected with subtype B.

Adjustment for confounders with regression analysis did not reveil a significant

Table 3.3 *Clinical diagnosis at presentation*

	Subtype A (n = 150)	Subtype B (n = 82)
Apnoea ± URTI	5.3	3.7
URTI	24.7	28.0
Bronchiolitis/pneumonia	3.3	2.4
With URTI	52.0	56.1
With apnoea and URTI	14.7	9.8

No significant differences between RSV subtype A and B were observed
URTI: upper respiratory tract infection
Numbers expressed as % unless stated otherwise

difference between subtype A and B for any of the disease outcome parameters such as impaired feeding, presence of wheezing, presence of retractions, SaO_2 and pCO_2, lower respiratory tract infection, ICU-admission and mechanical ventilation.

3.3.2 Analysis of the season 1993 - 1994 and comparison of both analyses

This period included 61 patients. The male/female ratio was 1.3 (35/26). We did not find a significant difference in the study population characteristics between subtype A and subtype B. No significant differences were found between subtype A and subtype B for all the observed variables.

The outcome of the analysis of the data from the season 1993 - 1994 was equal to the outcome of the analysis of the data from the period 1992 - 1995, covering three separate RSV seasons, except for pCO_2 and the presence of retractions.

3.4 Discussion

We investigated whether a relationship between clinical severity of RSV infection and subtype A or B could be demonstrated. The results of our study indicate that such a relationship does not exist.

Two analyses were performed: one on data from the whole period between 1992 and 1995, covering three RSV seasons, and one on data of the season 1993 - 1994. By comparing the outcome of one season with the outcome of three seasons, the possible effect of confounding variables from a particular season was ruled out.

As strong indicators for severity of infection in this study were used oxygen saturation (SaO_2), pCO_2, clinical diagnosis (upper respiratory tract infection versus lower respiratory tract infection), ICU admission and the need for mechanical ventilation. These indicators are strongly related to pulmonary dysfunction [10,15].

Table 3.4 *Characteristics of hospitalisation*

	Subtype A (n = 150)	Subtype B (n = 82)
Number of admissions	76.7	85.4
Mean length of stay (days)	10.4 ± 6.2	9.3 ± 4.8
Number of ICU admissions	34.0	23.2
Mean length of stay in ICU (days)	6.2 ± 8.3	5.9 ± 5.0
Mechanical ventilation	14.0	8.5

No significant differences between RSV subtype A and B were observed.
ICU: intensive care unit.
Numbers expressed as % unless stated otherwise.

None of the variables observed in the season 1993 - 1994 were significantly different between patients infected with subtype A and subtype B. This was also found in the analysis of the period 1992 - 1995, except for the presence of retractions and pCO_2. More retractions were noted in patients with subtype A than in patients with subtype B. However, the association did not hold after correcting for confounders using logistic regression analysis.

The other variable that showed a significant difference is pCO_2. This is due to a relationship between young age and high pCO_2, as has been demonstrated by Mulholland *et al* [16]. In the season 1994 - 1995 (a season with only subtype A infections) significantly more children younger than two months of age were admitted with RSV than in the two preceding epidemics. The mean pCO_2 of these children is significantly higher than the mean pCO_2 of children ≥ 2 months. This influences the mean pCO_2 for subtype A in the whole period covering the three seperate RSV-seasons. After correcting for age, the association between high pCO_2 and subtype A did not hold.

Several studies investigated the possible relationship between clinical severity of RSV infection and subtype. McConnochie *et al* conclude that subtype A is related to a more severe disease [9]. They analysed data from 157 patients with known subtype, using arbitrary cut-off values at which a variable was to be considered severe. Significant differences were observed in $pCO_2 > 45$ mm Hg, $SaO_2 < 87\%$ and respiratory rate > 72 min^{-1}. A significant difference was also observed between subtype A and subtype B regarding mechanical ventilation. Straliotto *et al* concluded that subtype B was related to a more severe disease, but their population was rather small: 29 patients [11]. The results of both these studies are conflicting with those from our study. David *et al* concluded that there is no difference in severity between subtype A and subtype B. They analysed their data using a severity index consisting of three groups: severe, moderate and mild [12].

In conclusion, the outcome of the analysis of the data obtained in our study indicate

that there is no relationship between clinical severity of RSV infection and subtype A or B. Hence it is not necessary to determine subtype at presentation, because there are no consequences for clinical management. The outcome of our study also implies that the development of a vaccine has to use a strategy which uses antigenic epitopes, which are shared by subtype A as well as by subtype B.

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CHAPTER 4

RISK FACTORS FOR RESPIRATORY SYNCYTIAL VIRUS ASSOCIATED APNOEA

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Abstract

Respiratory syncytial virus (RSV) infections are characterized by upper or lower respiratory tract diseases including bronchiolitis and pneumonia. Apnoea may be the first sign of disease in children with RSV infection. The aims of this study were the identification of independent risk factors for RSV associated apnoea and the prediction of the risk for mechanical ventilation in children with RSV associated apnoea. Medical records of children younger than 12 months of age admitted with RSV infection between 1992 and 1995 to the Sophia Children's Hospital, were reviewed. Demographic parameters, clinical features and laboratory parameters (SaO_2, pCO_2 and pH) were obtained upon admission and during hospitalisation. Children with and without apnoea were compared using univariate and multivariate logistic and linear regression analysis. One hundred and eighty-five patients with RSV infection were admitted. Thirthy-eight (21%) presented with apnoea. Patients with apnoea were significantly younger, had a significantly lower temperature, higher pCO_2 and lower pH and had on chest radiographs also more signs of atelectasis. The number of patients admitted to the ICU because of mechanical ventilation and oxygen administration was significantly higher in children with RSV associated apnoea. Apnoea at admission was a strong predictor for recurrent apnoea. The relative risk for mechanical ventilation increased with the number of episodes of apnoea: 2.4 (95% CI 0.8 - 6.6) in children with one episode of apnoea (at admission) versus 6.5 (95% CI 3.3 - 12.9) in children with recurrent episodes of apnoea.

Conclusions Age below 2 months is the strongest independent risk factor for RSV associated apnoea. Apnoea at admission increases the risk for recurrent apnoea. The risk for mechanical ventilation significantly increases in children who suffer from recurrent apnoea.

4.1 Introduction

Respiratory syncytial virus (RSV) has been identified as the most important cause of respiratory tract infections in infants and young children since its first isolation from an infant with lower respiratory tract infection in 1956 [3,6]. RSV, a member of the genus pneumoviridae from the family of paramyxoviridae, causes worldwide annual epidemics. Most infections have a mild course. However, a small number of infected children (0.5% to 2%) develop severe disease necessitating hospitalisation [5].

The major presenting symptoms in RSV infection are those of upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI), including bronchiolitis and pneumonia in infants younger than six months of age [9,11]. Apnoea may be the first sign of disease. The incidence of RSV associated apnoea in young infants is estimated at 16% to 20%. Suggested risk factors for apnoea include young age and prematurity [1,2,4]. The aetiology of apnoea however remains unknown. Furthermore, there is limited information about the need for mechanical ventilation after an initial episode of apnoea [12].

The objective of this study was *(i)* to look for independent risk factors for the development of RSV associated apnoea and *(ii)* to study the risk for mechanical ventilation in infants with RSV associated apnoea.

4.2 Patients and methods

4.2.1 Patients

Patients younger than 12 months of age who were admitted to the Sophia Children's Hospital Rotterdam due to RSV infection in the period between 1992 and 1995 were included in the study. The Sophia Children's Hospital is a university hospital with secondary and tertiary care function.

RSV infection was virologically confirmed by a positive direct immunofluorescent assay (DIFA) and/or a positive viral culture on HEp-2 cells performed on specimen obtained from nasopharyngeal washing.

4.2.2 Variables

Medical records were reviewed to collect demographic and clinical data. Demographic parameters included gender, age at diagnosis, gestational age, birthweight and the presence of any of the three following underlying diseases: congenital heart disease, bronchopulmonary dysplasia and T-cell immunodeficiency.

Clinical data obtained at presentation included information on impaired feeding , the presence of wheezing, the presence of retractions, respiratory rate and body temperature. Laboratory parameters obtained on admission included SaO_2, pCO_2 and pH. The arterial oxygen saturation (SaO_2) was measured transcuteanously by a pulse oximeter (Nellcor 100/200). pCO_2 and pH were measured on capillary blood samples.

4.2.3 *Diagnostic definitions*

Apnoea at admission was defined as the following: (*i*) apnoeic spells (a history of respiratory arrest with cyanosis) leading to hospitalisation, or (*ii*) an observation in the paediatric emergency room of respiratory arrest for a period longer than 20 seconds and/or bradycardia with accompanying cyanosis or oxygen desaturation below 90%. Recurrent apnoea was defined as apnoea during hospitalisation - observation (with a Nellcor 100/200, alarm set at oxygen saturation below 95% and/or heart rate below 100/min) of respiratory arrest for a period longer than 20 seconds and/or bradycardia with accompanying cyanosis - following a prior episode of RSV associated apnoea which led to hospitalisation. Upper respiratory tract infection was defined by coughing and/or rhinitis with no abnormalities on chest radiograph. Lower respiratory tract

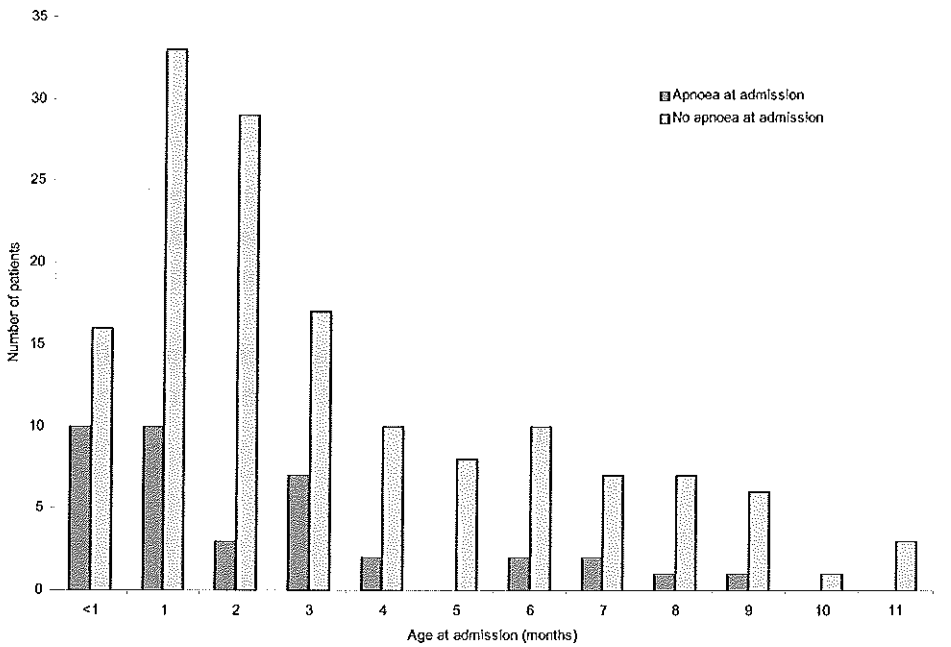

Figure 4.1 *The relation between age and RSV associated apnoea at admission in a total group of 185 patients hospitalised with RSV infection (1992 - 1995).*

infection was defined by clinical features (signs of URTI with tachypnoea, diffuse rhonchi, fine rales and wheezing) and the presence of atelectasis, hyperinflation, bronchial thickening and/or an infiltrate on chest radiograph. Chest radiographs were assessed by two radiologists. The following characteristics of hospitalisation were also documented: the duration of (ICU) admission, oxygen administration and mechanical ventilation. Indications for mechanical ventilation were to control apnoea, clinical deterioration or acidosis with severe hypercarbia ($pCO_2 > 8.0$ kPa).

4.2.4 *Statistical analysis*

Subjects were divided into two groups: group I (apnoea at admission) and group II (no apnoea). Tabular analysis was performed using the χ^2-test or the Fisher Exact Test when the value of any cell was below five. Mean values were compared using either the Student's *t*-test or the Mann Whitney-U test when data were not normally distributed. To identify risk factors for apnoea, the relative risk (RR) and 95% confidence interval were calculated from 2 x 2 tabels. A RR with the 95% CI including 1 indicates no significant association between a risk factor and apnoea. As the various factors were interrelated, all findings were corrected for gender, age at admission and gestational age using multivariate linear and logistic regression analysis to examine if the association between apnoea and a certain variable remained.

Patient characteristics - based upon the literature - which might play a role in the development of RSV associated apnoea were subsequently entered in a multivariate logistic regression model in order to assess their independent contribution to the probability of such an apnoea. A forward inclusion selection of variables was performed to identify the most important predictors for apnoea. Variables were entered until no remaining candidate variable met a significance level of 0.05 or below. The variable(s) in the final model were considered to be the strongest predictor(s) for RSV associated apnoea.

Statistical analysis was performed using the software program SPSS 6.1™ for Windows™. *P* - values ≤ 0.05 were accepted as significant.

4.3 Results

4.3.1 *Validity of the study*

In 179 children (97%) both DIFA and viral cultures were positive. In three patients only the DIFA was positive and in another three children only viral cultures were positive. Data on gender and postnatal age could be obtained in all patients. Data on

Table 4.1 *Characteristics of 185 patients hospitalised with RSV infection between 1992 and 1995*

	Group I (apnoea a admission) (n = 38)	Group II (no apnoea at admission) (n = 147)	RR (95% CI)
Patient demographics			
Boys/Girls (= RC)	20/18	99/48	0.6 (0.4 - 1.1)
Underlying disease	6	22	1.1 (0.4-2.8)
Gestational age (wks)	36.6 ± 3.8	37.6 ± 3.6	n.a.
< 32 weeks	4	10	1.6 (0.5 - 4.7)
Birthweight (gr)	2813 ± 7	2998 ± 1885	n.a.
Age (months	2.3 ± 2.5*	3.3 ± 2.8	n.a.
≤ 60 days	19	47	1.6 (1.1 - 2.3)
≤ 42 days	14	28	1.9 (1.2 - 3.2)
≤ 28 days	7	11	2.7 (1.2 - 6.3)
Weight at admission(gr)	4769 ± 1888	5855 ± 4290	n.a.
Findings on admission			
Impaired feeding	30	103	1.1 (0.9 - 1.4)
Presence of wheezing	15	47	1.4 (0.7 - 2.9)
Presence of retractions	21	80	0.9 (0.4 - 2.0)
Temperature (°C)	37.3 ± 1.2**	38.0 ± 0.9	n.a.
Respiratory rate (/min)	54 ± 21	51 ± 15	n.a.
Blood gas analysis			
SaO_2 (%)	85 ± 16*	90 ± 9	n.a.
pCO_2 (kPa)	7.4 ± 2.4*	6.5 ± 1.8	n.a.
pH	7.31 ± 0.13*	7.36 ± 0.	n.a.
Features on chest radiograph			
Atelectasis	18*	37	1.4 (1.0 - 1.9)
Hypertranslucency	20	73	1.1 (0.9 - 1.4)
Infiltrate	3	9	1.5 (0.5 - 4.5)
Bronchial thickening	16	45	1.2 (0.9 - 1.7)
Type of disease			
LRTI	28	109	1.0 (0.8 - 1.3)
URTI	9	38	RC
Characteristics of hospitalisation and disease cours			
Duration of hospitalisation (days)	10.7 ± 6.2	9.7 ± 5.5	n.a.
Number of ICU-admissions	31*	46	9.7 (4.0 - 23.7)
Duration of ICU stay (days)	7.2 ± 10.5	5.3 ± 4.2	n.a.
Oxygen administration	29*	80	2.7 (1.2 - 6.1)
Duration of O_2 adm. (days)	6.1 ± 5.9	5.3 ± 10.4	n.a.
Mechanical ventilation	14**	14	5.5 (2.3 - 13.1)
Duration of mech. vent (days)	6.2 ± 5.1	5.6 ± 3.6	n.a.
Apnoea during hospitalisation	18**	6	

*$p < 0.05$, ** $p < 0.005$*
Numbers expressed as absolute figures or mean ± standard deviation
NA: not applicable, RC: reference category

gestational age, birthweight and weight at admission could be acquired in 90%, 85% and 94%. Data on chest radiographs were obtained in all but one patient. Findings on admission were complete for more than 94% of the study population. SaO_2 was measured in 85%. Data on blood gas analysis were obtained in 88% of the study

population.

4.3.2 Apnoea at admission

Between 1992 and 1995, 185 children with RSV infection were admitted to the Sophia Children's Hospital. The clinical characteristics of these patients are summarized in Table 4.1. Thirty-eight children (21%) presented with apnoea. Eighteen of the 38 patients with apnoea at admission (47%) developed recurrent apnoea (RR 11.6, 95% CI 4.9 - 27.2). A history of apnoea of prematurity was documentated in only two (5%) children who presented with RSV associated apnoea.

Significant differences were found in mean age, mean pCO_2, mean pH, mean temperature and presence of atelectasis on chest radiograph. The age distribution is depicted in Figure 4.1. Characteristics on disease course and of hospitalisation are summarized in Table 4.1. More children with apnoea than without apnoea were admitted to the intensive care unit (RR 9.7, 95% CI 4.0 - 23.7), received oxygen administration (RR 2.7, 95% CI 1.2 - 6.1) and required mechanical ventilation (RR 5.5, 95% CI 2.3 - 13.1).

In further analysis, all findings were corrected for gender, age at admission and gestational age using multivariate linear and logistic regression analysis. This analysis demonstrated that the assocation between apnoea and respectively lower temperature, high pCO_2, low pH, the presence of atelectasis, ICU admissions and mechanical ventilation remained.

Table 4.2 shows the contribution of gender, age at admission, premature birth, weight at admission and type of disease to the risk for developing RSV associated apnoea. After a forward inclusion, age at admission remained the only significant variable contributing to the risk for apnoea. The probability of RSV associated apnoea

Table 4.2 *The contribution of gender, age at admission (months), premature birth, weight and type of disease to the risk for apnoea at admission (ß coefficient's estimate, SE standard error of the coefficient's estimate)*

	ß	SE	*p* - value
I Full model			
INTERCEPT	-0.89	0.64	
Gender	0.70	0.39	0.08
Age at admission (months)	-0.16	0.10	0.13
Premature birth	0.29	0.47	0.53
Weight on admission (gr)	-6.5×10^{-5}	0.00	0.56
LRTI	-0.05	0.44	0.90
II After forward inclusion of variables:			
INTERCEPT	-0.87	0.27	
Age at admission (months)	-0.17	0.08	0.04

Results are from a forward condition stepwise logistic regression analysis procedure

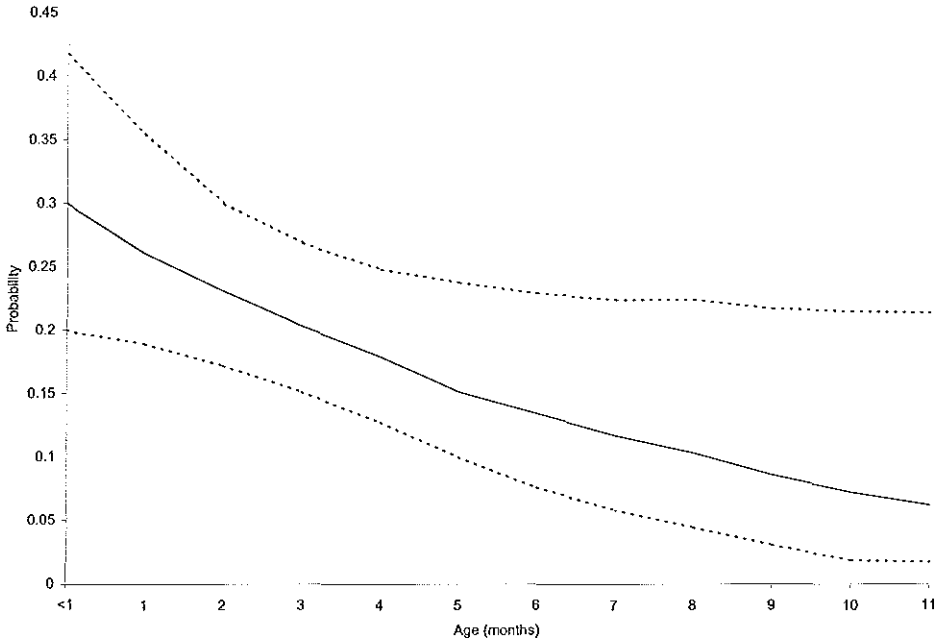

Figure 4.2 *The probability of developing RSV associated apnoea at admission as a function of age at admission (the continued line represents the probability, where the dashed lines represent the upper and lower limits of the 95% confidence interval)*

at admission, as a function of the child's age at admission, is depicted in Figure 4.2.

Twenty-four children developed an episode with RSV associated apnoea in hospital. Apnoea during hospitalisation occurred in 54% within the first day after admission and in 88% within two days after admission.

4.3.3 Apnoea and mechanical ventilation

The number of episodes with apnoea influenced the risk for mechanical ventilation. The relative risk for mechanical ventilation increased from 2.4 (95% CI 0.8 - 6.6) in patients with only (a history of) apnoea at admission to 6.5 (95% CI 3.3 - 12.9) in patients with recurrent apnoea.

Two patients died. One death, a term born 1 month old child, was contributable to apnoea at home. The other death, a term born 3 month old, suffered from gangliosidosis type I.

4.4 Discussion

Of all children admitted with RSV infection to the Sophia Children's Hospital

between 1992 and 1995, 21 % presented with apnoea. This is in agreement with other studies, reporting that the incidence of RSV associated apnoea varies between 16% and 20% [1,2,4].

Young age was related to the development of RSV associated apnoea as has been demonstrated previously [1,2]. Age remained the only independent risk factor for RSV associated apnoea after logistic regression analysis. Especially children younger than two months of age were at an increased risk for the development of RSV associated apnoea.

Children admitted with RSV associated apnoea - either at home or observed in the pediatric emergency room - were at increased risk for the development of recurrent apnoea. Patients with recurrent apnoea often required mechanical ventilation. This finding has implications for clinical management; infants with increased risk for recurrent apnoea should be closely monitored. The risk for RSV associated apnoea was highest in the first 48 hours after admission and diminished thereafter.

Prematurity was not related to RSV associated apnoea. This finding is in contrast with previous studies [1,2,4]. In these studies, it was also reported that a history of apnoea of prematurity was more frequently found in infants with RSV associated apnoea. This could not be confirmed in our study.

The aetiology of RSV associated apnoea remains unknown, although several theories have been proposed. Church *et al* suggested that severe clinical illness may predispose to RSV associated apnoea [4]. This may be due to a more severe degree of hypoxemia, secondary to severe lower respiratory tract disease. Another group of investigators reported that in patients with apnoea more signs of pulmonary parenchymal involvement, including an increased number of infiltrates, were found [1]. Our data indicate that there was no difference in SaO_2 and also no difference regarding type of disease between patients with and without (a history of) apnoea at admission after correcting with multivariate logistic regression analysis. However, we did find more atelectasis and a higher pCO_2 and an increased need for oxygen treatment in patients with apnoea. This may indicate a more severe disease state. The higher pCO_2 values and increased tendency to atelectasis could also be considered as a result of apnoea, and not as a causative factor.

Another explanation for apnoea is that respiratory syncytial virus alters the sensitivity of laryngeal chemoreceptors, thus causing a prolonged or even fatal apnoea when these receptors are stimulated [8,10]. Furthermore, the occurrence of apnoea is not related to RSV subtype, as was previously reported by our group [7].

A factor that most likely plays an important role in RSV associated apnoea is a immaturity of the respiratory control centre in the brain stem. In our study, only young age was independently related to apnoea, indicating that the degree of maturation of

the respiratory control center in young infants plays a role in RSV infection. This implies that RSV associated apnoea is of central origin.

4.4 References

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CHAPTER 5

PREDICTORS FOR A NORMAL CHEST RADIOGRAPH IN RESPIRATORY SYNCYTIAL VIRUS INFECTION

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SUBMITTED

Abstract

Respiratory syncytial virus (RSV) accounts for the majority of lower respiratory tract infections (LRTI) in infants and young children. A chest radiograph is frequently performed in infants with RSV LRTI. The aim of this study was to develop and validate a prediction model to estimate the probability for a normal chest radiograph by using easily obtainable diagnostic parameters in children with RSV infection. This prediction model may be applied to decide which patients do not have an indication for a chest radiograph.

The data of 287 children admitted with RSV infection or diagnosed as such in the outpatient department of the Sophia Children's Hospital between 1992 and 1996, were studied. The derivation set comprised 232 patients (1992 - 1995), the validation set contained 55 patients (1995 - 1996). A chest radiograph was designated as normal when atelectasis, hyperinflation and pulmonary infiltrates were absent. In order to develop a prediction model, patient history, clinical and laboratory variables were consecutively entered into a logistic regression model according to the diagnostic work-up in practice. Variables with p – values ≤ 0.10 were retained in the model. The predictive accuracy of the multivariable models was examined using the area under receiver operating curve (ROC-area).

In 202 (87%) patients from the derivation set a chest radiograph was performed. A normal chest radiograph could be predicted by increasing age, increasing birthweight, presence of rhinitis, absence of retractions and increasing SaO_2. The ROC-area was 0.80 in the derivation and validation set. This prediction model was transformed into a score chart.

Conclusions A normal chest radiograph can easily be predicted using a model including easy obtainable patient characteristics, clinical and laboratory variables. It may be a useful tool in the decisionmaking whether or not to perform a chest radiograph in patients with RSV-infections.

5.1 Introduction

Respiratory syncytial virus (RSV) accounts for the majority of lower respiratory tract infections in infants and young children [1]. The diagnosis of RSV infection is based upon clinical findings and virological confirmation. The chest radiograph is not diagnostic. Also, the spectrum of roentgenographic findings in RSV infection – atelectasis, hyperinflation or pulmonary infiltrate – does not necessarily correlate with severity of illness [2,3]. However, a chest radiograph is still performed in approximately 70% of all hospitalised infants visiting the pediatric outpatient department with signs and symptoms of RSV lower respiratory tract infection [4]. Hence, it would be interesting to identify a subgroup of RSV patients in which a normal chest radiograph can be predicted. Identification of these patients reduces the number of unnecessary chest radiographs.

The aim of this study was to develop and validate a prediction model to estimate the probability for a normal chest radiograph in RSV patients using easily obtainable diagnostic parameters documented from the patient's history, physical examination and laboratory results.

5.2 Patients and methods

5.2.1 Patients

The study population consisted of children younger than 12 months of age, who either visited the outpatient department or were admitted to the Sophia Children's Hospital with virologically confirmed RSV infection in two periods: 1992 – 1995 (derivation set) and 1995 – 1996 (validation set). Data from the first period were used to derive a prediction model (derivation set) and from the second period to evaluate the performance of the prediction model in an independent population (validation set). Virological confirmation was defined by a positive direct immunofluorescent assay (DIFA) and/or a positive viral culture on HEp-2 cells performed on specimens obtained from nasopharyngeal washings. Children with nosocomial RSV infections were excluded. Demographical, clinical and laboratory data obtained at admission were acquired by a review of the patient's medical chart as previously described [5]. In brief, these included gender, postnatal age, gestational age, birthweight, the presence of an underlying disease state, presence of impaired feeding, presence of wheezing and retractions, respiratory rate, body temperature, transcuteanously registered oxygen saturation measured by a Nellcor 100/200 and pCO_2 measured on capillary blood samples.

5.2.2 Chest radiography

Chest radiographs were assessed by one of two paediatric radiologists of our hospital. The outcome was classified as normal, atelectasis, hyperinflation or pulmonary infiltrate. Atelectasis was defined as a collapse of bronchioli without air bronchogram [6]. Hyperinflation was defined as a depression of the diaphragm below the 6th intercostal space of the anterior rib. A pulmonary infiltrate was defined as lobar, segmental or subsegmental opacities with discrete or irregular margins. A chest radiograph was designated as normal when atelectasis, hyperinflation and pulmonary infiltrates were absent.

5.2.3 Statistical analysis

The aim of the analysis was to discriminate between patients with a normal chest radiograph and patients without a normal chest radiograph. In the derivation set the probability of a normal chest radiograph was related to each potential predictor to identify candidate predictors using univariable logistic regression analyses (SPSS 8.0, Chicago, Illinois, USA, 1998). Variables with p-value ≤ 0.15 were consecutively entered in the multivariable models starting with the demographic, history and physical examination variables and subsequently extending with laboratory variables [7,8]. Variables with p-values ≤ 0.10 in the multivariable models were retained in the model. Missing values were imputated using the expectation and maximization method avaible in the standard SPSS software to increase statistical efficiency [9,10]. Such imputation is based on the correlation between each variable with missing values from all other variables. These correlations are estimated from the subjects from which all data were available.

Predictive accuracy of the multivariable models was distinguished in reliability and discrimination [8]. Reliability (goodness of fit) was assessed using the Hosmer & Lemeshow method [11,12]. The discrimination of each model was assessed using Receiver Operating characteristic (ROC) curves. The area under the curve (ROC area) is a suitable parameter to summarize the overall discriminative or diagnostic value of a model [8,12]. The ROC area can range from 0.5 (useless model, like a coin flip) to 1.0 (perfect discrimination). A value of 0.80 or greater can be interpreted as good [13]. The difference in ROC area (with 95% confidence interval) between models was used to estimate the added value of an extended model over a reduced model [14]. In these comparisons, correlation between models was taken into account as they were based on the same cases [15].

The final prediction model was transformed into a score chart. Scores for each

Table 5.1 *Comparison of demographical characteristics of patients in the derivation and in the validation set*

	Derivation set (n = 202)	Validation set (n = 51)
Age (months)	3.3 ± 2.9	4.7 ± 3.7
Birthweight (kg)	2.9 ± 0.9	3.2 ± 0.8
Gestational age (weeks)	37.4 ± 3.6	37.7 ± 3.0
Weight (kg)	5.5 ± 2.0	6.3 ± 2.4
Underlying disease state	15.8%	7.8%
Normal chest radiographs	37%	45%

predictor were derived from the logistic regression coefficients, multiplied by 10 and rounded to whole numbers.

5.3 Results

5.3.1 Study population

Between 1992 and 1995, 232 children were diagnosed with RSV infection. In all but six children both DIFA and viral culture were positive. In 202 children (87%) a chest radiograph was performed. Data of these children were used for the derivation set. Gestational age, birthweight and weight at presentation were obtained in 89%, 85% and 86% respectively. Clinical findings at presentation were present for at least 92% of the study population. The arterial oxygen saturation was measured in 82%, blood gas analysis was performed in 95%. The results of the analyses based on the complete subjects (n = 138) and on subjects after imputation, yielded no differences so that the imputated approach is presented. The same method was applied to the validation set.

Out of 202 children in whom a chest radiograph was performed, 75 (37%) had no abnormalities. 127 (63%) children had either atelectasis, hyperinflation and/or pulmonary infiltrate found on chest radiographs.

In 1995 – 1996, 55 children were diagnosed with a virologically confirmed RSV infection of which 93% had a chest radiograph. Table 5.1 summarizes the demographic characteristics of the derivation and the validation set, which yielded the same distribution except for age which was significantly higher in the validation set ($p = 0.04$).

5.3.2 Derivation of the prediction model

Table 5. 2 summarizes the results of the univariable analysis. Variables significantly associated with a normal chest radiograph were increasing postnatal age, increasing

Table 5.2 *Results from the univariable analysis on predictors for a normal chest radiograph (absence of atelectasis, hyperinflation and/or pulmonary infiltrate) in the derivation set. Figures are depicted as either means ± standard deviations or absolute numbers*

	Normal chest radiograph (n = 75)	Abnormal chest radiograph (n = 127)	*P* - value
Demographic characteristics and patient history			
Male gender	51	79	0.41
Postnatal age (months)	4.2 ± 3.4	2.7 ± 2.4	0.001
Gestional age (weeks)	37.7 ± 3.3	37.2 ± 3.8	0.35
Birthweight (gr)			0.10
Underlying disease state	8	24	0.12
Cough	67	116	0.64
Rhinitis	71	105	0.02
Impaired feeding	46	96	0.03
Apnoea at admission	11	26	0.44
Physical examination			
Pulse rate (/min)	145 ± 24	154 ± 27	0.03
Respiratory rate (/min)	52 ± 20	52 ± 15	0.97
Weight (kg)	6.1 ± 2.3	5.2 ± 1.8	0.01
Temperature (°C)	38.0 ± 0.8	37.8 ± 1.0	0.07
Retractions	28	79	0.001
Wheezing	22	52	0.10
Laboratory investigation			
SaO_2 (%)	94.1 ± 5.8	87.9 ± 12.3	< 0.001
pCO_2 (kPa)	5.7 ± 1.0	7.0 ± 2.1	< 0.001

birthweight, the presence of rhinitis, increased pulse rate, increasing body weight, decreasing body temperature, the absence of retractions, the absence of wheezing, increasing transcutaneously measured oxygen saturation and decreasing pCO_2. These variables were consecutively entered into a multivariable logistic regression analysis.

Table 5.3 presents the results of the multivariable logistic regression analysis. The initial model contained the significant variables postnatal age in months, birthweight in kg, the presence of rhinitis and the absence of retractions. The area under the ROC-curve for this model was 0.74 (SE 0.04) (Figure 5.1). After adding SaO_2 and pCO_2 to the model, only SaO_2 contributed significantly to the outcome of the chest radiograph. The ROC-area increased to 0.80 (SE 0.03). All models showed good reliability; the Hosmer & Lomeshew test was far from significant (data not shown).

5.3.2 Performance of the model

The logistic regression coefficients (the OR) of the final model from table 5.3 were transformed to a score chart (Table 5.5). Table 5.4 summarizes the performance of the prediction model in both the derivation and validation set. The original score ranged from -56 to 27, a correction factor of +56 was added to the prediction role to make the score range from 0 to 83 points. The value -64 represents the intercept from the logistic

Table 5.3 *Results from the multivariate logistic regression analysis on predictors for a normal chest radiograph*

Model	Model 1	Model 2
	Demographical characteristics and patient history	Model 1 + laboratory examination
Variables	OR (95% CI)*	OR (95% CI)*
Demographic characteristics, patient history and physical examination		
Age (months)	1.2 (1.1 – 1.4)	1.2 (1.1 – 1.3)
Birthweight (kg)	1.3 (1.0 – 1.6)	1.2 (1.0 – 1.6)
Presence of rhinitis	3.4 (1.0 – 10.9)	3.2 (1.0 – 11.1)
Absence of retractions	2.6 (1.4 – 4.8)	2.2 (1.2 – 4.3)
Laboratory examination		
SaO_2		1.8 (1.3 – 2.6)
ROC area (95% CI)	0.74 (0.66 - 0.82)	0.80 (0.74 – 0.86)

* *The log OR is equal to the logistic regression coefficient, which is used to make the scorings rule*
OR: Odds ratio, CI: confidence interval

Table 5.4 *Distribution of patients with and without a normal chest radiograph according to the calculated score using the diagnostic model from Table 5.3 in the derivation and validation set. Values represent absolute numbers (%).*

Score	Total	Normal chest radiograph	Abnormal chest radiograph
Derivation set			
≤ 20	6 (3)	1 (1)	5 (5)
21 – 30	18 (9)	1 (1)	17 (13)
31 – 40	33 (16)	2 (3)	31 (24)
41 – 50	47 (23)	15 (20)	32 (25)
51 – 60	60 (30)	28 (37)	32 (25)
61 – 70	34 (17)	24 (33)	10 (8)
≥ 71	4 (2)	4 (5)	0 (0)
All	202 (100)	75 (100)	127 (100)
Validation set			
≤ 20	0 (0)	0 (0)	0 (0)
21 – 30	3 (6)	0 (0)	3 (11)
31 – 40	7 (14)	3 (13)	4 (15)
41 – 50	11 (22)	3 (13)	8 (30)
51 – 60	12 (24)	8 (35)	4 (15)
61 – 70	15 (30)	8 (35)	7 (26)
≥ 71	2 (4)	1 (4)	1 (3)
All	50 (100)	23 (46)	27 (54)

regression model. From Figure 5.1, one can identify the best probability threshold (associated with the highest sensitivity and the lowest most acceptable false positive rate (FPR). For instance, a total sum score of 52 or greater was associated with a sensitivity of 70.7% and a FPR of 25.2. From Table 5.4, the corresponding sensitivities and FPRs are summarised for various probability categories. For example, in the derivation set the model placed 98 patients (49%) with a sum score of > 50 of which 56 indeed had a normal chest radiograph (correctly identifying 75%) and 42 had not

Table 5.5 *Score chart for calculating the probability for a normal chest radiograph in RSV infection*

												Score
												-64
Age (in months)												
0	1	2	3	4	5	6	7	8	9	10	11	
0	**2**	**3**	**5**	**7**	**9**	**11**	**13**	**14**	**16**	**18**	**20**	
Birthweight (kg)												
≤ 1.99		2.0 – 2.49		2.5 – 2.99		3.0 – 3.49		3.5 – 3.99		4.0 – 4.49	≥ 4.5	
2		**4**		**6**		**8**		**10**		**12**	**14**	
Rhinitis												
		Present						Absent				
		12						**0**				
Retractions												
		Present						Absent				
		0						**8**				
SaO_2 (%)												
≤ 74		75 – 79		80 84		85 – 89		90 – 94		≥ 95		
6		**12**		**18**		**25**		**31**		**37**		
										Sum score (add)		
										Correction factor (add to sum score)		56
										Total score		

(incorrectly identifying (FPR) 32%).

5.3.3 *Validation of the prediction model and use of the score chart*

The area under the ROC curve of the validation set was 0.81 (SE 0.07), suggesting identical performance compared to the derivation set.

For individual patients, the scores corresponding to the values of the predictors can be filled in on the score chart. For example, a one month old infant with a birthweight of 2750 grams, presence of rhinitis, presence of retractions and transcutaneously measured oxygen saturation of 94%, has a total sum score of 43. The selected score threshold will determine whether or not this total sum score is associated with a normal chest radiograph (sensitivity 90%, FPR 55%, figure 5.1).

5.4 Discussion

In clinical practice, a chest radiograph is performed in a large number of patients visiting the outpatient clinic with LRTI by RSV [4]. This study provides evidence for a

reduction in the number of chest radiographs by identifying patients with a probability for a normal chest radiograph. Independent predictors for a normal chest radiograph in this study were increasing postnatal age in months, increasing birthweight in kg., presence of rhinitis, absence of feeding difficulties, absence of retractions and increasing transcutaneously measured oxygen saturation. They can predict the outcome of the chest radiograph with good accuracy.

Age and birthweight were independent predictors for a normal chest radiograph. Young age, especially below 6 months of age, is a risk factor for severe RSV-disease which often includes pulmonary involvement as represented in an abnormal chest radiograph [16]. Low birthweight is associated with small lower airways. Thus with increasing age and increasing birthweight, the probability for a normal chest radiograph increases.

Physical examination of infants with RSV bronchiolitis often reveals retractions [3]. In this study, it suggests lower respiratory tract involvement yielding a decreased probability for a normal chest radiograph. Arterial oxygen saturation measured transcuteanously is a strong measure of severity of lower RSV infection [2,16-20]. Absence of hypoxia may thus indicate a normal chest radiograph.

The prediction model must serve as an additional tool in the diagnostic procedure from which a physician can determine which threshold one favours balancing the benefits of performing a chest radiograph and the non-benefits of missing abnormalities

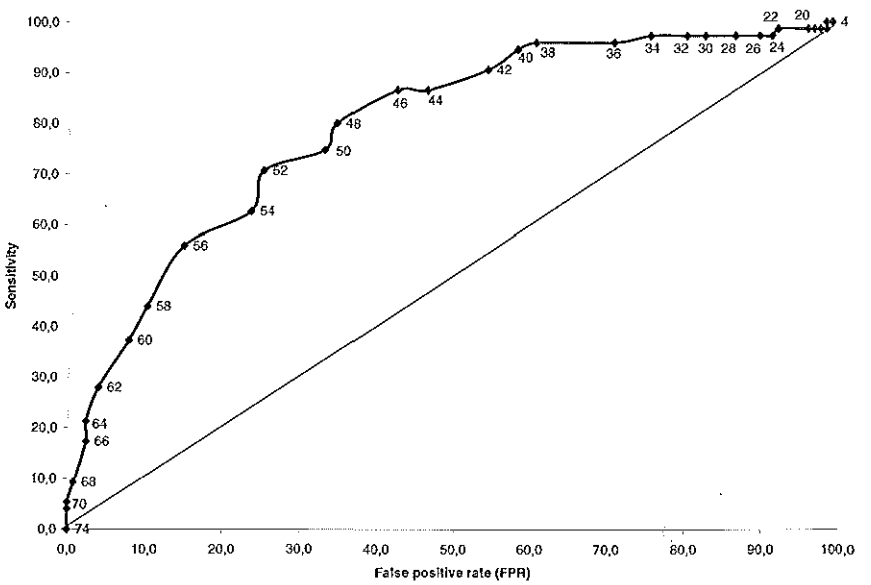

Figure 5.1 *ROC-curve derived from the multivariate logistic regression analysis on data from the derivation set for predictors for a normal chest radiograph.*

on chest radiograph. A total sum score of > 52 (Figure 5.1) is in our view associated with the best performance of the prediction model (the most balanced positive and negative predictive value, Figure 5.1). However, in approximately 25% of the patients an abnormal chest radiograph shall then be falsely predicted as normal. This must be judged in the light of clinical practice. A poor correlation between the clinical diagnosis of bronchiolitis and the presence of hyperinflation has been described [21]. Atelectasis is usually transient and resolvement starts within a few days. Within three months after initial diagnosis of respiratory tract infection, atelectasis has resolved completely [22]. However, atelectasis also may remain present and become a focus for bacterial superinfection. One group found atelectasis to be more common in combined bacterial and RSV infection than in infections with RSV alone. The authors suggested that chest radiographs are important in the assessment of which patients with bronchiolitis need to be treated with antibiotics [23]. However, other investigators reported that bacterial superinfection seldomly occurs [24,25].

We suggest to perform chest radiographs in children with RSV-LRTI if they have a low probability for a normal chest radiograph as can be calculated by our model, and if there is an unexpected deterioration in the patient's condition [26].

A limitation of our study is that not in all children a chest radiograph was performed. However, the reason for the omission of a chest radiograph will be the presence of a (very) mild disease. No differences in demographical and clinical characteristics between patients with a normal chest radiograph and patients with a missing chest radiograph were found. Therefore, this selection bias has not influenced our results.

The chest radiographs were assessed by one of two paediatric radiologists. No information on interobserver reliability between the two radiologists was available. This may have influenced especially the number of pulmonary infiltrates. One study reported a moderate interobserver coefficient for perihilear linear opacities or infiltrates [27].

Although the prediction model was validated, this model requires a prospective validation.

In conclusion, this study demonstrates that an increased probability for a normal chest radiograph in RSV infection is associated with increasing age, increasing birthweight, the presence of rhinitis, the absence of retractions, and increasing transcutaneously measured oxygen saturation. With the prediction model, the probability for a normal chest radiograph in the individual patient can be estimated. This model may be a useful tool in the decisionmaking whether or not to perform a chest radiograph. This will reduce exposure to radiation and costs associated with chest radiography.

Appendix

The exact formula to calculate the sum score is:

Sum score (normal chest radiograph): -6.43 + 0.18 * (age in months) + 0.21 (birthweight) + 1.18 * (rhinitis) + 0.80 (retractions) + 0.61 (transcuteanously measured oyxgen saturation).

For birthweight the following strata have been applied where 1 = ≤ 1.99, 2 = 2.00 – 2.49, 3 = 2.50 – 2.99, 4 = 3.00 – 3.49, 5 = 3.50 – 3.99, 6 = 4.00 – 4.49 and 7 = ≥ 4.50. The variable rhinitis is 1 when present and 0 when absent, the variable retractions is 0 when present and 1 when absent. For transcuteanously measured oxygen saturation the following strata have been applied where 1 = ≤ 74, 2 = 75 – 79, 3 = 80 – 84, 4 = 85 – 90, 5 = 90 – 94 and 6 = ≥ 95. The corresponding probability is calculated by (1 / (1 + exp(-(sumscore / 10)))) * 100.

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CHAPTER 6

LOW INCIDENCE OF NOSOCOMIAL RESPIRATORY SYNCYTIAL VIRUS INFECTIONS IN INFANCY

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Abstract

Objective To investigate the occurrence and clinical features of nosocomial RSV infections in comparison with community acquired (CA-RSV) RSV infections in the Sophia Children's Hospital in the period between 1992 and 1995 and to propose a guideline for the prevention of nosocomial RSV infections.

Design Retrospective.

Methods Children younger than 12 months of age with RSV infection were included. The diagnosis RSV infection was confirmed by a positive direct immunofluorescent assay (DIFA) and/or a positive viral culture on materials obtained from nasopharyngeal washes. A nosocomial RSV infection was defined by an infection which occurred more than five days after hospital admission for any underlying disease. Demographical and clinical data were studied and compared between children with nosocomial RSV infections and those with community acquired RSV infections.

Results 1260 children younger than 12 months of age were admitted during three RSV seasons. Thirty-four (2.7%) of these children developed a nosocomial RSV infection. The number of nosocomial RSV infections decreased during the study period. 232 children with a community acquired RSV infection were included. Significant differences between NA-RSV and CA-RSV were only found regarding birthweight (2.5 kg ± 0.9 versus 3.0 ± 0.9 for community acquired RSV-infection, $p = 0.004$), cough (64.7% versus 91.8%, $p < 0.001$) and impaired feeding (100% versus 68.9%, $p < 0.001$). All children with BPD and NA-RSV required mechanical ventilation.

Conclusion The number of nosocomial RSV infections decreased during the period 1992 - 1995. Nosocomial RSV infections are comparable to community acquired RSV infections in severity of disease. Based upon the literature, handwashing remains the best preventive approach.

6.1 Introduction

Respiratory syncytial virus (RSV) remains the most important cause of viral lower respiratory tract infections in infancy and has great health care impact on the elderly and immunocompromised patients [1]. This virus commonly causes nosocomial infections, which are infections acquired during hospitalisation and may result in a severe disease course with increased mortality rates in a general hospital population [2-4].

The percentage of nosocomial infections by RSV varied in American and European studies between 3.5% and 7% [4-8]. Very few data are available about the situation in The Netherlands.

The objectives of this study were (*i*) to estimate the percentage of nosocomial infections by RSV, (*ii*) to compare characteristics of nosocomial infections by RSV (NA-RSV) with community acquired RSV infections (CA-RSV), and (*iii*) to develop clinical guidelines for the prevention of nosocomial RSV infections.

6.2 Patients and methods

In this study, infants younger than 12 months of age diagnosed with a RSV infection between 1992 and 1995 – spanning three separate RSV seasons – at the Sophia Children's Hospital were included. The RSV-season starts October 1st and ends March 31st the following year. Demographical and clinical data were retrieved from the patient's medical chart [9]. The diagnosis RSV infection was virologically confirmed by a positive direct immunofluorescent assay (DIFA) and/or a positive viral culture on HEp-2 cells of materials obtained from nasopharyngeal washings. The total number of children younger than 12 months of age admitted to the Intensive Care Unit (ICU) and Medium Care Unit (MCU) of our hospital during the RSV season was determined.

The incubation period of RSV is approximately five days [10]. Hence, a nosocomial infection by RSV was defined as a RSV infection occurring more than five days after hospital admission for any other cause. For children with NA-RSV the time span was determined between date of admission for the underlying disease and virological confirmation of the RSV infection.

Patients were divided into two groups. Group I comprised all NA-RSV, whereas group II comprised all CA-RSV. Statistical analysis for dichotomous variables was performed using the χ^2 test (for 2 by 2 tables or 2 by *n* tables to estimate the significance of a trend) or the Fisher Exact test. The Mann Whitney-U test was applied for continous variables. All statistical analysis were carried out using SPSS 8.0 (Chicago, Illinois USA 1998). A p – value ≤ 0.05 was accepted as statistically significant.

6.3 Results

Between 1992 and 1995, 266 children were diagnosed with an infection by RSV. Thirthy-four of these children had a nosocomial infection by RSV. In the same period 1849 children younger than 12 months of age were admitted either at the ICU or the MCU of the Sophia Children's Hospital. From these infants 1260 were admitted during the RSV seasons. The percentage of NA-RSV was estimated at 2.7%. This percentage was 2.9% (12 out of 409 admissions) in the ICU and 2.6% (22 out of 851 admissions) in the MCU.

The percentage of children with NA-RSV decreased during the study period as is depicted in Figure 6.1. This decrease was highly significant ($p < 0.001$). Table 6.1 summarizes the characteristics of NA-RSV compared with CA-RSV. Patients with NA-RSV had a significantly lower birthweight (2.5 ± 0.9 kg) in comparison with patients with CA-RSV (3.0 ± 0.9 kg, $p = 0.004$). Approximately 35% of the children with NA-RSV occurred in children with congenital heart disease (CHD) or bronchopulmonary dysplasia (BPD). Other underlying diseases comprised gastro-intestinal diseases (21%) and neurologic diseases (18%).

Table 6.1 also summarizes the clinical features of patients with NA-RSV and CA-RSV. With the exception of significant differences between the two groups regarding

Table 6.1 *Comparison of children with nosocomial infections by RSV and community acquired infections by RSV in infants and young children*

	Nosocomial RSV infection (n = 34)	Community acquired RSV infection (n = 232)	*P* - Value
Demographic parameters			
Boys/girls	20/14	150/82	NS
Age (months)	3.7 ± 3.5	3.5 ± 2.9	NS
Gestational age (weeks)	36.2 ± 4.0	37.5 ± 3.6	NS
Prematurity (<37 weeks)	12	58	NS
Birthweight (kg)	2.5 ± 0.9	3.0 ± 0.9	0.004
BPD and CHD	12	33	0.002
Weight (kg)	4.9 ± 2.3	5.8 ± 3.8	NS
Clinical parameters			
Apnoea	2	38	NS
Cough	22	213	< 0.001
Rhinitis	29	204	NS
Impaired feeding	34	160	< 0.001
Retractions	13	11	NS
Wheezing	8	82	NS
Respiratory rate (/min)	52 ± 15	52 ± 17	NS
Pulse rate (/min)	139 ± 39	146 ± 36	NS
Temperature (° C)	36.7 ± 6.8	37.9 ± 1.0	NS
Laboratory parameters			
SaO_2 (%)	89.4 ± 7.9	90.3 ± 10.6	NS
pCO_2 (kPa)	6.7 ± 2.1	6.5 ± 2.0	NS

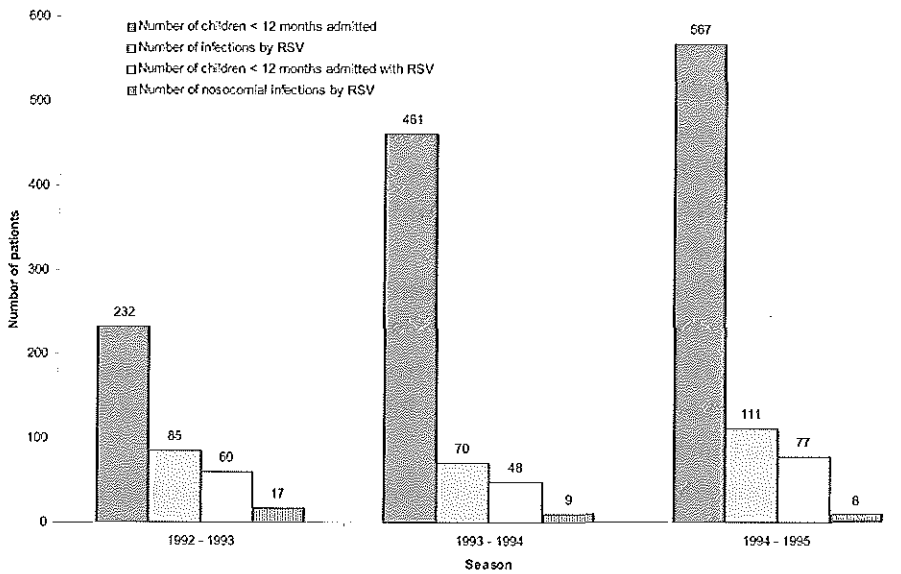

Figure 6.1 *Course in time of the number of nosocomial infections by RSV compared to the total number of infections by RSV and the total number of children younger than 12 months of age admitted to the Sophia Children's Hospital*

the presence of cough (64.7% versus 91.8%, $p < 0.001$) and impaired feeding (100% versus 68.9%, $p < 0.001$), no other differences were observed. The number of patients which required mechanical ventilation (NA-RSV 21% versus CA-RSV 13%) or oxygen supplementation (NA-RSV 50% versus CA-RSV 51%) was equally distributed among NA-RSV and CA-RSV. The number of deaths was significantly increased in the NA-RSV group.

All four children with BPD and NA-RSV required mechanical ventilation compared with two out of 16 children with BPD and CA-RSV ($p = 0.0005$). One out of eight children with CHD and NA-RSV required mechanical ventilation compared with two out of 17 children with CHD and CA-RSV (p = NS).

6.4 Discussion

The incidence of nosocomial RSV infections in infancy in the study was approximately 3% and decreased during the study period 1992 - 1995. Patients with a nosocomial infection by RSV did not experience an enhanced disease course compared with those with community acquired infections by RSV. Only children with BPD and NA-RSV required more often mechanical ventilation compared with children with BPD and CA-RSV. Hence, hospitalised children with BPD should be considered for prophylaxis against infections by RSV.

Infants with NA-RSV had a significantly lower birthweight and more impaired feeding than infants with CA-RSV. These differences may be a reflection of the underlying disease and the characteristics of the population of the Sophia Children's Hospital. We could not draw any conclusion about the prolonged hospital stay in patients with NA-RSV. It is difficult to determine precisely the number of extra hospital days attributable to NA-RSV since it is unclear which endpoints need to be applied (clinical improvement? Negative RSV test?).

Approximately 25% of NA-RSV suffered from CHD. However, children with CHD and NA-RSV did not suffer from a more severe disease course compared with children with CHD and CA-RSV. The recommendations from American studies to avoid hospital admission for patients with CHD during the RSV season are therefore not directly applicable for the Dutch situation [4].

The clinical presentation of children with NA-RSV was equal to that of children with CA-RSV. Therefore, clinical features that give evidence of a nosocomial infection by RSV are comparable to those of CA-RSV and include cough, rhinitis, dyspnoea and/or apnoea. The clinical disease course was also comparable between children with NA-RSV and CA-RSV. Selection bias as a result of an increased awareness of the occurrence of an infection by RSV during the season may influence these observations, although this could not be confirmed in this study.

Infection with RSV occurs through contact of the virus via the nose and eyes as a result of a large droplet aerosol or through self-inoculation after touching contamined surfaces on which RSV may survive for up to six hours [11]. Strict handwashing before and after any patient contact or after touching contaminated surfaces in the vicinity of a patient reduces the survival of RSV and thus remains the most important preventive approach [1,7].

Table 6.2 *Recommended approaches to prevent nosocomial infections by RSV*

I	Isolation of patients with infections by RSV - rapid viral diagnosis is essential - cohort nursing (RSV-ward or admission to a single-persons room)
II	Handwashing before and after contact with a patient - gloves are of no use, handwashing after removing gloves remains necessary - hands must be washed after touching contaminated surfaces such as furniture or toys present in the patient s room
	Use of gowns in patients with infections by RSV
	Per patient toys that may not be used by other patients
III	Hospital staff needs to follow appropiate guidelines when experiencing signs and symptoms of a cold; they need to use a mask, apply strict handwashing and may not take care of high-risk patients

The use of gloves may have a supplementary effect when compliance with handwashing is low [2,10]. However, survival of RSV on gloves is increased compared with bare skin. Hence, handwashing after removal of the gloves remains necessary. It is also advisable not to share toys between patients [11].

The use of gowns and masks remains controversial despite being intensively investigated. Two independent groups reported a beneficial effect associated with the use of gowns and masks [2,10]. Contrasting, one study included 162 children aged between two weeks and three years during two RSV-seasons. In the first season, gowns and masks where used whereas in the second season they were not. No significant differences in the number of NA-RSV was observed between the two years [12]. This observation was confirmed by other groups [1,4]. A possible explanation for this observation may be that RSV also infects through the eyes [11]. Masks do not cover the eyes, hence droplet-aerosols may reach the eyes unhampered. The use of combined eye-nose goggles may prevent this route of infection [13]. Isolation of patients with RSV on a special RSV-ward is a useful tool in decreasing the number of NA-RSV [10,14,15]. Rapid diagnosis of the infection should be applied before a patient is admitted to this RSV-ward or isolated in a single-persons room. Table 6.2 summarizes the general means of preventing nosocomial RSV infections.

Hospital staff form an important vector in transmitting RSV and may thus provide an important source of NA-RSV [3,16]. Consequently, they ought to respect all recommendations in order to prevent NA-RSV from occurring in a certain patient especially when they experience signs of a cold.

The Sophia Children's Hospital pursues guidelines which comprises isolation of patients with a virologically proven or a suspicion of an infection by RSV, handwashing before and after contact with a patient and the use of gowns in patients with RSV. As a result of these guidelines the number of NA-RSV in our hospital proved low and had decreased during the study period. However, no conclusion could be drawn about compliance with handwashing or the use of gowns as a result of the study design. A more strict appliance of the recommendations may result in a further decrease in NA-RSV.

In conclusion, the number of nosocomial infections by RSV in the period between 1992 and 1995 was approximately 3%. Infants with BPD and NA-RSV experienced enhanced disease course. For other infants, the disease course of NA-RSV was found comparable with that of CA-RSV.

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CHAPTER 7

PREDICTORS FOR THE DURATION OF RESPIRATORY SYNCYTIAL VIRUS ASSOCIATED HOSPITALISATION

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SUBMITTED

Abstract

Identification of one or more independent variables predictive for the duration of RSV associated hospitalisation may be useful for preventive and therapeutic approaches. A recent published prediction model ("Michigan model") for the duration of hospitalisation in RSV infection demonstrated good discrimination between children with and without an increased likelihood of a prolonged hospital stay ≥ 7 days, but required examination of it's generalizability to other centers. The Michigan model comprised the variables log weight, congenital heart disease, failure to thrive, premature birth, bronchopulmonary dysplasia, other pulmonary disease, miscellaneous conditions, early mechanical ventilation and early ribavirin treatment.The objective of this study was (*i*) to validate the Michigan model and (*ii*) to derive a prediction model for prolonged hospital stay ≥ 9 days using the Rotterdam database since duration of hospitalisation in The Netherlands is doubled when compared to the USA.

Methods Data of 177 children younger than 12 months of age admitted with virologically confirmed RSV infection to the Sophia Children's Hospital Rotterdam between 1992 and 1995, were used.

Results Mean duration of hospitalisation for the Rotterdam database was 10.3 (± 6.3) days with a median of 9 days. 138 (75%) patients had a hospital stay ≥ 7 days. The Michigan-model performed poorly in the Rotterdam database. The area under the ROC curve was 0.65 (SE 0.04). The Rotterdam prediction model (hospital stay ≥ 9 days) comprised weight and need for oxygen supplementation. The area under the Receiver Operating Characteristic curve of 0.65 (SE 0.04).

Conclusions Both the Michigan and the Rotterdam model could not predict the duration of hospitalisation as assessed by the combination of a high sensitivity with a low false positive rate. Prediction of the duration of RSV associated hospitalisation was not possible in this study as based upon demographical and clinical variables. Duration of RSV associated hospitalisation therefore is not a good outcome variable in multicenter trials which evaluate the results of therapeutic trials.

7.1 Introduction

For more than four decades, the respiratory syncytial virus (RSV) is known to be responsible for the great majority of lower respiratory tract infections in infants and young children. Despite its great impact, hospitalisation for RSV infection occurs in only a small minority of approximately 0.5% to 2% of these children [1].

The duration of hospitalisation may be related to severity of disease. The identification of one or more independent variables predictive for the duration of hospitalisation in RSV infection could be useful in preventive and therapeutic approaches. However, it seems not possible to predict the duration of RSV associated hospitalisation [2]. Very recently, Moler and Ohmit developed a model to predict a hospital stay ≥ 7 days. The parameters used in this model include patient weight, underlying disease state and early therapeutic interventions such as mechanical ventilation and ribavirin (Michigan-model) [3]. The model demonstrated good discrimination as assessed by the area under the Receiver Operating Characteristic (ROC) curve of 0.89. The authors proposed to examine the generalizability of their model to other centers. Since duration of hospitalisation for RSV infection differs significantly between Europe and North America , this validation is of interest in Europe [4].

This study was undertaken (*i*) to validate the Michigan-model for RSV hospitalised patients in a large university hospital in The Netherlands and (*ii*) to develop and validate our own model predicting the likelihood of a prolonged hospital stay greater than the median number of days admitted to our hospital.

7.2 Patients and methods

7.2.1 Patients

This study was performed as part of a clinical epidemiological study of RSV infections in young children. The design of the study has been described earlier [5]. In brief, children with a postnatal age below 12 months of age admitted with RSV infections to the Sophia Children's Hospital, Rotterdam between 1992 and 1996 were included. The study period spanned three RSV seasons from October 1st to April 1st the following year. The diagnosis RSV infection was virologically confirmed by a positive direct immunofluorescent assay (DIFA) and/or a positive viral culture on Hep-2 cells performed on specimen obtained from nasopharyngeal washings.

Demographical and clinical parameters were retrospectively obtained from the patient's medical chart [5]. Definitions applied by Moler and Ohmit were adapted [3].

Patients were diagnosed with failure to thrive when after correction for gestational age their weight plotted on standard Dutch growth charts was below the third percentile.

7.2.2 Validation of the Michigan-model

The Michigan-model was validated on the data of 1992 - 1995. This prediction model ("number one") comprised the variables: logarithm of the weight, congenital heart disease, failure to thrive, premature birth, bronchopulmonary dysplasia, other pulmonary diseases, miscellaneous conditions, early mechanical ventilation and early ribavirin (see the appendix for the exact rule).

Using this rule, for each patient the probability of prolonged hospital stay ≥ 7 days was calculated. The area under (AUC) the Receiver Operating Characteristics curve (ROC) was calculated. Also, the sensitivity, specificity, positive (PPV) and negative predictive value (NPV) were calculated for different cut-off points on the range of the prolonged hospital stay ≥ 7 days.

7.2.3 Derivation and validation of the Rotterdam-model

For the Rotterdam-model, the outcome was defined as a hospital stay greater than the median number of days (9 days). In the univariate analysis predictors for a hospital stay ≥ 9 days were identified by estimating the association (odds ratio (OR) and it's 95% confidence interval (CI)) between each potential predictor and the duration of hospitalisation.

Subsequently, all potential predictors with a p – value ≤ 0.10 were entered into a multivariable logistic regression model to evaluate which variables independently predicted a prolonged hospital stay ≥ 9 days. Variables with a probability ≤ 0.10 were retained in the multivariate model.

Reliability of the model was estimated (goodness of fit) using the Hosmer & Lemeshow method, and discrimination of the model was assessed using the area under the Receiver Operating Characteristic (ROC) curve. An ROC-area under the

Table 7.1 *Comparison of characteristics of hospitalisation between the Michigan and the Rotterdam database*

	Rotterdam database	Michigan database
Number of patients	177	802
Study period	1992 – 1995	1983 – 1992
Mean duration of hospitalisation (days)	10.3 ± 6.3	5.5 ± 7.2
Median	9 days	4 days
Mode	7 days	Not mentioned
Upper quartile	12 days	7 days
Mean age	3.1 ± 2.7 months	5.9 ± 5.5 months

curve (AUC) of 0.80 or greater indicates good discrimination of the model between patients with and without a hospital stay ≥ 9 days [6]. All statistical analyses were performed using SPSS 8.0™ (Chicago, Illinois, USA, 1998).

Data on gestational age, birthweight and weight at presentation were present in 90%, 86% and 94%, respectively of all patients. Clinical characteristics including details from physical examination were present for at least 93% of the patients. The arterial oxygen saturation was transcutaneously measured in 85% and blood gas analysis performed in 98% of the children.

Imputation of missing values using the expectation and maximization method available in standard SPSS software was applied in order to increase the statistical efficiency [7].

7.3 Results

7.3.1 Patient characteristics

185 infants were admitted to our hospital. Data on the duration of hospitalisation could be acquired in 177 infants. The mean duration of hospitalisation was 10.3 days (standard deviation 6.3 days) with a range of 1 to 38 days. The median was 9 days. A hospital stay ≥ 9 days was observed in 96 patients. Table 7.1 compares characteristics of hospitalisation from the Michigan database with the Rotterdam database. Duration of hospitalisation for the three seasons separately was comparable (data not shown).

7.3.2 Validation of the Michigan-model

In our database, 138 of the 185 patients had a hospital stay ≥ 7 days. The area under the ROC curve was 0.65 (95% CI 0.57 - 0.73). The validation of the Michigan-model for different thresholds or cut-off points of the hospital stay ≥ 7 days can be seen in Figure 7.1. For example, when a threshold for a hospital stay ≥ 7 days at 0.50 is used, then 44% of all patients with a hospital stay ≥ 7 days would be correctly identified (i.e. the sensitivity at this threshold). However, the false positive rate (FPR) would then be 33%. An increase in sensitivity was accompanied by an increase in FPR.

7.3.3 Derivation of the Rotterdam-model

Table 7.2 summarizes the results of the univariable analysis on all investigated predictors for a prolonged hospital stay ≥ 9 days. Variables significantly associated

Table 7.2 *Results from the univariate analysis on predictors for duration of hospitalisation ≥ 9 days (* $p \leq 0.15$). OR: odds ratio, CI: confidence interval*

	Hospital stay (days)		
	≥ 9 days (n = 96)	< 9 days (n = 81)	OR (95% CI)
Patient demographics			
Boys vs girls	29/67	34/47	0.6 (0.3 – 1.2)*
Age (months)	2.9 ± 2.7	3.2 ± 2.7	0.9 (0.7 – 1.3)
Gestational age (weeks)	37.6 ± 3.5	37.5 ± 3.7	1.0 (0.9 – 1.1)
Prematurity	24	24	0.8 (0.4 – 1.6)
Birthweight (kg)	2.9 ± 0.9	2.9 ± 0.8	0.9 (0.7 – 1.3)
BPD without prematurity	2	0	
CHD	9	3	2.7 (0.6 – 13.6)*
Prematurity + BPD	7	5	1.2 (0.3 – 4.6)
Weight (kg)	5.1 ± 1.9	6.3 ± 5.3	0.8 (0.7 – 1.0)*
Clinical findings at admission			
Apnoea's	23	14	1.5 (0.7 – 3.4)
Cough	85	74	0.7 (0.2 – 2.2)
Rhinitis	81	72	0.7 (0.3 – 1.7)
Impaired feeding	70	60	0.9 (0.5 – 1.9)
Wheezing	33	27	1.0 (0.5 – 2.0)
Retractions	53	43	1.1 (0.6 – 2.1)
Temperature (°C)	37.8 ± 1.0	37.7 ± 1.0	1.1 (0.8 – 1.5)
Pulse rate (/min)	151 ± 42	142 ± 33	1.0 (1.0 – 1.0)*
Respiratory rate (/min)	53 ± 20	50 ± 13	1.0 (1.0 – 1.0)
SaO_2 (%)	88.4 ± 11.3	90.1 ± 10.9	1.0 (1.0 – 1.0)
pCO_2	7.0 ± 2.2	6.3 ± 1.4	1.3 (1.0 – 1.5)*
Findings on chest radiograph			
Atelectasis	32	22	1.3 (0.7 – 2.)
Hyperinflation	58	41	1.5 (0.8 – 2.8)
Infiltrate	7	5	1.2 (0.3 – 4.6)
Characteristics of hospitalisation			
Mechanical ventilation	19	8	2.3 (0.6 – 6.0)*
Oxygen administration	65	42	2.0 (1.0 – 3.8)*

with prolonged hospital stay ≥ 9 days included gender, CHD, weight, pulse rate, SaO_2, mechanical ventilation and oxygen supplementation. These variables were consecutively entered into multivariable regression analysis. The independent predictors for prolonged hospital stay ≥ 9 days were weight and oxygen supplementation (Table 7.3). The Hosmer & Lemeshew test was far from significant (chi square 4.77, $p = 0.7814$), indicating good reliability of the model. However, the area under the ROC-curve was only 0.65 (95% CI 0.57 - 0.73). The performance of the model can be seen in Figure 7.1 For example, when a threshold at 0.20 is used, the sensitivity would be 40%. The false positive rate would then be approximately 15%.

7.3.4 Validation of the Rotterdam-model

Forty-two patients with infections by RSV were admitted in the period 1995 - 1996.

Table 7.3 *Results of the multivariate analysis on predictors for prolonged hospital stay ≥ 9 days*

Variable	OR (95% CI)
Weight (kg)	0.9 (0.7-1.0)
Oxygen supplementation	1.7 (0.9-3.3)
ROC area (95% CI)	0.65 (0.57 – 0.73)

Unfortunately, the false positive rate never decreased below 35% (Figure 7.1).

7.4 Discussion

This study validated a prediction model developed by Moler and Ohmit for the duration of hospitalisation in RSV infection [3]. In their population, severe infections were represented by a prolonged hospital stay for seven or more days (the upper quartile of duration of hospitalisation). The model had a good discriminative value with the area under the ROC-curve of nearly 0.90. When evaluated on the Rotterdam

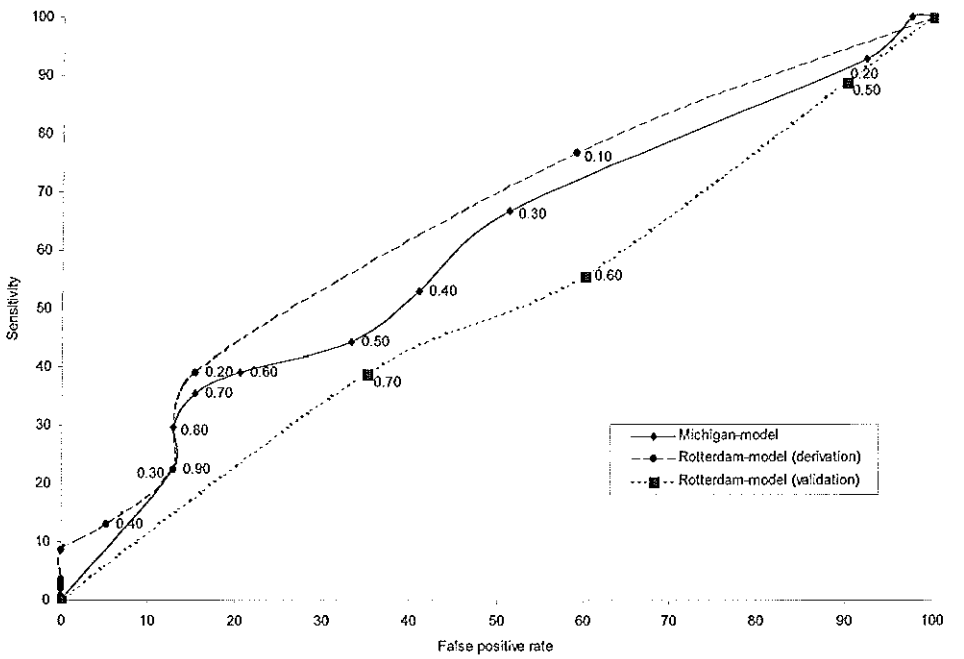

Figure 7.1 *Performance of the Michigan-model on data of infants younger than 12 months of age admitted between 1992 and 1995, and validation of the Rotterdam-model on data of infants younger than 12 months of age admitted in the season 1995 – 1996. The numbers next tot the markers are the cut-off probability thresholds*

database, the Michigan model demonstrated acceptable sensitivity (dependent upon the cut-off probability used), but the FPR did not decrease below an acceptable value. This might be explained by the longer duration of hospitalisation in The Netherlands compared to the United States. The upper quartile in the Michigan database is 12 days. Thus, in our institution, a cut-off at seven days does not necessarely correctly discriminate between "less severe" and "severe" infections. Secondly, the patients in our population were almost twice as young compared with the Michigan population. This difference in age may have influenced the severity of disease in the study population [8]. Severity of illness between the Michigan and the Rotterdam model was comparable for the number patients requiring mechanical ventilation (9.1% for the Michigan database versus 15.% for the Rotterdam database), but was not comparable for the number of ICU admissions (15.1% for the Michigan database versus 41.6% for the Rotterdam database). All of these differences may account for the inapplicability of the Michigan-model to our hospital. Therefore, we describe independent predictors for a hospitalisation ≥ 9 days (the median) in RSV infections for the Rotterdam database. Independent predictors for a hospital stay ≥ 9 days were weight and need for oyxgen supplementation. These variables are also associated with the severity of infections by RSV [9,10].

Unfortunately, when validated on data from a single RSV-season our model also failed to accurately predict the duration of hospitalisation and identify patients at risk for prolonged hospital stay with a low false positive rate. A reliable prediction of duration of hospitalisation in RSV infection seems not feasable. Differences in RSV subtype are not responsible for the observation that the duration of hospitalisation in children with RSV infections is unpredictable [11]. Parental-related factors or doctor-related factors not included in the model may influence the duration of hospitalisation. For instance, there may be an interobserver variability in the decision whether a patient has to remain hospitalised. The PICNIC study demonstrated that RSV associated duration of hospitalisation can be a useful outcome in clinical trials in case of a high interobserver reliability [12].

One could argue that the duration of hospitalisation in RSV infection may not be an adequate criterium for severity of RSV associated disease. However, many multicenter trials investigating the efficacy of preventive treatment such as RSV-IVIG (including 54 centers in the United States) and Palivizumab (including 139 hospitals from the United States, Canada and the United Kingdom) included duration of hospitalisation as an endpoint in the assumption that duration of hospitalisation reflects the severity of disease [13,14].

In conclusion a reliable prediction of the duration of RSV associated hospitalisation based upon demographical and clinical variables was not possible. Therefore, the

duration of hospitalisation in RSV infections may not be an adequate criterium for the severity of disease and outcome of clinical trials.

Appendix

The exact rule of the Michigan model is:
Probability for prolonged hospital stay ≥ 7 days = 1 / (1 + exp (- [score])), where the score is calculated by: 0.0905 + (-1.678 * log weight) + (1.456 * CHD) + (1.850 * FTT) + (0.922 * premature birth) + (1.633 * BPD) + (1.671 * other pulmonary disease) + (0.812 * miscellaneous conditions) + (3.213 * early mechanical ventilation) + (0.659 * early ribavirin).

The exact rule of the Rotterdam model is:
Probability for prolonged hospital stay ≥ 9 days = 1 / (1 + exp (- [score])), where the score is calculated by: 0.5954 + (-0.1367 * weight in kg) + (0.5502 * need for oxygen supplementation).

For dichotomous variables 1 should be filled in when variable is present where 0 should be filled in if variable is absent.

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CHAPTER 8

TREATMENT AND PREVENTION OF RESPIRATORY SYNCYTIAL VIRUS INFECTIONS

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Abstract

This review discusses the current knowledge on treatment and prevention of RSV infections in children. Unfortunately, effective therapy is not yet available for children with RSV infections. The efficacy of corticosteroids and bronchodilators has not yet been adequately documented. The use of ribavirin in patients with RSV infection is only indicated in a highly selected group of high risk patients with T-cell immunodeficiency. The results of studies on the efficacy of vitamin A, interferon and antibiotics showed disappointing results. Vaccination research has produced candidate vaccines such as the recombinant vaccine BBG2Na, a subunit vaccine PFP-2 and *coldpassaged-temperature sensitive* vaccines. However, phase III efficacy trials in infants, young children and the elderly are still lacking. Hence, it will take many years before effective vaccines will become available for routine use. Passive protection against infections by RSV can be conferred by the use of RSV hyperimmune globulin (RSV-IVIG) or by the administration of palivizumab, a monoclonal antibody. The efficacy of these two compounds has been demonstrated. The easy route of administration and the minor side effects of palivizumab make this drug a promising drug. However, large costs are involved. In addition, major differences have been reported in the prevalence of RSV lower respiratory tract infections in different countries, regions and even withing well-known high-risk populations. We therefore suggest that the development of local and regional guidelines, based on hospitalisation rates in high-risk infants and cost-benefit analysis studies is necessary.

8.1 Introduction

Respiratory syncytial virus (RSV) is a member of the genus *Pneumoviridae* of the family *Paramyxoviridae*. Its genome consists of linear, single stranded RNA and encodes ten proteins of which two of the transmembrane surface proteins (G and F proteins) are of most interest, since they are involved in viral pathology and infectivity [46]. The G protein is responsible for attachment of the virus to the host cell, the F protein is responsible for fusion of the virus with the host. Both F and G proteins induce neutralising antibodies [116]. Two major antigenic groups of RSV – subtypes A and B – may be identified by the use of monoclonal antibodies [127].

RSV is the worlds leading cause of viral respiratory tract infection in infants and young children [70]. The clinical spectrum extends from a mild upper respiratory tract infection to severe lower respiratory tract disease including bronchiolitis [79]. In the United States, RSV infection annually leads to approximately 100.000 hospital admissions with an estimated number of 4500 deaths [57].

RSV associated lower respiratory tract disease especially occurs in young children with a peak incidence between two and six months of age [12]. In some of these young infants a severe disease course may be observed. Children with underlying chronic diseases such as bronchopulmonary dysplasia, congenital heart disease and T-cell immunodeficiency or prematurely born infants have a higher risk for severe RSV infections [41, 51, 82].

RSV associated bronchiolitis results from an immunopathological process in which T-cells play a major role [13, 40]. Neutrophils and eosinophils are also involved in the RSV related disease process [33, 72]. Eosinophils become activated and subsequently release mediators which are thought to be responsible for wheezing during acute infection [72].

Currently, the treatment of RSV infections is mainly supportive. The therapeutic effects of corticosteroids, bronchodilators, ribavirin, and RSV immune globulin and the prevention of RSV infections by hyperimmune globulin and monoclonal antibodies have been investigated in many studies. The interpretation of the results of these studies and the implementation in clinical practice may vary considerably as was illustrated by a poll among members of the European Society for Paediatric Infectious Diseases [73]. Recent studies with new candidate vaccines have yielded promising results [37, 66, 71].

This review seeks to provide the reader with a comprehensive overview of the current knowledge on the treatment and prevention of RSV infections in infants and young children.

8.2 TREATMENT OF RESPIRATORY SYNCYTIAL VIRUS INFECTION

8.2.1 *CORTICOSTEROID THERAPY*

A large body of experimental data underscores the important role of the host immune response in RSV induced bronchiolitis. This has led to the hypothesis that a modulation of the immune response by the use of corticosteroids may have a beneficial effect on the outcome of children with RSV infections [91]. Data from studies carried out in the 1960's were conflicting and difficult to interpret [17,81,96,129]. Tal and co-workers described clinical improvement after the use of a combination of dexamethasone and salbutamol in a group of 32 infants with RSV infections [131]. However, the index group in this study was not only restricted to infants with acute bronchiolitis.

The intravenous use of hydrocortisone 1 mg/kg/day and subsequently prednisone 2 mg/kg/day orally in combination with albuterol inhalations was investigated in 50 infants younger than one year with a first episode of bronchiolitis [126]. In this study no clinical benefit was demonstrated. The efficacy of intramuscular dexamethasone (1 mg/kg) administered in 65 infants was compared with that of placebo given to 53 infants with an intial episode of wheezing [114]. The duration of oxygen therapy or time to resolution of symptoms did not decrease. This lack of benefit was also observed when the efficacy of oral dexamethasone was investigated [75]. Van Woensel *et al* studied the effect of oral prednisolone 1 mg/kg/day for seven days in 27 RSV positive infants [136]. They observed a significant reduction in symptom score in the first three days of the treatment with a non-significant tendency to treatment benefit in children who suffered from severe bronchiolitis. The duration of mechanical ventilation was not reduced in the treatment group as compared to the control group, whereas the duration of hospitalisation was significantly reduced in the treatment group [136].

Investigators have also studied whether corticosteroids prevent post-bronchiolitic wheezing. The use of either oral prednisone with β_2-agonists or nebulized budesonide did not reduce the prevalence of wheezing, respiratory symptom scores or the proportion of children requiring bronchodilators or steroids one year after the acute illness [109].

Corticosteroids are not effective and therefore not indicated in children with bronchiolitis or as part of a prophylactic therapy to prevent post-bronchiolitic wheezing. The Infectious Diseases Society of America accordingly advised against the use of corticosteroids [85].

8.2.2 *BRONCHODILATOR THERAPY*

Bronchodilators are frequently used in children with RSV infections because of the

similar disease presentation in infants with asthma and with RSV bronchiolitis. Airway obstruction in RSV induced bronchiolitis is characterised by necrosis and lysis of respiratory tract cells with edema of the submucosa, excessive mucus secretion with or without bronchial hyperreactivity and surfactant abnormalities associated with airway collapse [3]. ß-agonists do not only generate bronchodilatation, but also contribute to mucus clearance, production and release of surfactant [27]. Four studies have demonstrated beneficial effects of ß-agonists on the course of RSV induced bronchiolitis in infancy [4, 14, 74, 134]. A short term improvement in clinical scores was seen in children treated with ß-agonists [68]. The use of salbutamol may lead to a short-term reduction in the respiratory distress assessment instrument (RDAI) lasting approximately 30 minutes [74].Recently, a French group reported a significantly greater improvement in respiratory rate, accesory muscle an wheezing score in infants treated with 0.15 ml/kg/dose nebulised salbutamol [74]. Schuch *et al* demonstrated in a non-controlled study of 50 infants with RSV bronchiolitis a significant improvent in airway conductance as a result of treatment with a bronchodilator [125]. However, the study group was not restricted to first time wheezers. Also, the bronchodilator-response group was characterised by a stronger family history of atopy and chronic bronchitis. This may indicate that infants prone for atopic asthma were included in the study population.

Other investigators have failed to demonstrate a beneficial effect of bronchodilators or an improvement in RSV associated respiratory failure [16, 38, 39, 52, 62]. A randomised controlled trial in 62 patients between 2 and 24 months of age comprising four treatment groups (salbutamol 0.15 mg/kg/dos plus ipratropium bromide 125 µg or 250 µg in infants aged 6 months or more, salbutamol alone, ipratropium bromide alone or saline) demonstrated no significant change in oxygen saturation, change in clinical score or duration of hospitalisation [141]. Two randomised trials including 88 and 83 children respectively failed to demonstrate a significant improvement on oxygen saturation by the use of salbutamol [74]. ß-agonists were also reported to induce bronchospasms [62].

Racemic adrenaline provides a significant improvement in the clinical score and transcutaneously measured *carbon dioxide* tension in children treated with adrenaline as compared with the control group, although a significant difference in SaO_2 was only found immediately after nebulisation [78]. Ipratroprium bromide has no benefit as adjuvant therapy for bronchiolitis [60, 119, 120].

Flores *et al* [36] therefore justifiable concluded on the basis of an extensive meta-analysis that there is insufficient evidence to support the efficacy of $ß_2$-agonist therapy. However, bronchodilators may produce a modest short-term improvement in clinical scores as suggested in a study using the Cochrane database [69]. Currently, bronchodilators are frequently used as supportive treatment despite the lack of evidence-based support for this practice [73, 83].

8.2.3 Antiviral therapy

Ribavirin (Virazole ®) is the only drug registered for the treatment of children with RSV induced bronchiolitis. Ribavirin has been licensed by the American Food and Drug Association (FDA) since 1985. Ribavirin (1-ß-D-ribafuranosyl-1,2,4-triazole-3-carboxamide) is a virostatic synthetic nucleoside analogue [47]. In vitro it is active against a wide variety of DNA and RNA viruses such as herpes, cytomegalovirus and influenza A and B viruses [35]. After administration, the drug is phosphorylated into ribavirin triphosphate, which is the highly active metabolite. Ribavirin is subsequently catabolised to triazole carboxamide. This inactive metabolite is excreted in the urine [18].

Ribavirin is administered by a nebuliser. Administration of a large dosage of aerosolised ribavirin aerosols in a short period (6 mg per 100 ml water for a period of 2 hours three times a day) leads to an equal effect as the gift of a low dosage during a longer period (6 gram per 300 ml water for 18 hours a day) without an increased ribavirin concentration in the patient or an increased ribavirin concentrations in the environment [32].

Teratogenic, carcinogenic and mutagenic side-effects of ribavirin have previously been described in animal models [56]. Carcinogenic effects occurred after rats were chronically fed with high doses of ribavirin, whereas teratogenicity was only seen when ribavirin was administered during the first trimester of pregnancy [35]. Orally administered ribavirin may also inhibit red cell production [35]. However, very little side-effects were reported in human clinical studies. The drug was not detected in either plasma or red blood cell samples of health care workers [11].

8.2.3.1 Ribavirin in non-ventilated children

Hall and co-workers observed that the drug significantly improved the overall clinical score, decreased the number of lower respiratory tract associated symptoms and improved the arterial oxygen saturation in 33 infants of which seventeen were treated with ribavirin [50]. Taber *et al* and Rodriguez *et al* confirmed the observation of improvement in the clinical score and increased rate of improvement of oxygen saturation by the use of ribavirin [112, 130]. Barry *et al* found a significant decrease in cough and crepitations in children treated with ribavirin [6]. Beneficial effects of ribavirin were also observed in children with congenital heart disease or bronchopulmonary dysplasia [45, 49]. Conrad *et al* compared 33 infants treated with ribavirin with a retrospectively obtained control group [21]. The demographics of the treatment and control group were not fully comparable, since infants treated with ribavirin were significantly younger than the control group. Ribavirin significantly reduced the duration of

hospitalisation, mechanical ventilation and mean therapeutic intervention scores. The use of ribavirin did not lead to a significantly lower airway resistance or increased compliance [64]. In contrast to these observations, two recent studies failed to demonstrate a beneficial effect of ribavirin [80,93].

8.2.3.2 RIBAVIRIN IN VENTILATED CHILDREN

Three studies evaluated the efficacy of ribavirin in ventilated patients [87,90,124]. Smith and co-workers observed an improved arterial oxygen saturation, a significant reduction in duration of mechanical ventilation, duration of oxygen suppletion and duration of hospitalisation in ventilated infants with RSV infections, who were treated with ribavirin [124]. In two recent studies in 41 and 439 patients these observations could not be confirmed [87,90]. However, in these studies sterile water was used as placebo. Wheeler *et al* retrospectively compared two hospitals: in one hospital ribavirin was routinely used whereas in the other hospital only supportive therapy for RSV bronchiolitis was given [145]. No significant differences in mortality, duration of hospitalisation and duration of mechanical ventilation were observed.

The early studies demonstrated beneficial effects of ribavirin. However, these investigations were critisized on their methodology and use of subjective endpoints such as clinical score rather than major endpoints such as mortality, oxygen saturation and mechanical ventilation. Furthermore, several groups used aerosolized water which may induce bronchospasms as part of the placebo treatment [28]. Additional problems of ribavirin include high costs of the drug, difficulties with administration and the potential teratogenic and carcinogenic effects. The American Academy of Pediatrics have altered their recommendations from "should be used" to "may be considered" in 1996. These guidelines indicate treatment with ribavirin in high risk children (CHD, BPD, prematurity and age < 6 weeks), immunodeficient children and severely ill children with or without mechanical ventilation [2]. However, in our opinion there is not enough evidence that ribavirin has a beneficial effect in high risk children with CHD, BPD, prematurity or even in severely ill children requiring mechanical ventilation. A meta-analysis addressed the question whether ribavirin is useful in the treatment of RSV-infections [107]. These authors concluded that the relative risk for mortality was 0.42 (95% CI 0,13 – 1.44, p = NS). The risk for respiratory deterioration was 0.42 (95% CI 0.16 – 1.34, p = NS). They concluded that the use of ribavirin was not associated with a significant benefit. However, the individual trials may lack sufficient power to provide reliable estimates of the effects [108]. We therefore suggest to restrict administration of ribavirin to immunodeficient children, in which ribavirin may reduce the duration

of viral shedding of RSV [51,84].

The prevention of long term sequelae of RSV bronchiolitis after the use of ribavirin has also been investigated. Infants treated with ribavirin demonstrated significantly lower RSV-specific IgE responses (which may be responsible for the wheezing) with lower geometric mean titers than controls [115]. The use of ribavirin did not result in differences in lung function or in decreased respiratory symptoms after RSV bronchiolitis in treated versus non-treated infants five to six years after the initial illness [77]. Recently, Edell *et al* retrospectively demonstrated in a group of 22 infants younger than 6 months of age treated with ribavirin a significant reduction in the prevalence of reactive airway disease one year after bronchiolitis [30]. This effect was only detected in the group with "mild" reactive airway disease. However, the study subjects were not randomized for treatment and loss to follow-up was high (36 out of 77 hospitalised infants).

8.2.4 RSV-Immunoglobulin

Administration of aerosolised RSV-immunoglobulin in the cotton rat results in a strong reduction of viral titers in pulmonary tissue and clearance of detectable virus in most of the animals [97]. Furthermore, a much lower dose is needed to achieve similar effects when RSV-IVIG is administered topically instead of intravenously [104]. These observations in animal studies raised the possibility to use RSV-immunoglobulin in the treatment of RSV-bronchiolitis.

Rodriguez *et al* performed two clinical trials to investigate the efficacy of RSV-specific immunoglobulin (RSV-IVIG) in the treatment of lower respiratory tract infection by RSV [111, 113]. In the first study, RSV-IVIG was used in young children with BPD, chronic lung disease, congenital heart disease or prematurity < 32 weeks of gestation. There were no significant differences in duration of RSV-hospitalisation, ICU stay and mechanical ventilation between the treatment and the control group [113].

The second study enrolled previously healthy infants who were hospitalised with RSV infection [111]. Again, efficacy of RSV-IVIG could not be confirmed. Therefore, RSV immunoglobulin is not used as therapy for infections by RSV.

8.2.3 Other therapies

8.2.3.1 Vitamin A

Serum vitamin A levels were significantly decreased in children with RSV infections, thus possible contributing to a more severe course of infection. These data suggested

that vitamin A might be a useful adjunctive therapy [92, 106]. However, in a subsequent study oral administration of vitamin A did not decrease respiratory morbidity in children with acute RSV bronchiolitis [100].

8.2.3.2 Antimicrobial therapy

The risk of a secondary bacterial infection in patients with RSV bronchiolitis is very low [52]. Hence, the routine use of antibiotics in the treatment of RSV bronchiolitis is not warranted. The decision whether or not to start antibiotics generally depends on the clinical impression of the patient, increased white blood cell count (WBC) and elevated C-reactive proteïn. White blood cell counts greater than 15×10^9/l have been associated with bacteremia [133]. Although white blood cell counts may increase up to 20×10^9/l, serum CRP-levels in RSV-infection are usually low [118]. We suggest to restrict the use of antibiotics to those children in whom an unexpected clinical deterioration is seen accompanied by an increase in inflammatory parameters such as C-reactive protein.

8.2.3.3 Interferon

Interferon (IFN) is released by leukocytes in response to viral infections and may induce a state of resistance to replication in host cells [101] The interferon response to RSV infection in children was found to be minimal when compared to that by other respiratory viruses such as influenza [48].

Higgins *et al* conducted a double-blind, placebo-controlled study to investigate the clinical and prophylactic efficacy of IFN-2a [61]. Although a prophylactic effect was observed, the severity of signs and symptons nor the duration of illness was influenced by IFN-2a. Therefore, IFN-2a has little value in the treatment of infections by RSV [15].

8.3 Prevention of RSV infections

8.3.1 Vaccination

A formalin inactivated aluminum precipitated RSV vaccine was developed and tested in infants and children in the 1960's. Unfortunately, not only did this vaccine fail to protect against naturally acquired RSV infection, but on rechallenge with RSV it induced an immune-mediated response resulting in increased hospitalisation rates for lower respiratory tract pathology and increased mortality [37, 66, 71]. The pathophysiology of this processs was explored in animal models. In the cotton rat

formalin did not ablate all of the epitopes on the F and G glycoproteins, resulting in high levels of antibodies against the F and G protein [105]. This led to an aberrant immune response resulting in more severe pulmonary histopathology [98]. Subsequent studies showed that CD4+ T cells play a role in the enhanced pulmonary histopathology and that formalin inactived RSV vaccine induced low levels of major histocompatibility complex class I restricted cytotoxic T-cell activity as opposed to natural acquired RSV infection [20]. Formalin inactived RSV vaccine primed for a $T_{helper}2$ cell response, which is interleukin (IL) -4 and IL-10 dependent. Such a response has been associated with disease progression in mice [19]. Also, formalin inactived RSV-vaccine induced antibodies to the F protein, that were deficient in their fusion inhibiting activity [91].

The development of an effective vaccine against RSV still experiences several obstacles. A new vaccine has to protect against RSV subtypes A and B [76]. Also, RSV affects especially infants younger than six months of age with increased morbidity thus necessitating maternal or neonatal immunisation [140]. However, the presence of maternal antibodies may diminish the antibody response of infants on exposure with a RSV vaccine [31]. In addition, the ability to respond to viral surface glycoproteins appears to be low during the first six to nine months of life [22]. A vaccine has to protect for at least the first years of life. Finally, there may remain concern for an enhanced pulmonary disease course as was previously induced by the formalin inactived vaccine.

8.3.1.1 Recombinant vaccines

A newly developed recombinant fusion protein BBG2Na – RSV G protein fragment aa 130-230 with a conserved subgroup A specific protective epitope, lined up with the albumin-binding region (BB) of streptococcal protein G and a stretch of amino acids residues completely conserved in all known human A and B RSV isolates– was shown to be protective in mice and cotton rats [102]. Maternal immunisation resulted in a passive transfer of high levels of RSV-A antibodies to offspring, protecting them from RSV challenge for up to 14 weeks. Also, maternal antibodies did not suppress the effect of neonatal vaccination. A phase I trial with BBG2Na on healthy volunteers has been performed in our institution. The vaccine was highly immunogenic, well tolerated and resulted in few side effects (Power UF *et al*, Safety and immunogenicity of a recombinant subunit vaccine against RSV; abstract book "RSV after 43 years", Stuart, Florida 1999).

Other recombinant vaccines include those with a vector such as the recombinant adenovirus type 4,5 and 7 expressing either the F and G or both proteins, a recombinant bacteriophage displaying a chimeric coat protein fused to a protective epitope from the glycoprotein G or an optimized plasmid DNA-vector expressing the RSV fusion protein

(DNA-F) [7]. The bacteriophage induced a high level of RSV-specific antibodies in BALB/c mice. These candidate vaccines are still evaluated in animal models.

8.3.1.2 Subunit vaccines

Animal studies demonstrated that purified F and/or G protein protect immunised mice against subsequent challenge by RSV [116]. In humans, the use of Purified Fusion Protein-1 (PFP-1) did not prevent infection, although severe respiratory tract disease did not occur [9]. Importantly, when PFP-1 was administered intramuscularly, none of the recipients developed enhanced disease after vaccination or subsequent infection [135].

PFP-1 contains approximately 90% – 95% F –protein, whereas PFP-2 contains approximately 98% F-protein and to a lesser extent other virus proteins [143]. Administration of PFP-2 resulted in a good immune response and a significantly reduced number of lower respirator tract illnesses in childen with cystic fibrosis or bronchopulmonary dysplasia [99]. Other subunit vaccines include the RSV fusion protein with cholera toxin (CT) as an adjuvant or a subunit vaccine of purified fraction 21 of *Quillaja saponaria* with the fusion protein [137, 138].

8.3.1.3 Live vaccines

Since killed RSV vaccines produced paradoxical effects, attention has focussed on live attenuated RSV vaccines. A live RSV vaccine was shown to be ineffective [10]. Over the past decades, attenuated vaccines such as host range mutants, cold-passaged mutants (*cp*) and temperature sensitive (*ts*) mutants (RSV replicates at 37° but not in higher temperatures) were created [29]. A group of British investigators developed *ts* mutants of RSV strain A chemically mutagenized by growth in the presence of 5-fluorouracil (*ts*1A and *ts*19A) or mutagenised by growth in ICR 340 or ICR 372 (*ts*1B and *ts*19B) [86]. Intranasal administration of this candidate vaccine was evaluated in 20 healthy volunteers. The vaccine was able to produce a secondary immune response almost equal to wild type RSV, but with a reduction in morbidity. However, the authors concluded that "the residual pathogenicity of even the most modified mutant would not be acceptable in infants".

RSV strain A2 mutagenized twice with 5-FU in Vero cell monolayer cultures yielded a series of candidate vaccines designated *cpts*-248, *cpts*248/404 and *cpts*530/1009 [23, 67]. All three candidate vaccines protected (seronegative) chimpanzees against challenge with wild type RSV [23, 26, 67]. However, when evaluated in young children, important side-effects - especially in infants younger than two months of age - were seen including

Table 8.1 *Summary of the results from the three studies on the use of RSV-IVIG*

Study	RSV-IVIG (750 mg/kg)	Control group	% reduction	*P* - value
Groothuis et al (N Engl J Med 1993;329:1524-1530)				
Inclusion: children < 4 years with BPD, CHD or premature birth				
Sample size (n)	81	89		
All RSV-LRTI (n)	7	20	62	0.01
Moderate-to severe RSV-LRTI (n)	3	12	72	0.03
Number of hospitalisations (n)	6	18	63	0.02
Hospital days	43	128	63	0.02
ICU admissions (n)	1	6	82	0.12
Groothuis et al (Pediatrics 1995;95:463-467)				
Inclusion: premature infants with of without BPD				
Sample size (n)	58	58		
All RSV-LRTI (n)	4	14	71	0.01
Moderate-to severe RSV-LRTI (n)	1	10	90	0.006
Number of hospitalisations (n)	4	13	69	0.02
Hospital days	31	83	63	0.06
Prevent Study Group (Pediatrics 1997;99:93-99)				
Inclusion: premature infants with of without BPD				
Sample size (n)	250	260		
Number of hospitalisations (n)	20	35	41	0.046
Hospital days	60	129	52	0.045
Hospital days on oxygen	34	85	60	0.007
Hospital days classified as severe	49	106	54	0.049
ICU admissions (n)	8	12	31	0.41

upper respiratory tract symptoms such as nasal congestion (Karron RA *et al*, Evaluation of live recombinant RSV A2 vaccines in children over 6 months of age; abstract book "RSV after 43 years", Stuart, Florida, 1999). An important characteristic of this vaccine was the stability of the *temperature sensitivity* phenotype. In order to simulate the condition of human infants with maternally derived antibodies against RSV, some chimpanzees were intravenously pretreated with RSV immune globulin. Viral replication was highly restricted in both upper and lower respiratory tract after challenge with wild type RSV, although protective efficacy was not altered [23]. At this moment, it seems that it will take several years before one of these vaccines will become available for infants.

8.3.2 Passive immunisation

8.3.2.1 Intravenous immunoglobulins

Studies in the cotton rat demonstrated that adequate neutralising antibody titers provided protection from RSV infection and did not result in RSV associated disease [58]. Topical administration of lower dosages had equal effects [59]. In humans, neutralising

antibody titers between 1:200 and 1:400 correlate with less severe pulmonary infection [42]. However, standard immunoglobulins may not contain sufficient RSV neutralising antibody to ameliorate or prevent RSV-LRT disease [88]. This problem can be resolved by the use of RSV specific immunoglobulin [121,122].

The first study which investigated the effect of RSV-Ig was performed in 1993 and included 249 infants and children younger than four years of age with BPD, CHD or prematurity [41]. RSV-Ig was given up to a total of five monthly infusions during the RSV-season in either high dose (750 mg/kg) or low dose (150 mg/kg). Recipients of the high dose had a 62% reduction in incidence of RSV-infections and 72% reduction in moderate to severe infections (Table 8.1). The high dose group differed significantly from the control group in the number of hospitalisations and the duration of hospitalisation. The greatest benefit of RSV-Ig was found among preterm infants and infants with BPD. Adverse events occurred only in three percent and comprised a mild fluid overload, mild decreases in oxygen saturation and fever. Six deaths occurred which were non attributable to infusion of RSV-Ig. Five out of the six deaths were patients with CHD. These deaths were not direct attributable to RSV related disease. Three deaths were related to complications of cardiac surgery.

A subanalysis from this study included prematurely born infants with or without BPD [43]. The efficacy of high dose RSV-Ig (n = 58) was compared to that of placebo (n = 58). Again, RSV-Ig significantly reduced the number of moderate-to-severe RSV-LRTI's and the number of RSV-hospitalisations. Duration of hospitalisation nearly reached significant difference ($p = 0.06$). Adverse events were seen in 5% of the study population.

The second study investigating the use of RSV-IVIG in premature infants and infants with BPD was carried out by the PREVENT group in 1997 [103]. High dose RSV-IVIG 750 mg/kg (n = 250) was compared to placebo (n = 260). RSV-IVIG prophylaxis was associated with 41% reduction in RSV-hospitalisation and 53% reduction in total days of RSV hospitalisation (Table 8.1). Children with RSV-IVIG had a significantly reduced number of days on supplemental oxygen. The number of patients requiring mechanical ventilation did not differ between the two groups. Adverse events were infrequent and mild.

The outcomes of the two studies resulted in RSV-IVIG (RespiGam®) being licensed by the Food and Drug Administration (FDA) in January 1996 for use in the prevention of RSV infection in infants and children younger than 2 years of age with BPD or a history of premature birth (≤ 35 weeks). The recommendations were supported by the American Academy of Pediatrics with a minor modification of the history of prematurity (≤ 32 weeks). It was advised to defer measles-mumps-rubella-varicella vaccinees until 9 months after the last dose [5].

The beneficial effect of RSV-IVIG was demonstrated in a systematic review of all

published studies on RSV-IVIG [142]. It was found that RSV-IVIG effectively prevents hospitalisations and ICU-admissions by RSV, but does not result in a reduction of mechanical ventilation.

Currently, RSV-IVIG is not indicated in children with CHD. In a recent study, 202 children younger than four years of age with congenital heart disease or cardiomyopathy received 750 mg/kg RSV-IVIG, whereas the 214 controls received albumin [123]. Neither the number of RSV-associated RTI's, nor the number of hospital days for RSV-LRI differed between the two groups. No significant differences regarding ICU-admission and mechanical ventilation were observed between the two groups. In eight children, the infusion of RSV-IVIG resulted in unanticipated cyanosis necessitating immediate cardiac intervention.

Several drawbacks are associated with the use of RSV-IVIG. The drug has to be administered intravenously, with occasional problems to obtain intravenous access. Also, several hours of hospital admission are required. The volume which has to be administered can be considerable using the recommended dosage of 15 ml/kg. RSV-IVIG interferes with routine vaccinations. One of the major drawbacks is the costs associated with the use of RSV-IVIG. Costs per patient per season range from approximately $2000 to $ 6700 dependent upon the patients weight. The costs for immunisation will exceed the costs of hospitalisation avoided as a result of prophylaxis for most infants [95]. In other words, the use of RSV-IVIG may not result in net savings in medical resources [55]. The net cost of prophylaxis with the use of RSV-IVIG seems to be the lowest for preterm infants with BPD and age < 3 months [95].

Robbins *et al* calculated the Number Needed to Treat (NNT) based upon data from the above mentioned studies [110]. These authors concluded that RSV-IVIG should not be routinely administered in premature infants younger than 6 months of age without BPD since 62.5 infants need to be treated with RSV-IVIG in order to prevent one hospital admission [110]. Twelve infants with BPD need to be treated in order to prevent one hospital admission.

8.3.2.2 Monoclonal antibodies

Monoclonal antibodies are used to distinguish between the two known subtypes of respiratory syncytial virus, designated A and B. In animal studies monoclonal antibodies directed at the F-protein were responsible for fusion of the virus to its host and protection against infection in the absence of exacerbation of disease [132, 139]. Intranasal administration of these monoclonal antibodies resulted in clearance of the virus from the lungs [25].

8.3.2.2.1 IgG MONOCLONAL ANTIBODIES

Johnson and coworkers developed a humanised IgG monoclonal antibody against RSV, designated MEDI-493 [65]. MEDI-493 was initially administered intravenously in prematurely (≤ 35 weeks gestation) born infants younger than 6 months of age and infants with BPD younger than 24 months of age in various dosages and compared to 0.9% saline as a placebo [128]. Few adverse events were observed. In a consecutive study, intramuscularly administered MEDI-493 yielded similar results [117]. Monthly intramuscular injections of 15 mg/kg resulted in mean serum concentrations above 40 µg which in animal models is associated with a 99% reduction in pulmonary virus titer.

The clinical efficacy of MEDI-493 (also known as palivizumab or Synagis®) was investigated by the Impact-RSV Study Group [63]. A randomized double blind, placebo controlled trial was performed in 139 centers in the United States, Canada and United Kingdom which included 1502 children with a history of premature birth ≤ 35 weeks and younger than 6 months of age, and infants with BPD younger than 24 months of age. They received either 15 mg/kg palivizumab or placebo every 30 days. The results showed a 55% reduction in RSV hospitalisations for all infants. No deaths were attributable to the use of palivizumab. The reduction in RSV hospitalisations was highly significant in the group of preterm infants (RSV hospitalisation rate of 1.9% for the palivizumab group versus 8.1% for the placebo group indicating a 78% reduction, $p < 0.001$) but borderline significant in the BPD group (RSV hospitalisation rate of 7.9% for the palivizumab group versus 12.8% for the placebo group indicating a 39% reduction, $p = 0.038$). The number of RSV hospitalisation days per 100 children (36.4 days in the palivizumab group and 62.6 days in the placebo group, $p < 0.001$) and days with increased supplemental oxygen per 100 children (30.3 days in the Palivizumab group and 50.6 in the placebo group, $p < 0.001$) were significantly decreased in the palivizumab group. The number of ICU admissions was not significantly different between the treatment and the control group (1.3% in the palivizumab group and 1.3% in the placebo group, $p = 0.23$). The number of infants requiring mechanical ventilation was also not altered by palivizumab. Few adverse events were reported: mainly fever and injection site reaction were observed. The mean serum concentrations of palivizumab were above 40 µg/ml.

The American Academy of Pediatrics recently recommended the use of palivizumab according to the following indications [1]:

- children with chronic lung disease (CLD) younger than 2 years who have required medical therapy for their CLD within 6 months before the RSV-season
- children younger than 12 months of age at the start of the RSV season born after

a gestational age of 28 weeks or less
- children younger than 6 months of age at the start of the RSV season born after a gestational age between 28 and 32 weeks
- children younger than 6 months of age at the start of the RSV season born after a gestational age between 32 and 35 weeks with risk factors such as a predisposition for respiratory complications, day care attendance, surgery for a non-cyanotic congenital heart defect, exposure to tobacco smoke and living a long distance from the hospital.

Palivizumab has several advantages over RSV-IVIG. It can easily be administered and does not interfere with normal vaccinations. However, one major disadvantage is the high costs. Annual costs are approximately $4500 per patient per season [89]. The number-need-to-treat (NNT) with palivizumab in order to prevent one RSV hospitalisation was calculated at 17 regardless the underlying disease state. This results in a total cost of more than $77.000,- to prevent one hospital admission. In addition, the observation that palivizumab appears to be less effective in the highest risk group is of concern. The NNT for patients with BPD and for patients born before 32 weeks of gestation can be calculated from the IMpact study and is 20 and 21 respectively. The costs in order to prevent one hospital admission would then be approximately $90.000 and $95.000. This indicates that palivizumab is more effective in infants born with a gestational age of 32 – 35 weeks (NNT 12) [89].

Local guidelines for palivizumab have to be developed since the efficicacy of palivizumab will depend on the number of prematurely born infants, rehospitalisation rates and duration of hospitalisation [53]. For instance, rehospitalisation rates in several studies for preterm born infants vary between 3% and 25% [53]. These aspects have to be taken into account when the economical effects of palivizumab are investigated in order to balance the efficacy of prophylaxis in the local high risk population and the costs.

Other IgG-monoclonal antibodies are currently under study and/or tested in healthy volunteers and may be future candidates for phase III studies in high-risk infants with infections by RSV [24, 34].

8.3.2.2.2 IgA MONOCLONAL ANTIBODIES

HNK20 is an IgA-monoclonal antibody directed against two subunits of the F glycoprotein. HNK20 significantly reduces the virus titer in both upper and lower respiratory tract of mice and Rhesus monkeys [141]. Thus far, results of human studies HNK20 have not been published.

8.4 Conclusions

8.4.1 Treatment of RSV infection

Currently no effective therapy is available for children with infections by RSV. Treatment of RSV infection therefore remains mainly symptomatic. Because of their lack of efficacy corticosteroids, vitamin A, interferon and antibiotics are not indicated in patients with infections by RSV. Although the routine use of bronchodilators is not yet supported by evidence-based data, one may consider its use in the individual patient. Routine use of ribavirin as a therapy in high risk children with infections by RSV is not indicated any more. Administration of this drug may only be considered in the patient with a T-cell immunodeficiency.

8.4.2 Prevention of RSV infection

Research on the prevention of RSV infections has focused on active and passive immunisation. Interesting candidate vaccines include the recombinant vaccine BB2GNa, the subunit vaccine PFP-2 and cold passaged temperature sensitive (*cpts*) candidate vaccines such as *cpts*-248, *cpts*248/404 and *cpts*530/1009. The recombinant vaccine BB2GNa and the subunit vaccine PFP-2 seem to be effective against both RSV-A and RSV-B leading to adequate levels of neutralising antibody titers in both animal and human studies. Further studies are necessary to investigate whether or not these candidate vaccines are truly effective and can be safely applied in infants.

Passive immunisation may be achieved by the administration of RSV immune globulin (Respigam®) and monoclonal antibodies (palivizumab/Synagis®) in infants born prematurely (≤ 32 weeks) and children with BPD. RSV-IVIG should be used with great caution given the high costs and difficulties with the intravenous route of administration. RSV-IVIG is contra-indicated in patients with (cyanotic) CHD. Palivizumab is the most recent novel compound licensed for prevention of infections by RSV. However, large costs are involved when palivizumab is routinely used in high-risk infants. Hence, cost-effectiveness analyses which need to take into account local and regional differences in RSV hospitalisation rates in the high risk population are necessary in order to determine the appropriate indications for the use of palivizumab. Furthermore, a European trial on the efficacy of palivizumab may be considered since the severity of infections by RSV may differ between the USA and Europe [8]. Using data from the USA in European cost-effectiveness analysis may be misleading.

8.5 References

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CHAPTER 9

LONG TERM EFFECTS OF RESPIRATORY SYNCYTIAL VIRUS BRONCHIOLITIS IN INFANCY: A QUANTITATIVE REVIEW

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ACTA PEDIATR
(ACCEPTED)

Abstract

One of the major questions regarding long-term side effects of bronchiolitis by respiratory syncytial virus (RSV) is whether or not it induces asthma in later life. In this quantitative review the data of 10 controlled studies are analysed.

Methods Follow-up studies of RSV bronchiolitis published between January 1978 and December 1998 were identified through a MEDLINE search. Studies were selected if *(i)* postnatal age at the time of the inital illness was below 12 months, *(ii)* all children were hospitalised for RSV bronchiolitis, *(iii)* the diagnosis RSV was virologically confirmed in all cases, and *(iv)* a control group was used.

Results Six studies met all selection criteria. Up to five years of follow-up after RSV bronchiolitis in infancy, 40% of children reported wheezing as compared to only 11% in the control group ($p < 0.001$). Between five and ten years of follow-up 22% of the bronchiolitis group reported wheezing against 10% of the control group ($p = 0.19$) The incidence of recurrent wheezing as defined by three or more wheezing episodes also decreased with increasing years of follow-up: at five or more years of follow-up the difference between the RSV group and the control group was no longer significant. Furthermore, the presence of either a personal and/or a family history of either atopy and/or asthma did not differ between the two groups. *Conclusions* Wheezing is common after RSV bronchiolitis in infancy. It may persist for for more than 5 years of follow-up. However, no significant difference between the RSV-bronchiolitis and the control group was observed regarding *recurrent* wheezing by five years of follow-up. No significant difference between the RSV bronchiolitis and the control group were found regarding a personal history of atopy, a family history of atopy and/or asthma. Therefore it seems unlikely that RSV bronchiolitis is a cause of atopic asthma in later life.

9.1 Introduction

In the late 50's, Wittig *et al* described an association between viral bronchiolitis in infancy and asthma in later life. Over the past decades, numerous reports were published investigating a possible relationship. These studies suggested that acute viral bronchiolitis may lead to wheezing during childhood [1-6]. Respiratory syncytial virus (RSV) is the most important pathogen in respiratory tract infection in infants and young children and the major causative agent in bronchiolitis. By the age of two years, approximately 80% to 90% of all children has experienced at least one episode of infection by RSV. Most of these infections are characterised by a mild disease course. However, 0.5% to 2% of children develop severe lower respiratory tract infection requiring hospitalisation [7-9].

Wheezing in early childhood is a heterogenous condition [10]. A distinction can be made between wheezing as a result of atopic asthma (when there is a personal history of atopy and often a family history of asthma and/or atopy) and non-atopic transient wheezing [10]. The term asthma represents a condition characterised by chronic airway inflammation leading to repeated wheezing episodes, shortness of breath and cough which is (partially) reversible [1,11].

Studies investigating the possible relationship between RSV bronchiolitis in infancy and wheezing and asthma in later life cannot be easily compared because of methodological differences. However, the existence of a relationship between RSV bronchiolitis in infancy and recurrent wheezing or asthma in later life may have great implications for patient management and for further research. We therefore performed a quantitative review to determine *(i)* the course of wheezing, *(ii)* the incidence of atopy in children with RSV bronchiolitis and *(iii)* the association between a preceding RSV bronchiolitis and the development of recurrent wheezing in children hospitalised for RSV bronchiolitis in infancy.

9.2 Materials and methods

9.2.1 Study protocol

Studies were selected if they met the following criteria: (*i*) the age of all patients was below 12 months at the time of the initial illness, (*ii*) children were hospitalised for RSV bronchiolitis, (*iii*) the diagnosis RSV infection was virologically confirmed, and (*iv*) a control group was used. The inclusion criteria were defined prior to the screening of the literature.

9.2.2 Definitions

The definition of wheezing was adapted from the authors of included studies. It was documentated by history taking using a questionnaire ("a whistling sound coming from the chest"). Recurrent wheezing was defined by at least three episodes of wheezing verified by a physician, or the use of bronchodilators in the year preceeding the follow-up study.

Atopy was defined by the presence of allergic rhinitis and/or urticaria and/or rashes in response to food, inhalation antigens, or drugs and/or an elevated IgE level. A positive skin prick test (SPT) was defined as positive when the diameter of the wheal was 3 mm or greater. Outcomes of interest were wheezing, recurrent wheezing, personal history of atopy, positive SPT's and a family history of asthma and/or atopy.

9.2.3 Data sources

Controlled follow-up studies of bronchiolitis due to respiratory syncytial virus through a MEDLINE search of articles published from January 1978 to December 1998 using PubMed (http://www.ncbi.nlm.nhi.gov) were identified. Both prospective and retrospective case control studies and longitudinal cohort studies were included. Combinations of the keyword RSV and following keywords were used: bronchiolitis, wheezing, recurrent wheezing, asthma, atopy, bronchial hyperreactivity, follow-up and long term effects. These keywords were combined with viral bronchiolitis in a second search. Additional studies were identified from review articles and references of the retrieved articles.

9.2.4 Analysis

Summary data were collected from tabular and written presentation in the publications after a thorough review. When a study was designed as a longitudinal follow-up study, only the last year of follow-up year was included for analysis. This applied to Pullan '82 (last two years of follow-up) [13], Sigurs '95 (third year of follow-up) [15], Osundwa '93 (second year of follow-up) [16], and for Sly '89 (fifth year of follow-up) [18].

For each individual study, Odds Ratios (OR) and their 95% confidence intervals (95% CI) and p - values using the χ^2 test were calculated. It was attempted to combine the results of the individual studies. With the Mantel-Haenzel method a pooled OR and it's 95% CI was calculated. If a p - value was ≤ 0.05, it was accepted as statistically significant. The odds ratio and its 95% confidence interval were logarithmically

Table 9.1 *Descriptive data on study population and study design*

	First author (reference)									
	Sims (12)	Mok (16)	Pullan (13)	Murray (17)	Sigurs (14)	Osundwa (15)	Welliver (18)	Sly (19)	Welliver (20)	Sigurs (21)
Year of publicati	1978	1982	1982	1992	1995	1993	1993	1989	1986	1994
Total number of RSV patients	35	100	130	73	47	70	43	48	38	34
Study type	P, CC	P, CC	P, CC	P, CC	P, LC	R, CC	P, C	P, C	P, C	P,C
Control group (N)	35[1]	200[1]	111[1]	73[1]	93[2]	70[1]	--	--	--	--
Duration of follow-up (year)	8	7	10	5.5	1,2 and 3	2	7-8	1,2,3,4 and 5	4	2
Bronchiolitis as first wheezing episode	Unknown	Unknown	Unknown	Yes	Unknown	Unknown				
Data on:										
wheezing	+	+	+	+	+		+		+	+
asthma	+	+	+		+	+	+	+		+
atopy	+[4]	+	+	+[3]	+		+[3]			+[3]
famil history of asthma and/or atopy	+	+	+		+		+			

P prospective; R retrospective; CC case control; LC longitudinal cohort; C case only

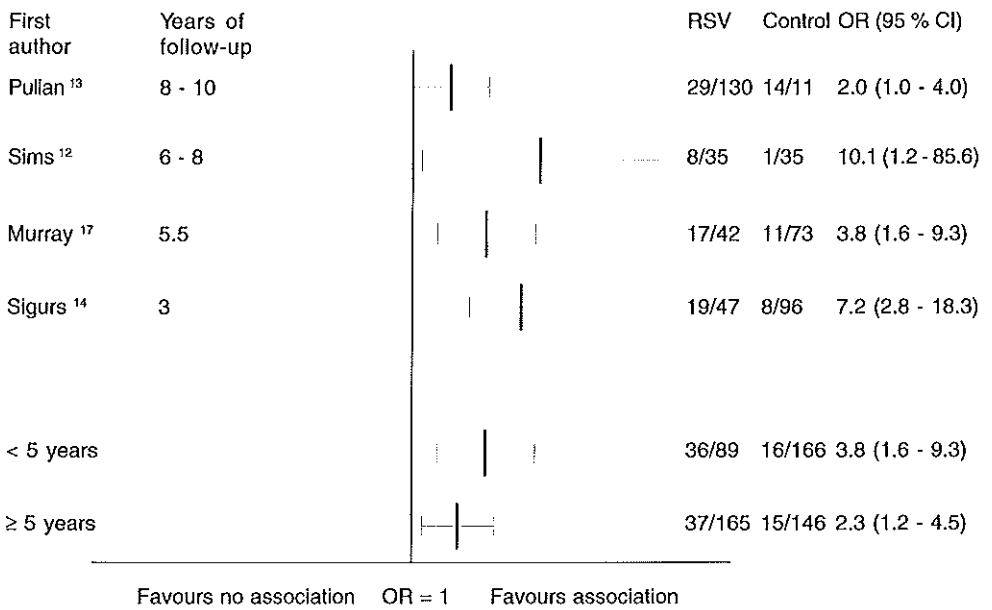
[1] *Included the use of a retrospective control group*

[2] *Prospectively followed control group*

[3] *Skin prick test performed*

[4] *Results on a personal history of atopy, family history of atopy and/or asthma and number of positive skin prick tests were reported in a later study which was used for analysis (Sims et al, Br Med J 1981, pages 2086 - 2088)*

Figure 9.1 *Relationship between RSV bronchiolitis in infancy and wheezing during (early) childhood. The thick vertical line represents the odds ratio, the small vertical lines represent the 95% confidence interval of the odds ratio. For the pooled OR, no heterogeneity was observed. YFU: years of follow-up.*



transformed for graphic presentation.

Heterogeneity between studies was tested to determine whether one pooled OR might be assumed for all studies. Homogeneity between studies is assumed when the p – value exceeds 0.05.

9.3 Results

9.3.1 Study identification

From 30 identified publications investigating the outcome of RSV bronchiolitis, only four articles fully met the selection criteria (Table 9.1) [12-15]. These were included for analysis. Two studies included also infants with viral agents other than RSV, but performed a subanalysis in which only the RSV (+) infants were compared with the controls [16,17]. These numbers were used for analysis. Four other studies satisfied all criteria except for the presence of a control group [18-21].

Data on the number of children with one or more of the outcomes of interest studied were easily retrievable. Some difficulties occurred for wheezing either one or two years before follow-up with the studies by Sims and Murray [12,17]. For Sims *et al*, the number of children with current wheezing was calculated by 18 (cumulative wheezing) minus 10 not experiencing wheezing episodes in the last two years before follow-up. The

number of RSV positive patients with wheezing in the study by Murray *et al* was calculated as follows: 39.5% for 42 RSV positive children available for analysis equals 17. For *recurrent wheezing*, only the number on asthma was used from the study by Sigurs *et al* since recurrent wheezing in their study yielded it not being verified by a physician [14].

9.3.2 Wheezing

The percentage of children with wheezing after RSV bronchiolitis in infancy declined with increasing years of follow-up. In the first five years after RSV bronchiolitis in infancy, 40% of the children experienced wheezing against 11% in the control group (OR 3.8, 95% CI 1.6 – 9.3 ($p < 0.001$)). At five to ten years of follow-up, the odds ratio decreased to 2.3 (95% CI 1.2 – 4.5, $p = 0.004$) (Figure 9.1). For the pooled OR's, no heterogeneity was observed.

All numbers presented represented "current" wheezing: wheezing episodes either one or two years before follow-up.

9.3.3 Recurrent wheezing

When investigating the percentage of patients with recurrent wheezing, in the first

Figure 9.2 *Relationship between RSV bronchiolitis in infancy and recurrent wheezing during (early) childhood. The thick vertical line represents the odds ratio, the small vertical lines represent the 95% confidence interval of the odds ratio. For the pooled OR, no heterogeneity was observed. YFU: years of follow-up.*

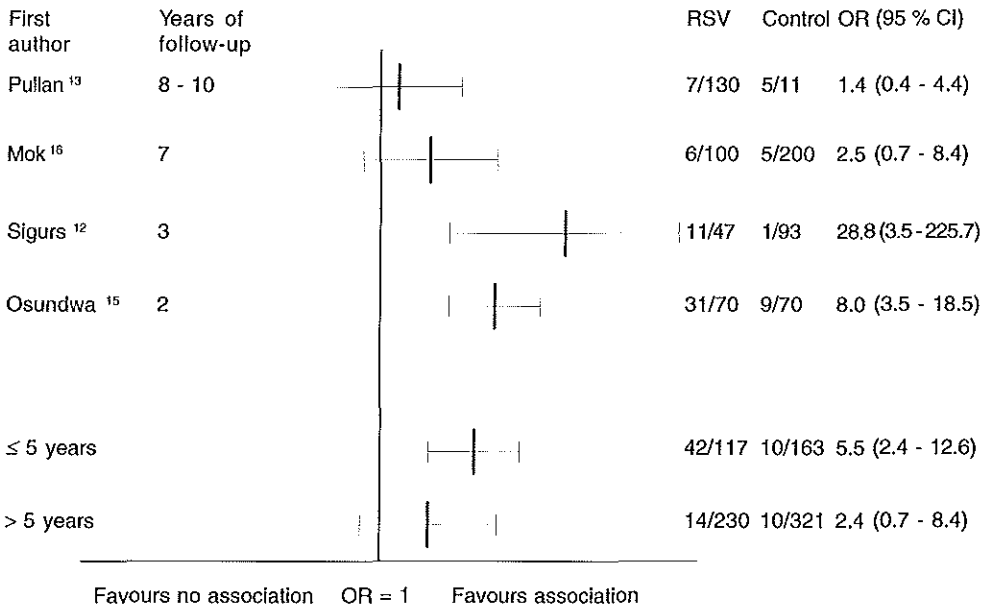


Figure 9.3 *Relationship between RSV bronchiolitis in infancy and a personal history of atopy. The thick vertical line represents the odds ratio, the small vertical lines represent the 95% confidence interval of the odds ratio. For the pooled OR, no heterogeneity was observed. YFU: years of follow-up.*

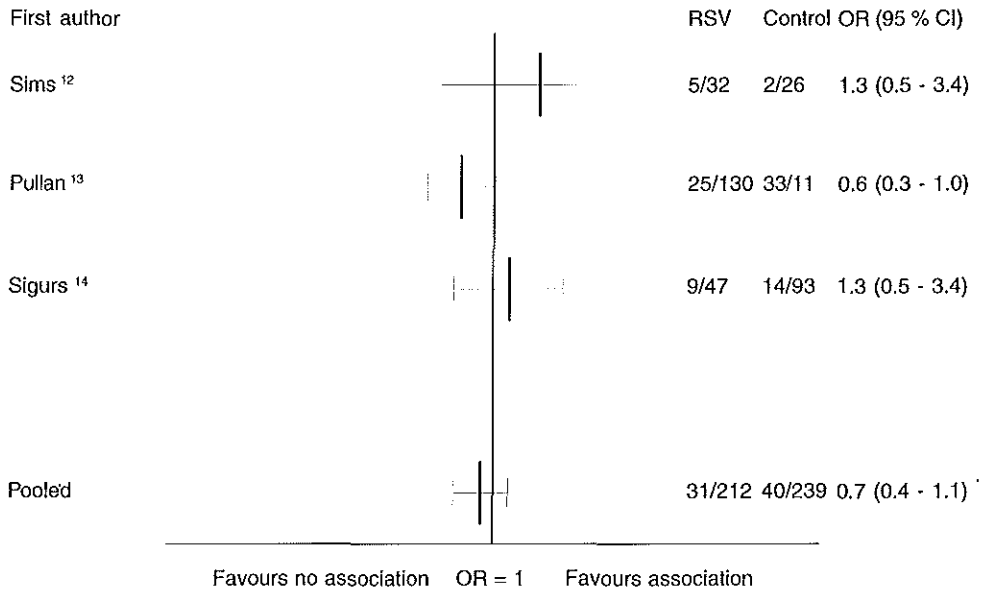
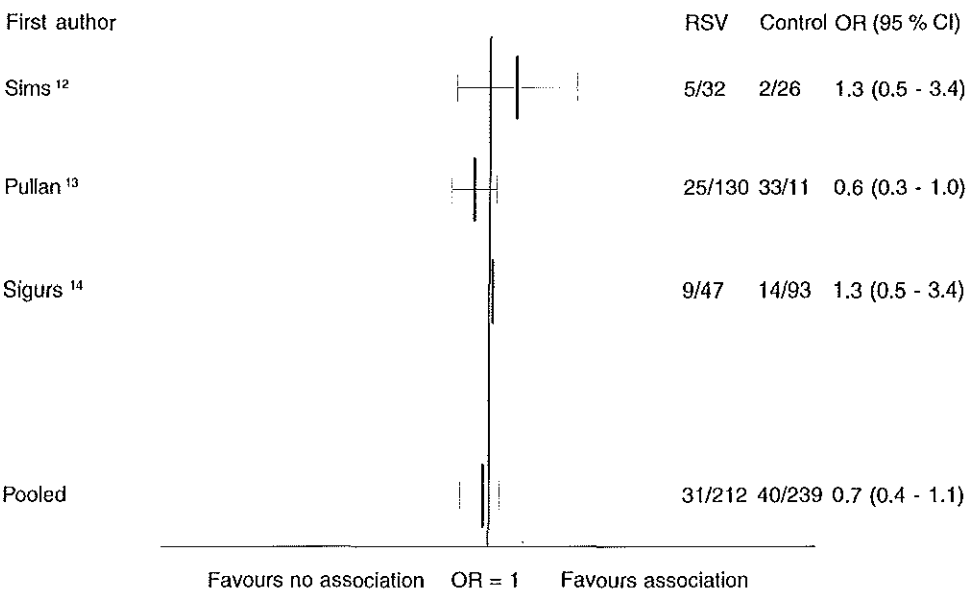


Figure 9.4 *Relationship between RSV bronchiolitis in infancy and a positive family history of asthma and/or atopy. The thick vertical line represents the odds ratio, the small vertical lines represent the 95% confidence interval of the odds ratio. For the pooled OR, no heterogeneity was observed. YFU: years of follow-up.*



five years after the initial illness a significant difference was observed between the RSV bronchiolitis and the control group (45% in the bronchiolitis group as compared to 13% for the control group, $p < 0.001$). By five years of follow-up or more, there was no clear difference in the prevalence of recurrent wheezing in the RSV bronchiolitis group and in the control group (Figure 9.2). For the pooled OR's, no heterogeneity was observed.

Numbers from the studies by Osundwa and Sigurs represent cumulative numbers at two or three years of follow-up. The numbers from the studies by Mok and Pullan represent current recurrent wheezing at seven and eight to ten years of follow-up.

9.3.3 Personal atopy

Figures 9.3 describes the relation between RSV bronchiolitis and personal history of atopy at the time of follow-up. Four studies investigated the relationship between the presence of a personal history of atopy and RSV bronchiolitis [12-14,16]. One group found no relationship between RSV bronchiolitis and atopy, but gave no actual figures [16]. The study by Sigurs *et al* investigated the number of infants with atopy prior to the bronchiolitis, whereas the studies by Pullan *et al* and by Sims *et al* defined a personal history of atopy by either atopic dermatitis, allergic rhinitis or a history of allergy up to the age of eight or ten years.

The occurrence of a personal history of atopy was not significantly different between the RSV bronchiolitis and the control group (15% as compared to 12% for the control group).

No conclusion could be drawn about the relationship between RSV bronchiolitis in infancy and the number of positive SPT's at follow-up since the homogeneity test yielded a p – value < 0.05. This indicated that the results of the studies demonstrated heterogeneity and thus cannot be combined.

9.3.4 Family history of asthma and atopy

Significant differences between the RSV bronchiolitis and the control group were neither observed with respect to the presence of a family history of atopy and/or asthma (Figure 9.4). For all pooled OR's, no heterogeneity was observed.

A secondary analysis was performed, adding four studies lacking a control group but fullfilling all other criteria [18-21]. The outcome of this analysis was comparable (data not shown) with regard to wheezing, recurrent wheezing, personal history of atopy and a positive family history of atopy and/or asthma.

9.4 Discussion

In this quantitative review the course and development of (recurrent) wheezing was described in infants with RSV. Wheezing episodes persisted for five to ten years of follow-up. The percentage of patients with *recurrent* wheezing decreased with increasing years of follow-up. After five years of follow-up there was no significant difference between the RSV bronchiolitis group and the control group. No significant difference between the RSV bronchiolitis and the control group regarding a personal history of atopy was observed. No association between RSV bronchiolitis in infancy and a family history of asthma and/or atopy was observed.

Several studies investigated the relationship between RSV bronchiolitis and long term effects such as wheezing and the development of recurrent wheezing or asthma in later life. Unfortunately, methodological differences, such as differences in duration of follow-up and lack of a control group, between various studies complicated the performance of a straightforward meta-analysis. Therefore, the term quantitative was introduced so that the prevalence of lower respiratory tract symptoms after RSV bronchiolitis in infancy and trends in outcome parameters over time could be examined by this method [22].

This analysis demonstrated that wheezing is a feature after RSV bronchiolitis in infancy and that it may persist for five or more years of follow-up. At eight to ten years of follow-up a non-significant difference between the RSV bronchiolitis and the control group was observed by one group [13]. The cause of wheezing due to RSV bronchiolitis may be multifactorial. Many factors may explain wheezing after RSV bronchiolitis as was extensively reviewed by Balfour-Lynn [23]. It remains to be determined which of these factors are responsible for wheezing after viral bronchiolitis in infancy.

The prevalence of recurrent wheezing after RSV bronchiolitis in infancy decreased with increasing years of follow-up. At five years of follow-up, no clear difference between cases and controls was found. Recurrent wheezing was defined as three or more episodes of wheezing verified by a physician or the use of bronchodilators in the year preceding follow-up. This definition may also apply for the definition of asthma. However, there is increasing debate about the definition of asthma [24]. It is understood that there are different wheezing phenotypes. In some children wheezing during infancy resolves in the early school years. These wheezing episodes do not seem to be associated with increased IgE levels. In other children with persistent wheezing or children in whom the respiratory symptoms occur for the first time around five years of age, these symptoms may be the first manifestation of asthma. This wheezing may be an IgE mediated reaction [10,24-26].

Children are predisposed to develop "classic" asthma – to be called atopic asthma

- when there are signs of atopy - defined by a positive history for eczema, increased serum IgE levels, eosinophilia and/or positive skin prick tests - and a genetic predisposition (a positive family history for asthma) [11,25]. This analysis demonstrated that there was no difference between the RSV bronchiolitis group and the control group regarding the presence of a personal history of atopy and that there was no clear association between RSV bronchiolitis in infancy and a positive family history of atopy and/or asthma. This indicates that children with a history of RSV bronchiolitis in infancy are not more predisposed to develop atopic asthma than children without such a history.

It has been observed that RSV infection may result in allergic sensitization to food or inhalant allergens. The study by Sigurs *et al* provided evidence for an increased allergic sensitization as measured by an increased number of positive SPT's to inhalant allergens [14]. A later study by this group demonstrated that this allergic sensitization was associated with higher anti-RSV IgA titres [27]. Further evidence for allergic sensitization was found in the animal model. A possible explanation may be the induction of allergen-specific IL-2 responsiveness of lymphocytes after RSV infection [28]. However, others provided conflicting results [29]. It was concluded that only during the first year of life RSV infections slightly promoted aeroallergen sensitization in non-hospitalised children, though no clinical manifestations were apparent. Nonetheless, this possible allergic sensitization of children with a history of RSV bronchiolitis during infancy requires further study.

This study provides further evidence to the long term morbidity associated with RSV. However, many aspects on RSV remains unknown. For instance, it is still unknown as to how RSV bronchiolitis during infancy causes wheezing during (early) childhood. Identification of inflammatory markers produced during the acute illness that may be found in children with recurrent wheezing may provide some elucidating evidence. Furthermore, just four studies from all 30 identified used a control group. This indicates that in fact there are just a few studies from which the long time prognosis of RSV bronchiolitis in infancy can be estimated.

The interpretation of this analysis may be complicated by several factors. Studies used for analysis are heterogenous and cases and controls may vary in different characteristics. It was attempted to minimize these differences by defining a number of inclusion criteria prior to the screening of the literature. Furthermore, only a small number of suitable studies were identified using many different definitions of outcome variables. The study by Sigurs *et al* was the only one to use a prospectively followed control group whereas the others used a retrospective control group. The use of a prospectively followed control group is preferable, because then the true development of lower respiratory tract symptoms can be estimated.

Since is was not possible to extract clear definitions of wheezing from the included studies, the definition used by the various authors was adapted in order to avoid the elimination of studies that could contribute to this quantitative review. Characteristics not available for this analysis since they were not (frequently) mentioned in included studies may be confounding variables. For instance, socio-economic status and parental smoking are risk factors for wheezing in later life [30,31]. Only published studies retrieved through a MEDLINE/PubMed search were used in this quantitative review. This may implicate a publication bias. It may be possible that negative studies on the relationship between RSV bronchiolitis and asthma in later life may have not been published.

The analysis was restricted to infants hospitalised for RSV bronchiolitis, since it was assumed that only cases of bronchiolitis with lower respiratory tract involvement demonstrate pulmonary problems which may result in long term morbidity. Infection in children with a mild disease course is most likely restricted to upper respiratory tract involvement only.

In conclusion, the outcome of this quantative review demonstrated that children continued to wheeze after RSV bronchiolitis in the first year of life. This wheezing persisted for up to five years of follow-up or more. However, after five years of follow-up, no difference in recurrent wheezing between the RSV bronchiolitis and the control group was observed. No clear relationship was found between RSV bronchiolitis and the presence of a personal history of atopy. Also, no significant association was found regarding a family history of atopy or asthma between the bronchiolitis and control group. Therefore it seems unlikely that the occurrence of RSV bronchiolitis in the first year of life is a cause of atopic asthma in later life.

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CHAPTER 10

SUMMARY AND CONCLUSIONS

10.1 Introduction

Respiratory syncytial virus (RSV) is the most important cause of viral respiratory tract infections in infants and young children. This thesis describes the results of epidemiological and clinical studies on infections by RSV. The investigations were carried out at the Sophia Children's Hospital (SCH), Rotterdam, a university hospital with secondary and tertiary care functions. Between 1992 – 1995, 266 children younger than 12 months of age diagnosed with virologically confirmed RSV infections ("Rotterdam database") were included in the reports described in this thesis. Thirty-four of these 266 children had a nosocomial RSV infection. 185 of the 232 children with community acquired infections by RSV were admitted to the SCH. The data collection included demographical, clinical and laboratory parameters, as well as characteristics of hospitalisation.

In **Chapters 1 and 2,** pathophysiological and clinical characteristics of RSV infection are reviewed. The pathogenesis of RSV infection is complex and influenced by both humoral and cellular immune responses. Studies in humans and animals have shown that both CD8 positive cytotoxic T cells as well as CD4 positive T helper cells are involved in the elimination of RSV. Two major subsets can be identified within the T helper cells: T_H-1 and T_H-2 cells. T_H-1 cells take part in the cellular immune response whereas T_H-2 cells are associated with the production of immunoglobulins and the induction of eosinophilia. Both subsets are activated in children with RSV infections. In addition, T-cells may also contribute to the development of immunopathology. Other cells that are involved in the immune response include neutrophils, eosinophils and mast cells with subsequent production of various inflammatory mediators. A hypothetical model of the complex pathogenesis of RSV infections is presented in Chapter 2 (page 28).

Further research on the pathophysiology of RSV infections is required in order to provide a better understanding of the disease and its clinical presentations. A longitudinal cohort study may reveal whether infants are prone for a T_H 1- response or a T_H 2- response once infection by RSV occurs. It would be interesting to investigate whether a genetic predisposition for either one of these two responses may be identified. Genetic polymorphisms have already been elucidated as important determinants in the susceptibility and severity of other infectious diseases. For instance, a polymorphism in the promotor region of the gene encoding for plasminogen activator inhibitor (PAI)-1 has recently been associated with outcome in children with meningococcal sepsis [1]. Polymorphisms in cytokine genes or other components involved in the extent of the inflammatory process in infections by RSV may co-determine the outcome of disease.

10.2 Subgroup variation in infections by RSV

Two subtypes of RSV can be identified by the use of monoclonal antibodies on the basis of differences in the G-protein. These subtypes, A and B, can cocirculate but may also circulate independently from each other during an epidemic. Several investigators have studied the presence of a possible relationship between the clinical severity of infections by RSV and the subtype. The results of the Rotterdam study on the relationship between the clinical severity of infections by RSV and subtype A or B are described in **Chapter 3**. Between 1992 and 1995, the majority of RSV infections (64.7%) was caused by subtype A whereas 35.3% of the infections was due to subtype B. We could not detect a relationship between the clinical severity of infections by RSV and the subtype. One may therefore conclude that new vaccines need to protect against subtype A as well as against subtype B. The F-protein may be used as a target for further vaccines because of the great degree of homology of this protein in the two subtypes.

The results of our study are compared with those of 20 other studies (Table 10.1). In eleven studies no relationship was detected between the clinical severity and subtype A or B, whereas the results of eight investigations suggest that subtype A is associated

Table 10.1 *Studies on the relationship between clinical severity of infections by RSV and subtype A or B*

Study number	First author (reference)	Study period	Country	Number of isolates (A/B)	Methods[1]	Results[2]
1	Hendry [42]	82 – 82	USA	51/34	R/ND	=
2	Mufson [43]	81 – 86	USA	155/51	R/D	A
3	Hendry [44]	83 – 85	USA	125/92	R/ND	=
4	Taylor [45]	74 – 88	UK	328/169	R/D	A
5	Monto [46]	65 – 71	USA	64/29	R/ND	=
6	McConnochie [47]	76 – 81	USA	95/62	P/D/MV	A
7	McIntosh [48]	85 – 87	Austrialia	337/107	P/D	=
8	Hall [49]	89 – 91	USA	858/351	R/D	A
9	Wilson [50]	87 – 88	Canada	45/24	R/D	=
10	Wang [51]	93	Canada	102/250	P/D/MV	=
11	Salomon [52]	87 – 88	Argentina	23/93	RND	A
12	Tsutsumi [53]	80 – 89	Japan	77/52	R/ND	=
13	Heikkinen [54]	87 – 92	Finland	192/134	P/D	A
14	Russi [55]	85 – 87	Uruguay	19/22	P/D	=
15	Walsh [56]	88 – 91	USA	134/131	P/D/MV	A
16	Mufson [57]	78 – 88	USA	319/86	R/D	A
17	Stark [58]	85 – 88	USA	176/21	R/D	=
18	Mlinaric [59]	91	USA	8/10	R/D	=
19	Straliotto [60]	90	Brazil	22/7	R/D	B
20	Hornsleth [61]	93 – 95	Danmark	31/54	P/D	B
21	Kneyber	92 – 95	Netherlands	150/82	R/D/MV	=

[1] *R indicates retrospective study, P indicates prospective study; ND indicates that the indicators for severity and/or a scoring model were not described, whereas D indicates that they were documented*

[2] *A indicates subtype A more severe, whereas B indicates subtype B more severe and "=" indicates no difference in severity*

with a more severe disease course. For instance, studies 1, 8 and 13 found more cases of bronchiolitis, a larger number of patients with intensive care admission and an increased risk for wheezing and oxygen therapy associated with subtype A. Studies 7, 10 and 17 did not find a difference between subtype A and B in important estimates of disease severity such as mechanical ventilation and supplemental oxygen. Study 14 did not provide details on mechanical ventilation or oxygen supplementation but also found no relationship between the clinical severity and subtype A or B.

In our opinion, conclusions about a possible relationship between severity of infection and RSV subtype A or B may only be drawn based on objective outcome variables and multivariate analysis. Confounding variables may be identified and reliable conclusions about independent variables may be detected. Only four studies including the one presented in Chapter 3 used multivariate analysis. Two of these studies (6 and 15) which were performed in the same population concluded that subtype A was associated with more severe disease. In study 10 and in our study no relationship could be found between subtype A or B and severity of RSV infections.

The results of meta-analyses on the relationship between the severity of infections by RSV and subtypes A or B are presented in Figures 10.1 and 10.2. Studies were included if they met the following criteria: (*i*) age below 24 months of age and (*ii*) virological confirmation of the RSV infection (Table 10.2). Outcome parameters of interest were oxygen supplementation and mechanical ventilation. The Mantel-Haenzel pooled odds ratio and 95% confidence interval for oxygen supplementation was 1.2 (0.8 – 1.8)

Table 10.2 *Characteristics of studies included for meta-analyses on the relationship between the clinical severity of RSV infections and the subtype*

First author	Number of isolates (A/B)	Population	Age of inclusion	Outcomes studied[1]
Walsh [57]	134/131	Hospital	< 24 months	MV
Straliotto [61]	22/7	Hospital	< 12 months	O_2, MV
Stark [59]	101/16	Hospital	Unknown	O_2, MV
McConnochie [43]	95/62	Hospital	< 24 months	MV
McIntosh [42]	337/107	Hospital	Unknown	O_2, MV
Heikkinen [55]	192/134	Hospital and outpatient	Unknown	O_2, MV
Taylor [46]	328/169	Unknown	Unknown	O_2, MV
Kneyber	150/82	Hospital and outpatient	< 12 months	O_2, MV

[1] *O_2 oxygen supplementation, MV mechanical ventilation*

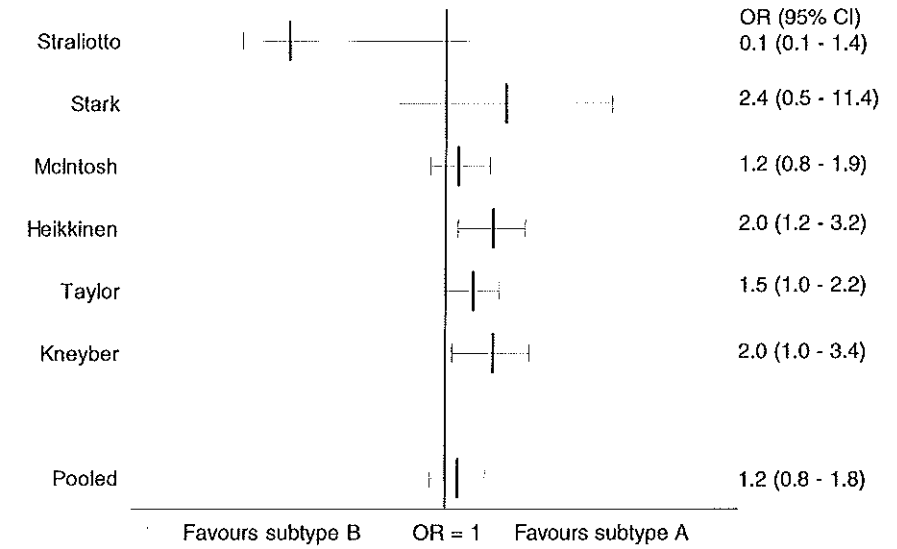


Figure 10.1 *Results from a meta-analysis on the relation between clinical severity of RSV infection and subtype with oxygen supplementation as outcome parameter. OR Odds ratio. For references of the included studies, see Table 10.2*

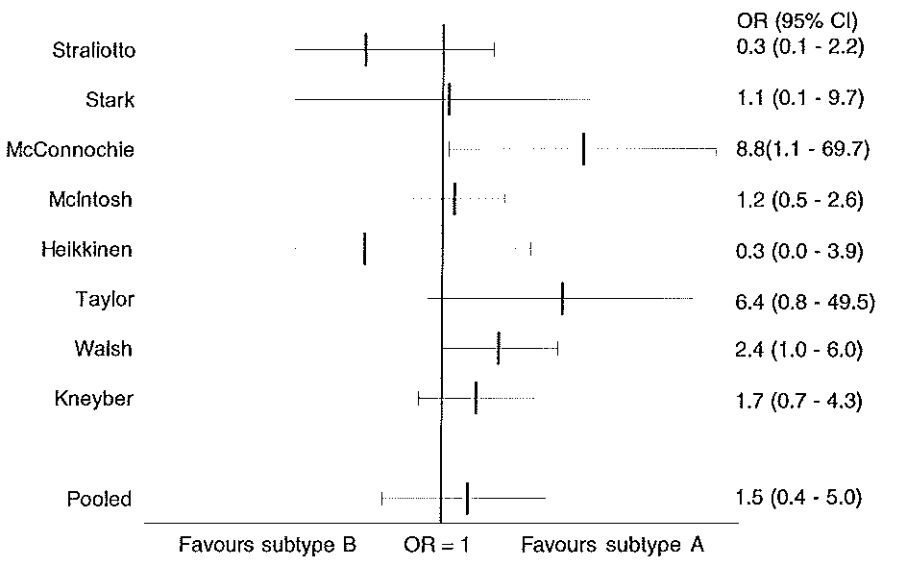


Figure 10.2 *Results from a meta-analysis on the relation between clinical severity of RSV infection and subtype with mechanical ventilation as outcome parameter. OR Odds ratio. For references of the included studies, see Table 10.2*

(Figure 10.1). This indicates the absence of an association between oxygen supplementation and subtype A or B. The same conclusion could be drawn with respect to mechanical ventilation (OR 1.5, 95% CI 0.4 – 5.0, Figure 10.2). No heterogeneity was observed for both analyses.

The data presented above conclude that there is no relationship between the clinical

severity of infections by RSV and subtype A or B.

10.3 Apnoea in infections by RSV

Apnoea may be the first sign of disease in children with infections by RSV. The results of a study on risk factors for RSV associated apnoea in a hospitalised population, on risk factors for mechanical ventilation once RSV associated apnoea occurred and the risk for recurrent apnoea are presented in **Chapter 4**. Apnoea at admission was defined as a history of respiratory arrest with cyanosis, or an observation in the paediatric emergency room of respiratory arrest for a period longer than 20 seconds and/or bradycardia with accompanying cyanosis or oxygen desaturation below 90%.

Twenty-one percent of the patients hospitalised with RSV infections presented with apnoea. These children were significantly younger, had a lower body temperature, higher pCO_2 and more often atelectasis on chest radiographs than patients without apnoea. Apnoea at admission increased the risk for recurrent apnoea by almost 12 times. The occurrence of recurrent apnoea increased the risk for mechanical ventilation by nearly seven times. The great majority of recurrent apnoea occurred within 24 to 48 hours after admission. Logistic regression analysis showed that young age - especially in infants below two months - was the only independent predictor for RSV associated apnoea. Hence, cardiorespiratory monitoring of infants with RSV infection seems warranted during the first 48 hours of admission. However, well-defined criteria are necessary in order to prevent excessive use of monitoring and the induction of unnecessary parental fears associated with monitoring [2]. We conclude from our study that apnoea at admission and age $\leq$ 2 months necessitate cardiorespiratory monitoring in children with infections by RSV. Further support for the age criterium is provided by the observation that in our database all children with a first episode of apnoea occurring during hospitalisation were $\leq$ 2 months of age. Previous studies suggest that prematurely born infants - especially those born with a gestational age of below 32 weeks – are also candidates for cardiorespiratory monitoring [3-6]. Table 10.3 summarizes the number of patients without apnoea at admission that have to be monitored (the Number Needed to Treat, NNT) in order to detect one patient with a first episode of apnoea occurring during hospitalisation. The NNT is calculated as follows: 100/[Number of apnoea's in a certain population (%) – number of apnoea's one allows to miss, which is usually 0%]. For instance, in our study population the number of first apnoea during hospitalisation is 6 (4.1%). The NNT would then be 100/4.1% equals 25 patients. This indicates that 25 patients have to undergo cardiorespiratory monitoring in order to detect one patient with a first episode of RSV associated apnoea during hospitalisation. Two from the six patients with a first RSV

Table 10.3 *Efficacy of cardiorespiratory monitoring in respiratory syncytial virus infection*

Number of infants with RSV associated apnoea at admission	38/185
Number of infants with RSV associated apnoea during hospitalisation	24/185
Number of infants with apnoea at admission and during hospitalisation	18/185
Number of infants with first episode of apnoea occutring during hospitalisation	6/185

Monitor indication	Apnoea/ group size	Missed apnoea's when indication applied	NNT
All infants without apnoe at admission	6/147	0	25
Age ≤2 months and/or gestational age ≤ 32 weeks	6/115	0	19
Age ≤2 months and/or gestational age ≤ 34 weeks	6/118	0	20
Age ≤2 months and/or gestational age ≤ 37 weeks	6/126	0	21
Age ≤2 months	6/101	0	17
Gestational age ≤ 37 weeks	3/50	3	17
Gestational age ≤ 34 weeks	2/21	3	11

NNT: number-needed-to-treat

associated apnoea during hospitalisation required mechanical ventilation.

The cause of RSV associated apnoea remains unclear. In our study, only young age was independently associated with RSV associated apnoea. We therefore suggest that underlying immaturity of the respiratory system is a major risk factor in RSV associated apnoea. Severe lower respiratory tract illness does not predispose to RSV associated apnoea. RSV may alter laryngeal chemoreceptors as has been described in humans [7]. Apnoea may then occur when these receptors are stimulated. Pickens and co-workers described a mixed character of RSV-associated apnoea (being both obstructive and of central origin) [8]. They observed that apnoea coincided with swallowing and coughing and therefore suggested that airway protective reflexes may suppres the ventilatory drive.

Continuation of the characterisation of RSV associated apnoea with polysomnographic studies in a hospitalised population is warranted to identify type, pathophysiology and recovery from RSV associated apnoea. Also, a future large multi-center study is necessary to provide additional data on criteria for cardiorespiratory monitoring in children with infections by RSV.

10.4 Chest radiography in infections by RSV

Chest radiographs are performed in approximately 70% of hospitalised children with infections by RSV [9]. Hyperinflation, atelectasis and pulmonary infiltrates are commonly seen. However, no correlation has been found between roentgenographic findings and severity of illness [10]. The results of a study of predictors for a normal chest radiograph are presented in **Chapter 5**. Using these predictors, a subgroup of patients with a high probability for a normal chest radiograph – defined as the abscence of atelectasis, hyperinflation or pulmonary infiltrate – may be identified. In 202 patients, a chest radiograph was performed. Seventy-five of these children had a normal chest radiograph. The prediction model for a normal chest radiograph comprised increasing age, increasing birthweight, presence of rhinitis, abscence of retractions and increasing SaO_2. The area under the Receiver Operating Characteristic (ROC)-curve was 0.80, indicating good discrimination of the model between normal and abnormal chest radiographs. The prediction model was transferred into a score chart to facilitate assessment of the probability for a normal chest radiograph.

What are the indications for chest radiography in infections by RSV? Dawson and co-workers suggested that intensive care admission, unexpected deterioration in the patient's condition or the presence of underlying pulmonary or cardiac disorders were good indicators to perform a chest radiograph [11]. Eriksson and co-workers suggested that the presence of atelectasis is strongly suggestive of a bacterial superinfection by *Streptococcus pneumoniae* and *Haemophilus influenzae*. Careful examination of their data leads to the conclusion that these indications apply especially to patients older than 12 months of age [12]. We suggest to perform chest radiographs in patients with a sum score less than 52 as can be calculated by our prediction model, and in patients with unexpected clinical deterioration. With these indications, the number of chest radiographs may be significantly reduced. Prospective validation is required in order to establish true applicability and generalizability of our model to other populations.

In university and general hospitals in The Netherlands chest radiographs are often performed in children with infections by RSV. However, the results of our study show that performance of a chest radiograph in children with infections by RSV is only indicated in selected patients.

10.5 Nosocomial infections by RSV

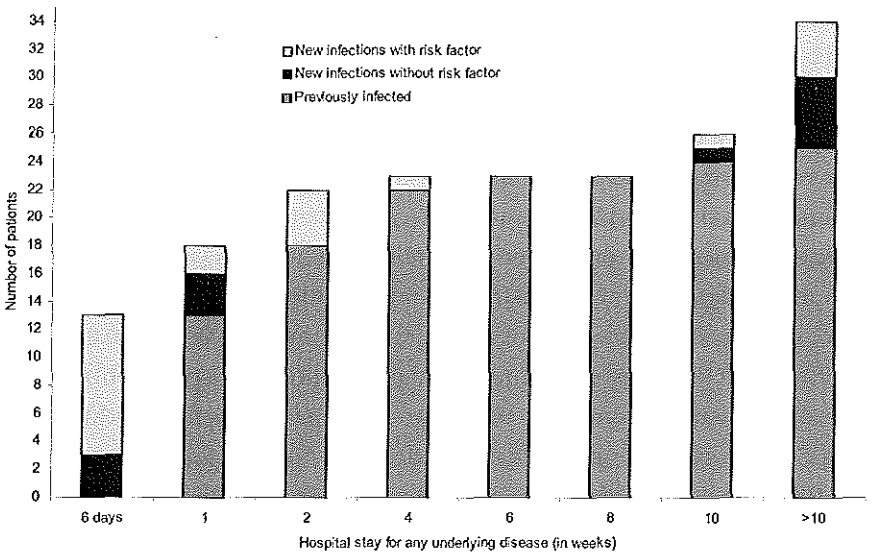
RSV has been identified as an important nosocomial pathogen leading to an increased morbidity and mortality especially in very young children. Approximately

4% to 7% of all hospitalised children will develop a nosocomial infection by RSV [15]. The increased morbidity is reflected by an increase in the occurrence of bronchiolitis, prolonged hospitalisation and an increased mortality among patients with underlying diseases such as congenital heart disease (CHD), bronchopulmonary dysplasia and prematurity [13,14]. Especially children with CHD have a significantly increased risk for mortality after acquisition of a nosocomial infection by RSV [15].

Chapter 6 presents the results from a study on nosocomial infections by RSV (NA-RSV). The number of NA-RSV was investigated and characteristics of NA-RSV were compared with community acquired RSV infections (CA-RSV). The percentage of nosocomial infections by RSV (number of children with NA-RSV divided by the number of hospitalised children younger than 12 months of age during the RSV seasons in the three year study period) was 2.7%. The number of nosocomial infections by RSV decreased over the three years. This is probably a result of the appropriate use of guidelines on prevention of nosocomial infections by RSV in our hospital. These guidelines include handwashing by hospital personnel and the isolation of RSV-positive patients or cohort-caring of these patients. The risk for the acquisition of a nosocomial infection by RSV was correlated with the duration of hospitalisation for any underlying disease (Figure 10.3).

The clinical picture of patients with nosocomial infections by RSV was comparable

Figure 10.3 *Number of nosocomial infections by RSV compared with the duration of hospitalisation for any underlying disease*



with that of community acquired infections by RSV. The significant differences in mean birthweight and impaired feeding between NA-RSV and CA-RSV were in our opinion a reflection of the specific population of our hospital and not due to a more severe disease course in patients with NA-RSV. Other characteristics of the clinical presentation and hospitalisation were comparable between children with NA-RSV and those with CA-RSV. All four children with BPD and NA-RSV required mechanical ventilation. We conclude that with the exception of patients with BPD, nosocomial RSV infections in this study are not characterised by a more severe disease course when compared to community acquired RSV infections.

10.6 Duration of hospitalisation in children with infections by RSV

Estimation of disease severity in infections by RSV may aid physicians to decide when to initiate specific therapies, or identifies patients prone for a severe disease course so that they may be subjects for preventive approaches. Moler and Ohmit have recently developed a prediction model for the duration of hospitalisation in RSV infection. The model showed good discrimination as assessed by the area under the ROC-curve [16]. They hypothesized that prolonged hospitalisation ≥ 7 days would discriminate the most severe infections with a mean duration of hospitalisation in their database of four days. The model predicted a hospital stay ≥ 7 days by weight, presence of congenital heart disease, presence of failure to thrive, prematurity, presence of BPD, presence of other pulmonary diseases, miscellaneous conditions, early mechanical ventilation and early ribavirin. Their model was validated on our database set. The results of this study are described in **Chapter 6**. The validation demonstrated that the model could not adequately predict a prolonged hospital stay ≥ 7 days as determined by the combination of a high sensitivity and a low false positive rate (FPR). Furthermore, it was argued that for our institution a cut-off at seven days would not necessarily provide a good discrimination between less severe and severe infections given the two-fold longer duration of hospitalisation for our patients compared with the Michigan-database. A good discrimination may be better represented by the median. Thus, a prediction model for prolonged hospitalisation ≥ 9 days (which represented the median in our database) was constructed. Independent predictors were weight and need for oxygen supplementation. The ROC of the model was low (0.65). When validated on a single RSV season, the prediction of duration of hospitalisation proved inadequate. We conclude from these data that a reliable prediction of duration of hospitalisation is currently impossible and hence may not adequately represent the severity of RSV infections. This observation has important implications since many multicenter trials used duration of hospitalisation as an outcome parameter for clinical

response.

10.7 Treatment and prevention of infections by RSV

10.7.1 Treatment of infections by RSV

Research on the treatment of RSV infections has focused on the use of corticosteroids, bronchodilators and antiviral drugs.

Corticosteroids would seem attractive agents in the treatment of infections by RSV because of the increasing evidence that RSV associated disease is an immunopathological process. However, various studies failed to demonstrate efficacy of corticosteroids in the treatment of infections by RSV [17]. Furthermore, corticosteroids do not prevent recurrent wheezing after RSV bronchiolitis. Hence, the use of corticosteroids is not warranted.

Bronchodilators are commonly used in patients with infections by RSV due to similarity in clinical features between RSV bronchiolitis and asthma. The results from several studies are conflicting [18]. There is no conclusive evidence to support the efficacy of bronchodilators [19]. At this moment, it would seem that an initial treatment with bronchodilators may be considered in the individual patient. When the patient shows clinical improvement, continuation of bronchodilators may be considered.

Ribavirin currently remains the only antiviral drug for RSV infection. Controversy about its use has arisen since promising results from early studies performed in the '80s were contradicted by results from recent, methodologically more sound studies. High costs and possible teratogenic and carcinogenic side-effects further contribute to this controversy. Hence, ribavirin is seldomly used nowadays [20]. Prescription of ribavirin should only be considered in the immunocompromised patient (T-cell immunodeficiency) with a severe disease course. RSV immune globulin should not be used for the treatment of RSV infections [18]. The occurrence of secondary bacterial infections is low [21]. As a consequence, therapeutic trials failed to identify any benefit of antibiotics [22,23]. Therefore, routine use of antibiotics is not warranted.

10.7.2 Prevention of infections by RSV

Prevention of RSV infections may be achieved through active and passive immunisation. Vaccination trials with formalin-inactived, alum precipitated RSV vaccin carried out in the '60s were complicated by serious side-effects. Vaccinees developed severe disease with increased mortality rates on subsequent challenge by wild type RSV. Nevertheless, continued research on novel vaccines has yielded several (potential)

candidate vaccines such a BBG2Na, Purified Fusion Protein (PFP)-2 vaccine and attenuated cold-passaged, temperature sensitive mutants such as cpts-248, cpts-248/404 and cpts-530/1009. The immunogenicity of these candidate vaccines has been demonstrated in the animal model [24-26]. PFP-2 has been tested in humans and was found not to reduce RSV associated respiratory tract illness [27,28]. Cpts-vaccines were effective in children over 6 months of age, but caused nasal congestion in one and two month old infants [29]. The development of a vaccine against RSV may be hindered by the presence of maternal antibodies which may depress the infant's immune response, and the low ability of young infants to respond to viral surface glycoproteins.

Research on passive immunisation has produced two licensed drugs: RSV immune globulin (RSV-IVIG) and humanised monoclonal antibodies directed against the F protein (Palivizumab). The PREVENT-study included prematurely born infants and children with BPD for treatment with RSV-IVIG or placebo. The study demonstrated a 41% reduction of RSV-hospitalisation and a 53% reduction in total days of RSV hospitalisation associated with RSV-IVIG [30]. The use of Palivizumab in children younger than 24 months of age with BPD and infants younger than six months of age with a history of a premature birth (≤ 35 weeks) resulted in a 55% reduction in RSV hospitalisations in a high risk population (a 78% reduction for the prematurely born infants and a 39% reduction in the BPD group). A reduction of 47% of RSV-hospitalisations was observed for infants born before a gestational age of 32 weeks, whereas a reduction of 80% was observed for infants with a gestational age between 32 and 35 weeks. This latter group constitutes the greatest portion of prematurely born infants with a gestational age below 37 weeks. The use of palivizumab did not alter the number of infants requiring mechanical ventilation.

Indications for administration of Palivizumab may be influenced by regional or (inter)national differences in RSV associated disease burden. These differences include number of prematurely born infants with or without chronic lung disease, severity of RSV associated disease and rehospitalisation rate for high-risk patients. Rehospitalisation rates may vary between 3% and 25% [31]. Limitations of a widespread use of RSV-IVIG include high costs and intravenous administration. A benefit of Palivizumab over RSV-IVIG is the intramuscularly route of administration. The use of RSV-IVIG seems to be outdated with the availability of Palivizumab. However, cost-effectiveness analyses have to be performed in order to generate appropiate indications for the use of Palivizumab. These analyses may result in the appropiate Number Needed to Treat acceptable for patients and doctors in relation to the costs and burden of treatment.

10.8 Long term effects of RSV infection

Over the past decades, the presence of a relationship between RSV infection in early childhood and asthma in later life has been studied extensively. This issue is still subject of intensive debate. Two hypotheses prevail: *(i)* RSV bronchiolitis and asthma are two different entities with comparable clinical manifestations, and *(ii)* RSV bronchiolitis identifies the predisposed host who subsequently develops asthma after bronchiolitis in early childhood. We performed a quantitative review to investigate the association between a preceding RSV bronchiolitis and the development of recurrent wheezing in children in time (**Chapter 9**). Controlled studies in hospitalised patients younger than 12 months of age with virologically proven RSV bronchiolitis were identified through a Medline search of prospective and retrospective case control or longitudinal cohort studies published between January 1978 and December 1998. Thirthy publications were identified, of which only six met all inclusion criteria.

The odds ratio for wheezing decreased from 3.8 (95% CI 1.6 – 9.3, $p < 0.001$) in the first five years after RSV bronchiolitis in infancy to 2.3 (95% CI 1.2 – 4.5, $p = 0.004$) at five to ten years of follow-up. Recurrent wheezing – defined as three or more episodes of wheezing verified by a physician, or the use of bronchodilators in the year preceeding follow-up – was not associated with RSV bronchiolitis in infancy at five or more years of follow-up (OR 2.4, 95% CI 0.7 – 8.4). No association between RSV bronchiolitis and the occurrence of a personal history of atopy or a family history of atopy and/or asthma was found. Hence, it was concluded that the occurrence of RSV bronchiolitis in the first year of life is not a cause of atopic asthma in later life.

The results of our quantitive review are supported by an increased understanding of the pathogenesis of RSV infections. Characteristics of asthma and bronchiolitis are presented in Table 10.4 . Whereas in asthma CD4 positive T cells are the predominant lymphocytes, an abundance of CD8 positive T cells is observed in RSV bronchiolitis [32]. Also, eosinophils are important in the pathogenesis of asthma, but these cells may

Table 10.4 Comparison between RSV bronchiolitis and asthma

	RSV bronchiolitis	Asthma
T - cells	CD8 (+)	CD4 (+)
T-helper subset	Both T_H-1 and T_H-2	T_H-2
Role of eosinophils	+	+++
Personal history of atopy	-	+++
Family history of atopy and/or asthma	-	+

only play a contributary role in the pathogenesis of RSV bronchiolitis [33]. Furthermore, whereas in asthma a dysbalance in the T_H-1/T_H-2 paradigm is found in favour of T_H-2 lymphocytes, RSV also induces a T_H-1 response.

IgE plays an important role in the pathogenesis of asthma [34]. However, serum IgE is not elevated after RSV bronchiolitis [35]. Although RSV-specific IgE may be found in nasopharyngeal secretions or BAL samples during the acute infection, the appearance of RSV-specific IgE was found not to be related to recurrent wheezing at the age of seven to eight years [36]. A similarity between the two clinical entities is the observation of increased concentrations of eosinophilic cationic protein (ECP). However, the measurement of ECP during RSV bronchiolitis does not contribute to the prediction whether children will develop bronchial obstruction or asthma [37]. Other viral infections may also induce increased ECP levels [38].

The pulmonary function after RSV bronchiolitis including FVC, FRC, inspiratory airway resistance, peak expiratory flow resistance and respiratory system compliance, were not decreased when compared to controls [39,40]. However, it was found that infants who develop bronchiolitis had evidence for pre-existing diminished lung function [41]. These pre-existing abnormalities in lung function may function as a risk factor for wheezing associated lower respiratory tract illnesses during the first year of life [34,42].

Children with RSV bronchiolitis should be followed for at least ten years in order to investigate which inflammatory markers that are produced during the acute illness - measured in nasopharyngeal washings – may be responsible for (recurrent) wheezing.

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CHAPTER 11

SAMENVATTING EN CONCLUSIES

11.1 Introductie

Het respiratoir syncytieel virus (RSV) is de belangrijkste verwekker van virale luchtweginfecties bij zuigelingen en jonge kinderen. In dit proefschrift worden de resultaten van klinisch-epidemiologisch onderzoek naar RSV infecties bij jonge kinderen beschreven. Het onderzoek werd verricht in het Sophia Kinderziekenhuis te Rotterdam, een academisch kinderziekenhuis met zowel secundaire als tertiaire zorg. De studie periode liep van 1992 tot en met 1996 ("Rotterdam database"). 266 kinderen jonger dan 12 maanden werden gediagnostiseerd met een virologisch bewezen RSV infectie. Van deze kinderen hadden 34 een nosocomiale RSV infectie. Van de 232 kinderen met een buiten het ziekenhuis verworven RSV infectie werden er 185 opgenomen in het ziekenhuis. Demografische en klinische parameters werden verkregen uit de medische status.

In **Hoofdstuk 1 en 2** worden de klinische en pathofysiologische aspecten van RSV infecties beschreven. De pathogenese van een RSV infectie is complex en wordt beïnvloed door zowel de humorale als de cellulaire immuniteit. Uit onderzoek verricht bij mens en dier volgen aanwijzingen dat zowel CD8 positive cytotoxische T lymfocyten als CD4 positive T helper cellen betrokken zijn bij het elimineren van RSV. Binnen deze T helper cellen kunnen twee subgroepen onderscheiden worden: T_H-1 en T_H-2 cellen. T_H-1 zijn betrokken bij de cellulaire immuunrespons, T_H-2 cellen zijn betrokken bij de produktie van antilichamen en de inductie van eosinofilie. Beide subgroepen zijn geactiveerd in kinderen met een infectie door RSV. De T-cellen kunnen echter ook bijdragen aan het ontstaan van immuunpathologie. Neutrofiele granulocyten, eosinofiele granulocyten en mestcellen worden eveneens geactiveerd en produceren ontstekingsmediatoren. Een hypothetische grafische weergave van de complexe pathogenese in RSV infecties kan gevonden worden in hoofdstuk 2 (pagina 28).

Om het ziekteproces beter te kunnen begrijpen is uitgebreid onderzoek naar de pathofysiologie gewenst. Een longitudinale cohort studie kan inzicht verschaffen welke kinderen genetisch gepredisponeerd zijn voor een T_H-1 respons en welke voor een T_H-2 respons wanneer een RSV infectie zich voordoet. Zogenaamde genetische polymorfismen zijn belangrijk in het bepalen van gastheer vatbaarheid en ernst van een infectieziekte. Zo is recent aangetoond dat een polymorfisme in de promotorregio van het gen dat codeert voor Plasminogeen Activitor Inhibitor (PAI)-1 de overleving van kinderen met een meningococcensepsis bepaalt [1]. Polymorfismen in genen welke voor cytokines coderen of voor andere onderdelen van het ontstekingsproces in RSV infecties bepalen mogelijk mede de ernst van het beloop van de ziekte.

11.2 Subtype variatie

Met behulp van monoclonale antilichamen kunnen twee subtypes van RSV onderscheiden worden: A en B. Antigene variatie in met name het G-eiwit is hiervoor verantwoordelijk. Beide subtypes circuleren zowel onafhankelijk als gezamenlijk gedurende een epidemie. De ontdekking van de twee subtypes heeft geleid tot diverse studies waarin een mogelijke relatie tussen subtype van het virus en de klinische ernst van de infectie werd onderzocht. De resultaten van een studie naar een dergelijke relatie worden beschreven in **Hoofdstuk 3.** Subtype A kwam vaker voor dan subtype B (subtype A 150 (64.7%) vs 82 (35.3%) subtype B). Wij vonden geen relatie tussen de klinische ernst van de RSV infectie en subtype. Derhalve concludeerden wij dat nieuwe vaccins dienen te beschermen tegen zowel subtype A als subtype B. Het F-eiwit is voor een zeer groot deel gelijk in beide subtypen. Zodoende komt dit eiwit in aanmerking als antigene determinant van een vaccin.

De resultaten van onze studie en van twintig andere studies zijn samengevat in Tabel 10.1. Uit elf onderzoekingen bleek geen verschil in de klinische ernst tussen beide subtypen. Daarentegen bleek uit acht studies dat een ernstiger verloop van de infectie geassocieerd is met subtype A. Zo bleek bijvoorbeeld in de studies 1, 8 en 13 dat

Tabel 11.1 *Studies naar de relatie tussen klinische ernst van een RSV infectie en subtype A of B*

Studie nummer	Eerste auteur (referentie)	Studie periode	Land	Aantal isolaten A/B)	Methoden[1]	Resultaten[2]
1	Hendry [43]	82 – 82	VS	51/34	R/NB	=
2	Mufson [44]	81 – 86	VS	155/51	R/B	A
3	Hendry [45]	83 – 85	VS	125/92	R/NB	=
4	Taylor [46]	74 – 88	GB	328/169	R/B	A
5	Monto [47]	65 – 71	VS	64/29	R/NB	=
6	McConnochie [48]	76 – 81	VS	95/62	P/B/MV	A
7	McIntosh [49]	85 – 87	Australië	337/107	P/B	=
8	Hall [50]	89 – 91	VS	858/351	R/B	A
9	Wilson [51]	87 – 88	Canada	45/24	R/B	=
10	Wang [52]	93	Canada	102/250	P/B/MV	=
11	Salomon [53]	87 88	Argentinië	23/93	R/NB	A
12	Tsutsumi [54]	80 89	Japan	77/52	R/NB	=
13	Heikkinen [55]	87 92	Finland	192/134	P/B	A
14	Russi [56]	85 87	Uruguay	19/22	P/B	=
15	Walsh [57]	88 91	VS	134/131	P/B/MV	A
16	Mufson [58]	78 88	VS	319/86	R/B	A
17	Stark [59]	85 88	VS	176/21	R/B	=
18	Mlinaric [60]	91	VS	8/10	R/B	=
19	Straliotto [61]	90	Brazilië	22/7	R/B	B
20	Hornsleth [62]	93 – 95	Denemarken	31/54	P/B	B
21	Kneyber	92 – 95	Nederland	150/82	R/B/MV	=

[1] *R: retrospectieve studie, P: prospectieve studie; NB geeft aan dat de parameters voor ernst van ziekte en/of eens scoringsmodel niet zijn beschreven, terwijl B weergeeft dat deze parameters wel zijn beschreven*

[2] *A geeft aan dat subtype A met een ernstiger ziektebeloop was geassocieerd, terwijl B aangeeft dat dit voor subtype B geldt en "=" geeft aan dat er geen verschil in klinische ernst tussen beide subtypes werd gevonden*

bronchiolitis, intensive care opname, wheezing en zuurstofbehoefte significant vaker geassocieerd waren met subtype A. In de studies 7, 10 en 17 werd géén verschil gevonden in belangrijke parameters van ziekte zoals beademing en zuurstofsuppletie tussen patiënten met een infectie door subtype A of subtype B. De auteurs van studie 14 beschreven niet in getal de bestudeerde parameters van ziekte. Er werd geen verschil in de klinische ernst tussen subtype A en subtype B gevonden.

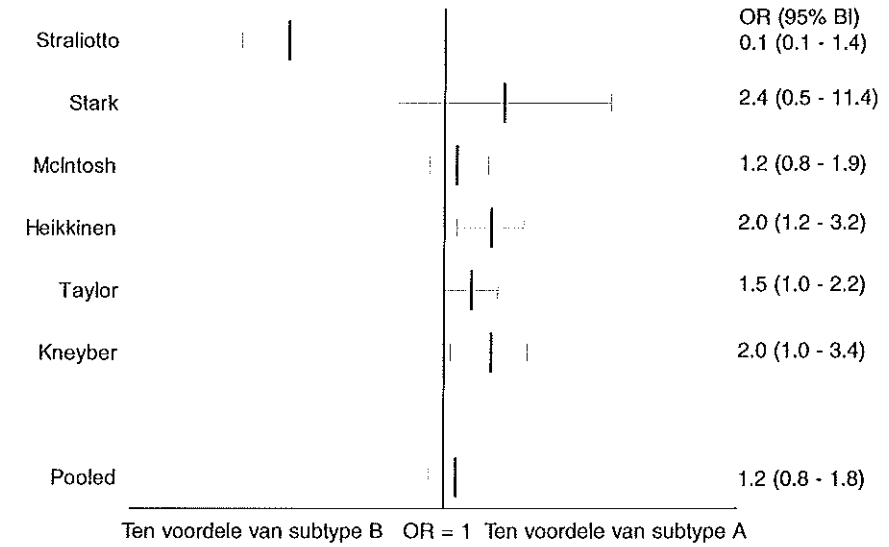
Ons inziens kunnen conclusies over een mogelijke relatie tussen RSV subtype en ernst van een infectie alleen getrokken worden indien objectieve parameters van ziekte geanalyseerd zijn. Daarnaast dient een multivariate analyse toegepast te worden om het effect van verstorende variablen uit te sluiten. Slechts vier studies hebben een dergelijke analyse toegepast, waaronder onze studie beschreven in hoofdstuk 3. Twee van deze vier studies (studie 6 en 15) waren gebaseerd op dezelfde patiëntenpopulatie. Hierin werd geconcludeerd dat subtype A geassocieerd is met een ernstiger verloop. In studie 10 en onze eigen studie bleek er geen relatie aanwezig te zijn tussen subtypes A en B en ernst van de RSV infectie.

De resultaten van de meta-analyses naar de relatie tussen klinische ernst van een RSV infectie en het subtype van het virus worden weergegeven in figuur 11.1 en figuur 11.2. Studies werden geïncludeerd indien aan de volgende voorwaarden was voldaan: (*i*) leeftijd jonger dan 24 maanden en (*ii*) de diagnose RSV infectie was virologisch bevestigd (tabel 10.2). De uitkomst parameters waren zuurstofsuppletie en beademing. De Mantel-Haenzel gepoolde odds ratio en het 95% betrouwbaarheidsinterval voor zuurstofsuppletie bedroeg 1.2 (0.8 – 1.8) (figuur 10.1). Dit houdt in dat er geen verband bestaat tussen RSV subtype en de behoefte tot zuurstofsuppletie. Een vergelijkbare

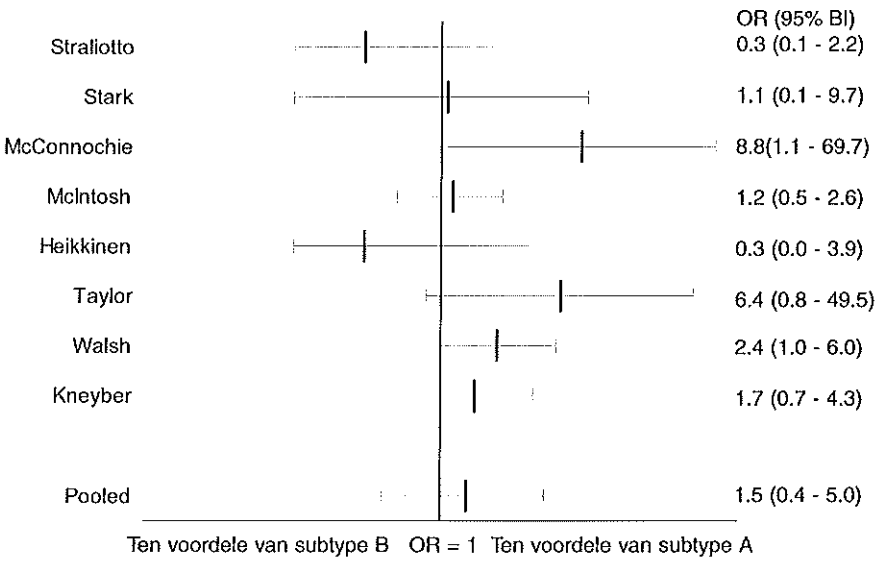
Tabel 11.2 *Karakteristieken van de studie geïncludeerd voor de meta-analyses naar de relatie tussen klinische ernst van een RSV infectie en subtype A of B*

Eerste auteur	Aantal isolaten (A/B)	Populatie	Leeftijdscriterium	Uitkomsten [1]
Walsh	134/131	Klinisch	< 24 maanden	B
Stratiotto	22/7	Klinisch	< 12 maanden	O_2, B
Stark	101/16	Klinisch	Onbekend	O_2, B
McConnochie	95/62	Klinisch	< 24 maanden	B
McIntosh	337/107	Klinisch	Onbekend	O_2, B
Heikkinen	192/134	Klinisch en poliklinisch	Onbekend	O_2, B
Taylor	328/169	Onbekend	Onbekend	O_2, B
Kneyber	150/82	Klinisch en poliklinisch	< 12 maanden	O_2, B

[1] *B: beademing, O_2: zuurstofsuppletie*



Figuur 11.1 *Resultaten van de meta-analyse naar de relatie tussen de klinische ernst van een RSV infectie en subtype A of B, met als uitkomst parameter zuurstofsuppletie. OR: Odds ratio. Voor referenties van de gebruikte studies wordt verwezen naar Tabel 11.2*



Figuur 11.2 *Resultaten van de meta-analyse naar de relatie tussen de klinische ernst van een RSV infectie en subtype A of B, met als uitkomst parameter beademing. OR Odds ratio. Voor referenties van de gebruikte studies wordt verwezen naar Tabel 11.2*

conclusie kon worden getrokken met betrekking tot beademing (OR 1.5, 95% CI 0.4 – 5.0, figuur 10.2). In beide analyses werd geen heterogeniteit gevonden.

De hierboven genoemde gegevens geven sterke aanwijzingen op dat er **géén** relatie bestaat tussen klinische ernst van een RSV infectie en subtype A of B.

11.3 Apnoe in RSV infectie

Het optreden van een apnoe – gedefiniëerd als een ademstilstand met een tijdsduur van 20 seconden of langer en/of een bradycardie met cyanose – kan het eerste teken zijn van een RSV infectie. De resultaten van een studie naar risicofactoren voor apnoe bij een RSV infectie in een gehospitaliseerde populatie, het risico op beademing indien een apnoe optreedt en het risico op een recidief apnoe worden beschreven in **Hoofdstuk 4**. Van alle opgenomen kinderen presenteerde 21% zich met een apnoe. Deze kinderen waren significant jonger, hadden een lagere lichaamstemperatuur, hogere pCO_2 en vaker atelectase op de thoraxfoto vergeleken met kinderen zonder een apnoe. Een apnoe bij binnenkomst confereerde een bijna twaalfvoudig verhoogd risico op een recidief apnoe. Het optreden van een recidief apnoe resulteerde in een ruim zesmaal verhoogd risico op beademing. Het meerendeel van de apnoes trad 24 tot 48 uur na ziekenhuisopname op. Na logistische regressie analyse bleek leeftijd, met name ≤ 2 maanden, de enige onafhankelijke risicofactor voor het optreden van een apnoe bij patiënten met een RSV infectie. Cardiorespiratoire bewaking van deze kinderen is dan ook geïndiceerd gedurende de eerste 48 uur na ziekenhuisopname. Om medische overconsumptie te voorkomen dienen strikte criteria voor bewaking opgesteld te worden [2]. Uit onze studie kan worden geconcludeerd worden dat dit de volgende criteria betreft: apnoe bij binnenkomst in het ziekenhuis en/of leeftijd ≤ 2 maanden. Ook bleek dat de kinderen die zich niet presenteerden met een apnoe, doch in het ziekenhuis hun eerste episode van apnoe doormaakten, allen ≤ 2 maanden oud waren. Op basis van de literatuur kan ook prematuriteit (met name een zwangerschapsduur ≤ 32 weken) als criterium toegevoegd worden [3-6]. In tabel 11.3 wordt het aantal kinderen aangegeven dat cardiorespiratoir bewaakt dient te worden om een eerste apnoe tijdens ziekenhuisopname (dus geen apnoe bij binnenkomst) te voorkomen. Deze Number Needed to Treat (NNT) analyse wordt als volgt berekend: 100/[het aantal apnoes in een bepaalde populatie (%) – het aantal apnoes dat men zich veroorlooft om te missen, (dit is 0%)]. Zes (4.1%) kinderen maakten in het ziekenhuis hun eerste apnoe door. De NNT bedraagt dan 100/4.1% is 25 kinderen. Dit betekent dat 25 kinderen cardiorespiratoir bewaakt dienen te worden om bij één patient een eerste apnoe te ontdekken. Twee van de zes patiënten met een eerste apnoe tijdens ziekenhuisopname behoefden beademing.

De oorzaak van een apnoe bij patiënten met een RSV infectie is vooralsnog onduidelijk. Gesteld kan worden dat onderliggende onrijpheid van het ademhalingscentrum een belangrijke risicofactor vormt voor een apnoe tijdens een RSV infectie. Dit gegeven is gebaseerd op onze bevinding dat alléén leeftijd onafhankelijk geassocieerd is met een apnoe. Een ernstig verloop van de infectie geeft

Tabel 11.3 *Effect van cardiorespiratoire bewaking in RSV infecties*

Aantal kinderen met een RSV infectie met een apnoe bij opname	38/185
Aantal kinderen met een RSV infectie met een apnoe gedurende ziekenhuisopname	24/185
Aantal kinderen met een RSV infectie met een apnoe bij opname en gedurende opname	18/185
Aantal kinderen met een RSV infectie en alleen een apnoe gedurende ziekenhuisopname	6/185

Monitor indicatie	Apnoe/ Groeps-grootte	Aantal gemiste apnoes wanneer indicatie niet toegepast	NNT
Alle kinderen zonder apnoe bij opname	6/147	0	25
Leeftijd ≤ 2 maanden en/of zwangerschapsduur ≤ 32 weeks	6/115	0	19
Leeftijd ≤ 2 maanden en/of zwangerschapsduur ≤ 34 weeks	6/118	0	20
Leeftijd ≤ 2 maanden en/of zwangerschapsduur ≤ 37 weeks	6/126	0	21
Leeftijd ≤ 2 maanden	6/101	0	17
Zwangerschapsduur ≤ 37 weken	3/50	3	17
Zwangerschapsduur ≤ 34 weken	2/21	3	11

NNT: number-needed-to-treat

géén aanleiding tot het optreden van een apnoe tijdens een RSV infectie . Bij de mens is aangetoond dat verandering van chemoreceptoren in de larynx kan resulteren in een versterkte respons en tot een apnoe wanneer deze receptoren worden gestimuleerd [7]. Pickens en collega's beschreven het karaker van een apnoe in een RSV infectie als gecombineerd (zowel centraal als obstructief) [8]. Zij observeerden dat een apnoe gepaard ging met slikken en hoesten, en concludeerden hieruit dat beschermende reflexen in de luchtwegen de ademhaling onderdrukken.

Het verder karakteriseren door middel van polysomnografie van een apnoe tijdens een RSV infectie is noodzakelijk om meer inzicht in de pathofysiologie van dit proces te verkrijgen. Een grote multicentrische studie is nodig om aanvullende criteria voor cardiorespiratoire bewaking op te kunnen stellen.

11.4 Thoraxfoto's bij patiënten met RSV infecties

Bij ruim 70% van alle kinderen die worden opgenomen in het ziekenhuis in verband met een RSV infectie wordt een thoraxfoto gemaakt [9]. Hyperinflatie, atelectasen en/of infiltraten zijn frequente bevindingen. Er bestaat echter geen relatie tussen deze bevindingen en het klinisch beeld [10]. De resultaten van een studie naar voorspellers voor een normale thoraxfoto bij patiënten met een RSV infectie worden beschreven in **Hoofdstuk 5**. Een groep van patiënten met een hoge waarschijnlijkheid op een normale thoraxfoto – gedefiniëerd als de afwezigheid van hyperinflatie, atelectasen en infiltraten – kan met behulp van dit voorspellingsmodel onderscheiden worden.

In onze populatie werd bij 202 kinderen een thoraxfoto gemaakt. Hiervan waren er 75 niet afwijkend. Een normale thoraxfoto kon voorspeld worden op basis van een toename in leeftijd en geboortegewicht, de aanwezigheid van rhinitis, de afwezigheid van intrekkingen en een toename in SaO_2. Het oppervlak onder de Receiver Operating Characteristic (ROC) curve was 0.80, hetgeen aangeeft dat het model een goed onderscheid biedt heeft tussen normale en abnormale thoraxfoto's. Het model werd getransformeerd naar een score kaart om de berekening van de waarschijnlijkheid op een normale thoraxfoto te vergemakkelijken.

Men kan zich afvragen wat de indicaties zijn om een thoraxfoto te maken bij kinderen met RSV infecties. Dawson en collega's noemden intensive care opname, aanwezigheid van onderliggend hart- of longlijden en een onverwachte achteruitgang in de klinische conditie van de patiënt [11]. Eriksson en collega's suggereerden dat de aanwezigheid van atelectasen zeer suggestief is voor een RSV infectie in combinatie met een bacteriële superinfectie met pathogenen zoals *Streptococcus pneumoniae* en *Haemophilus influenzae*, zodat een thoraxfoto noodzakelijk zou zijn [12]. Echter, dit geldt met name voor patiënten ouder dan 12 maanden. Wij stellen voor een thoraxfoto te maken bij patiënten met een somscore lager dan 52, of indien er een onverwachte achteruitgang is in de klinische conditie van de patiënt. Het aantal thoraxfoto's kan significant gereduceerd worden met deze criteria. Prospectieve validatie van het model is noodzakelijk om de toepasbaarheid op andere locaties te toetsen.

In kinderafdelingen in Nederland worden vaak thoraxfoto's gemaakt bij zuigelingen en peuters met een RSV infectie. De resultaten van onze studie geven echter aan dat dit slechts geïndiceerd is in een beperkt aantal patiënten.

11.5 Nosocomiale RSV infecties

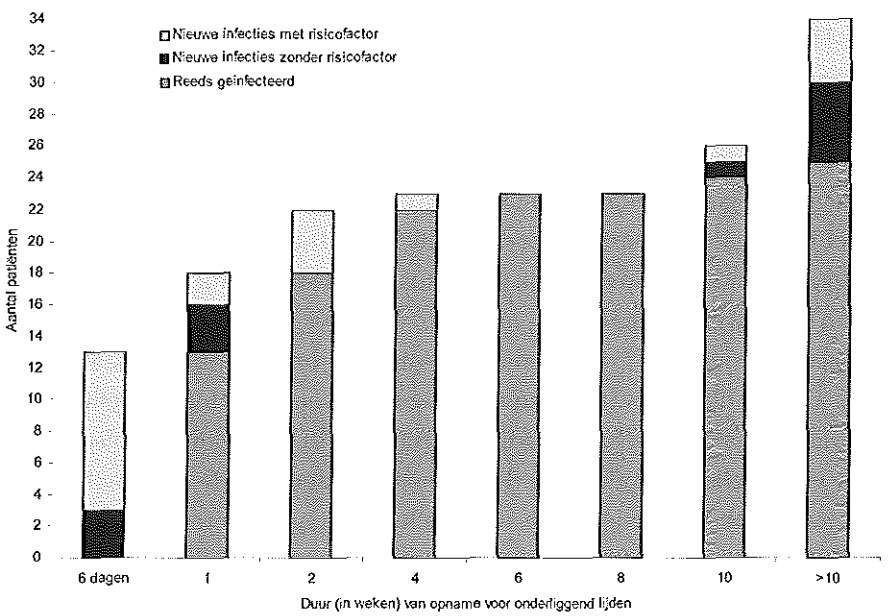
RSV wordt beschouwd als een belangrijke verwekker van nosocomiale infecties. Deze kunnen leiden tot een toename in morbiditeit en sterfte onder met name jonge

kinderen. Ongeveer 4% tot 7% van alle gehospitaliseerde kinderen maakt een nosocomiale RSV infectie door [15]. Een nosocomiale infectie door RSV zou vaker aanleiding geven tot een bronchiolitis en/of pneumoniën of een verlengde opnameduur. Een toegenomen mortaliteit wordt gezien onder kinderen met een congenitale hartziekte, bronchopulmonale dysplasie of prematuriteit [13,14]. Met name de combinatie van een congenitale hartziekte en een nosocomiale RSV infectie geeft aanleiding tot een verhoogde sterftekans [15].

In **Hoofdstuk 6** worden de resultaten beschreven van een studie naar nosocomiale RSV infecties (NA-RSV). Het aantal NA-RSV werd bestudeerd en karakteristieken werden vergeleken met die bij zuigelingen met een buiten het ziekenhuis verworven RSV infectie (CA-RSV). Het percentage patiënten met een NA-RSV (het aantal kinderen met een NA-RSV gedeeld door het totaal aantal opgenomen kinderen jonger dan 12 maanden gedurende het RSV seizoen) bedroeg 2.7% en vertoonde per seizoen een dalende tendens. Deze daling is waarschijnlijk een gevolg van de toepassing van specifieke richtlijnen ter preventie van nosocomiale RSV infecties in ons ziekenhuis. Deze richtlijnen omvatten handenwassen door ziekenhuispersoneel en de isolatie van RSV geïnfecteerde patiënten. Het risico op het verkrijgen van een NA-RSV gerelateerd aan de duur van opname voor onderliggend lijden is weergegeven in figuur 11.3.

Het klinisch beeld van patienten met NA-RSV bleek vergelijkbaar met dat van CA-

Figuur 11.3 *Aantal nosocomiale RSV infecties vergeleken met de duur van opname voor onderliggend lijden*



RSV. Het significante verschil tussen beide groepen met betrekking tot geboortegewicht lijkt het gevolg van de algemene ziekenhuispopulatie van ons ziekenhuis in plaats van een werkelijk gevolg van NA-RSV. De karakteristieken van ziekenhuisopname waren ook vergelijkbaar tussen NA-RSV en CA-RSV. Alle kinderen (n = 4) met bronchopulmonale dysplasie (BPD) en een NA-RSV werden beademend. Dit in tegenstelling tot kinderen met BPD en een buiten het ziekenhuis verworven RSV infectie, waarvan er slechts twee beademingsbehoeftig waren. Wij concludeerden dat met uitzondering van patiënten met BPD en een nosocomiale RSV infectie, nosocomiale RSV infecties in onze studie niet gepaard gaan met een ernstiger ziekteverloop dan buiten het ziekenhuis verworven RSV infecties.

11.6 Opnameduur in RSV infectie

Het gebruik van een voorspellingsmodel kan helpen bij de beslissing wanneer bepaalde therapiën voorgeschreven moeten worden. Ook kunnen patiënten geïdentificeerd worden die in aamerking komen voor preventieve maatregelen. Recent hebben Moler en Ohmit een model ontwikkeld waarmee de opnameduur voor patiënten met een RSV infectie voorspeld kon worden [16]. Zij veronderstelden dat aan de hand van een opnameduur ≥ 7 dagen een onderscheid gemaakt kon worden was tussen ernstige en niet ernstige infecties. De gemiddelde opnameduur in hun studie bedroeg vier dagen. Voorspellers voor een opnameduur ≥ 7 dagen waren gewicht, congenitaal hartlijden, failure to thrive, prematuriteit, bronchopulmonale dysplasie, longlijden anders dan BPD, diverse ziekten, vroege noodzaak tot beademing en vroege start van ribavirine. Het model had een goed onderscheidend vermogen zoals bepaald door het oppervlak onder de ROC curve. Het model werd gevalideerd op onze populatie. De resultaten hiervan zijn beschreven in **Hoofdstuk 7**. Het model bleek een slechte voorspeller aangezien er geen goede balans was tussen de sensitiviteit en het aantal vals positieven. De gemiddelde opnameduur in onze studie was twee keer zo groot als die van het Michigan databestand, zodat wellicht geldt dat een drempel van zeven dagen geen goed onderscheid mogelijk maakt tussen ernstige en niet ernstige infecties. De mediaan is dan wellicht een betere parameter. Derhalve werd een model ontwikkeld waarmee een opnameduur ≥ 9 dagen (de mediaan in onze populatie) kon worden voorspeld. Onafhankelijke variabelen waren gewicht en zuurstofsuppletie. Echter, het oppervlak onder de ROC curve bedroeg slechts 0.65. Uit de validatie bleek dat het een slecht voorspellend model was. Wij concludeerden dat de opnameduur van kinderen met een RSV infectie niet te voorspellen is en niet de ernst van een infectie weerspiegelt. Deze bevinding heeft belangrijke implicaties aangezien in veel multicentrisch onderzoek de opnameduur als uitkomst parameter werd gebruikt om

de klinische respons te bepalen.

11.7 Behandeling en preventie van RSV infecties

11.7.1 Behandeling van RSV infecties

Onderzoek naar de behandeling van RSV infecties heeft zich gericht op de toepassing van corticosteroiden, bronchodilatoren en antivirale medicatie.

Gezien het toenemende inzicht dat bronchiolitis een immuunpathofysiologisch proces is, lijkt het gebruik van corticosteroiden voor de hand liggend. De effectiviteit van corticosteroïden is echter niet aangetoond [17]. Ook zijn corticosteroïden onwerkzaam als profylacticum tegen het herhaald wheezing dat vaak optreedt aansluitend aan een RSV bronchiolitis. Derhalve wordt het gebruik van corticosteroiden afgeraden.

Vanwege de klinische overeenkomsten met astma waarbij bronchodilatoren zeer effectief zijn, worden bronchodilatoren frequent gebruikt bij kinderen met een RSV infectie. De resultaten van diverse studies zijn tegenstrijdig en leveren geen bewijs voor het effect van bronchodilatoren [18,19]. Bij de individuele patiënt kan een proefbehandeling worden overwogen. Bij voldoende effect kan de therapie zonodig gecontinueerd worden.

Ribavirine is het enige antivirale middel dat geregistreerd is voor het gebruik onder kinderen met RSV infecties. Controverse over het gebruik is ontstaan nadat de aanvankelijk gunstige resultaten van studies uit de jaren '70 en '80 werden weersproken door negatieve resultaten uit recente studies. Bovendien waren de studies uit de jaren '70 en '80 methodologisch niet sterk. Hoge kosten en mogelijke teratogene en carcinogene bijwerkingen van ribavirine hebben eveneens bijgedragen aan de nog steeds bestaande controverse. Ribavirine wordt tegenwoordig nauwelijks meer gebruikt [20]. Het gebruik dient alléén overwogen te worden bij de immuungecompromitteerde patiënt met een ernstig ziekte verloop.

RSV immuunglobuline heeft geen therapeutische waarde [18]. Een bacteriële superinfectie komt zelden voor [21]. Er werd geen therapeutisch effect gevonden van het routinematig gebruik van antibiotica ter preventie van een superinfectie [22,23].

11.7.2 Preventie van RSV infecties

Preventie van RSV infecties kan door middel van actieve en passieve immunisatie. In de jaren '60 had vaccinatie met een formaline geïnactiveerd vaccin dramatische gevolgen. Kinderen die het vaccin kregen, ontwikkelden na infectie met wild type RSV een ernstiger ziekteverloop met toename in sterfte. Op basis van jarenlang onderzoek

zijn recent een aantal interessante potentiële vaccins beschikbaar gekomen zoals BBG2Na, PFP-2 vaccin en een verzwakt *cold-passaged temperature sensitive* mutant vaccin zoals cpts-248, cpts 248/404 en cpts-530/1009. De immunogeniciteit van deze vaccins is in diermodellen aangetoond [24-26]. PFP-2 vaccins zijn getest bij de mens. Vaccinatie resulteerde niet in een reductie van het aantal respiratoire ziekten door RSV [27,28]. Cpts-vaccins waren wel effectief bij kinderen ouder dan zes maanden, maar veroorzaakten neusverstopping bij zuigelingen jonger dan twee maanden [29]. De ontwikkeling van een vaccin tegen RSV kan bemoeilijkt worden door de aanwezigheid van maternale antistoffen die de immuunrespons van de zuigelingen kunnen onderdrukken, en het verminderd vermogen van zuigelingen om te reageren op virale glycoproteïnen in vaccins.

Onderzoek naar de effectiviteit van passieve immunisatie heeft geleid tot de registratie van twee middelen: RSV immuunglobuline (RSV IVIG) en gehumaniseerde monoclonale antilichamen gericht tegen het F eiwit (palivizumab). In de PREVENT-studie werden prematuur geboren kinderen en kinderen met BPD profylactisch behandeld met RSV-IVIG of placebo. Het gebruik van RSV-IVIG leidde tot een reductie van 41% van het aantal RSV geassocieerde ziekenhuisopnames, evenals een reductie van 53% van het totaal aantal dagen in het ziekenhuis [30]. Het gebruik van palivizumab bij kinderen jonger dan 24 maanden met BPD, en bij prematuur geboren (≤ 35 weken) kinderen jonger dan 6 maanden resulteerde in een reductie van 55% van het aantal RSV geassocieerde ziekenhuisopnames (78% reductie in de groep prematuur geborenen en 39% reductie in de BPD groep). Een reductie van 47% werd gezien in het aantal RSV geassocieerde ziekenhuisopnames bij kinderen geboren voor een zwangerschapsduur van 32 weken, terwijl bij kinderen geboren na een zwangerschapsduur van 32-35 weken een reductie van 80% werd gevonden. Deze laatste groep vormt de grootste groep prematuur geboren kinderen met een zwangerschapsduur ≤ 37 weken. Het gebruik van palivizumab leidde niet tot een daling van het aantal kinderen dat beademing nodig had.

De indicaties voor het gebruik van palivizumab worden sterk beïnvloed door regionale en (inter)nationale verschillen in de gevolgen van infecties door RSV. Verschillen zijn bijvoorbeeld het aantal prematuur geboren kinderen met of zonder chronisch longlijden, de ernst van de RSV infectie en het percentage risico-patiënten dat opgenomen dient te worden. Dit percentage variëert van 3% in meer recente studies tot 25% in de oorspronkelijke publicaties [31]. Toepassing van RSV-IVIG op grote schaal wordt beperkt door de hoge kosten en de intraveneuze toedieningsvorm. Palivizumab is daarentegen makkelijker toe te dienen (intra-musculair). Het gebruik van RSV-IVIG lijkt achterhaald sinds het beschikbaar komen van palivizumab. Echter, kosten-baten analyses zijn noodzakelijk om de juiste indicaties voor het gebruik van palivizumab

vast te stellen. Uit deze analyses volgt de Number Needed to Treat die acceptabel is in relatie tot de kosten en nadelen van prophylaxe voor zowel patiënten als dokters.

11.8 Lange termijn effecten van RSV infecties

De mogelijke aanwezigheid van een relatie tussen RSV bronchiolitis op jonge leeftijd en het ontstaan van astma op latere leeftijd is intensief bestudeerd en het onderwerp van langdurig en heftig debat. Twee hypothesen zijn voorgesteld: *(i)* RSV broncholitis en astma zijn twee verschillende entiteiten met een vergelijkbaar klinisch beeld, of *(ii)* RSV bronchiolitis identificeert de gepredisponeerde gastheer die na het doormaken van de bronchiolitis astma ontwikkeld. De resultaten van een kwantitatieve review naar een mogelijke relatie worden beschreven in **Hoofdstuk 9**. Gecontroleerde follow-up studies met gehospitaliseerde patiënten jonger dan 12 maanden met een virologisch bewezen RSV infectie, werden geanalyseerd. Met behulp van Medline werden 30 retrospectieve en prospectieve case control of longitudinale cohort studies gevonden die gepubliceerd waren tussen januari 1978 en december 1998. Slechts zes studies voldeden aan alle inclusie criteria.

De odds ratio voor wheezing daalde van 3.8 (95% CI 1.6 – 9.3, $p < 0.001$) gedurende de eerste vijf jaar na een RSV bronchiolitis, naar 2.3 (95% CI 1.2 – 4.5, $p = 0.004$) bij vijf tot tien jaar follow-up. Recurrent wheezing – gedefinieerd als drie of meer episoden van wheezing vastgesteld door een arts of het gebruik van bronchodilatoren in het jaar voorafgaande aan follow-up – was niet geassocieerd met RSV bronchiolitis op zuigelingenleeftijd na vijf of meer jaren follow-up (OR 2.4, 95% CI 0.7 – 8.4). Daarnaast werd geen associatie gevonden tussen RSV bronchiolitis op de zuigelingenleeftijd en het voorkomen van een positieve anamnese voor atopie of een positieve familie-anamnese voor atopie of astma. Wij concludeerden dat het doormaken van een RSV bronchiolitis op de zuigelingenleeftijd geen oorzaak is van astma op latere leeftijd.

Tabel 11.4 *Vergelijking tussen RSV bronchiolitis en astma*

	RSV bronchiolitis	Astma
T - cellen	CD8 (+)	CD4 (+)
T-helper groepen	Zowel T_H-1 als T_H-2	T_H-2
Invloed van eosinofielen	+	+++
Atopie in de voorgeschiedenis	-	+++
Familie-anamnese positief voor atopie en/of astma	-	+

De resultaten van onze kwantitatieve review worden ondersteund door de toegenomen kennis over de pathogenese van RSV infecties. Karakteristieken van astma en bronchiolitis worden samengevat in tabel 11.4. CD4 positieve T cellen zijn een belangrijke component bij astma, terwijl bij infecties door RSV CD8 positieve T cellen een belangrijke rol hebben [32]. Voorts vervullen in tegenstelling tot de situatie bij in astma, eosinofielen in RSV bronchiolitis geen hoofdrol [33]. Een dysbalans van het T_H-1/ T_H-2 paradigme in het voordeel van T_H-2 wordt gezien bij kinderen met astma, terwijl RSV ook een T_H-1 respons induceert.

IgE speelt een belangrijke rol in de pathogenese van astma [34]. Het serum IgE gehalte is echter niet verhoogd in RSV bronchiolitis [35]. Ofschoon RSV-specifiek IgE werd gevonden in nasopharyngeaal spoelsels of bronchoalveolaire lavages gedurende de acute infectie, bleken de waarden hiervan niet voorspellend voor het optreden van recurrent wheezing op de leeftijd van zeven tot 10 jaar [36]. Een overeenkomst tussen astma en RSV bronchiolitis betreft de verhoogde concentraties van eosinofiel cationic protein (ECP). Het bepalen ervan gedurende een RSV bronchiolitis blijkt niet voorspellend te zijn voor welke kinderen wel en welke kinderen niet broncho-obstructieve klachten op latere leeftijd ontwikkelen [37]. Andere virale infecties kunnen ook aanleiding geven tot een verhoogde ECP concentratie [38].

De long functie gemeten bij kinderen die een RSV bronchiolitis hadden doorgemaakt(zoals FVC, FRC, luchtwegresistentie, peak flow en compliance), bleek niet verminderd te zijn ten opzichte van controles [39,40]. Wel werd aangetoond dat kinderen die een RSV bronchiolitis doormaakten een initieel lagere longfunctie hadden [41]. Deze pre-existente afwijkingen in longfunctie kunnen een risicofactor zijn voor lagere luchtweginfecties met wheezing gedurende het eerste levensjaar [34,42].

Kinderen die een RSV bronchiolitis doormaken dienen ten minste tien jaar gevolgd te worden om daarmee te onderzoeken welke ontstekingsmediatoren die gedurende de acute ziekte geproduceerd worden (gemeten in neusspoelsels), verantwoordelijk zijn voor (recurrent) wheezing na de RSV bronchiolitis.

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CHAPTER 12

95% CI	95% confidence interval
ADCC	Antibody dependent cell mediated cytotoxicity
ADH	Antidiuretic hormone
AUC	Area under the curve
BPD	Bronchopulmonary dysplasia
CA-RSV	Community acquired RSV infection
CCA	Chimpanzee Coryza Agent
CDCC	Complement dependent neutrophil-mediated cytotoxicity
CHD	Congenital heart disease
CLD	Chronic lung disease
Cpts	Cold passaged temperature sensitive
CRP	C-reactive protein
CTL	Cytotoxic T lymphocytes
DIFA	Direct immunofluorescence assay
ECP	Eosinophilic cationic protein
ELISA	Enzym-linked immunosorbent assay
HIV	Human immunodeficiency virus
ICAM	Intercellular adhesion molecule
ICU	Intensive care unit
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IL	Interleukin
IRF	Interferon regulatory factor
IVIG	Intravenous immunoglobulin
LRTI	Lower respiratory tract infection
LTC_4	Leukotriene C4
Mab	Monoclonal antibody
MCU	Medium care unit
MHC	Major histocompatibility class
MIP	Macrophage inflammatory protein
mRNA	Messenger ribonucleic acid
NA-RSV	Nosocomial acquired RSV infection
NK	Natural killer cells
Nm	Nanometer
NNT	Number Needed to Treat
OR	Odds ratio
PAF	Platelet activating factor
PCR	Polymerase chain reaction

pHOSP7	Probability for a hospital stay > 7 days
RANTES	Regulated on activation, normal T cell expressed and presumable secreted
RNA	Ribonucleic acid
ROC	Receiver Operating Characteristic
RR	Relative risk
RSV	Respiratory Syncytial Virus
SP	Surfactant protein
$T_{H}1$	T helper 1 cells
$T_{H}2$	T helper 2 cells
TNF	Tumour necrosis factor
URTI	Upper respiratory tract infection
WBC	White blood cell count

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