Respiratory Syncytial Virus Infections in Young Children

Risk Assessment and Prevention

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Respiratory Syncytial Virus Infections in Young Children

Risk Assessment and Prevention

Infecties met het respiratoir syncytieel virus bij jonge kinderen

Risicobepaling en preventie

Proefschrift

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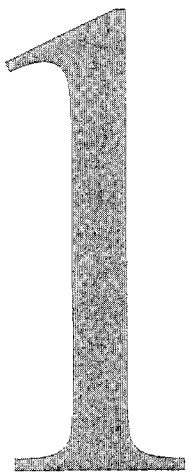
Voor Renatha, Simone en Matthijs

Contents



Introduction

Virology

In 1956 Morris et al. described an infectious agent, chimpanzee coryza agent (CCA), that caused coryza-like symptoms in chimpanzees.[6] Soon after this initial description, a virus indistinguishable from CCA was isolated in two infants with severe lower respiratory tract illness.[7] Because of the potential to cause respiratory tract infections and because of the characteristic syncytial cytopathogenic effect in living cell cultures, the virus was renamed respiratory syncytial virus (RSV).[8] Since than, extensive research has revealed the important role of RSV in lower respiratory tract infections in infants and young children.[9-13]

Human RSV is a nonsegmented single stranded negative-sense RNA virus, belonging to the genus *Pneumovirus* of the family of *Paramyxoviridae*.[14] This family also includes parainfluenza viruses 1-4, mumps virus, measles virus, and the recently discovered human metapneumovirus.[15]

The virion consists of a lipid bilayer envelope and a nucleocapsid containing the genome. The genome consists of 15,222 nucleotides that encode for ten viral proteins. Three of these proteins are expressed on the surface of the virus: the F, G, and SH proteins. The F protein mediates virus penetration into host cells and syncytium formation by membrane fusion of host cells. The G protein mediates virus attachment to host cells. The function of the SH protein is still unknown. The F and G proteins are the two major immunogenic proteins.

Based on reactions with monoclonal antibodies two groups of RSV can be distinguished, group A and group B.[16 17] These two groups have approximately 25% sequence homology. The antigenic difference is primarily caused by variation in the G protein, which shows only 1% to 7% homology.[18] The F protein on the other hand is much more conserved, and shows approximately 50% homology.

Epidemiology

RSV causes respiratory tract infections in all age groups. Severe disease, necessitating hospitalisation, most often occurs in infants, immunocompromised persons, and the elderly.[19 20] Approximately 70% of all children encounter their first infection before the age of one year, and almost all children are infected before the age of two years.[21] Approximately 0.5% to 2% of all infants are hospitalised because of severe RSV disease.[22] Of these, 8% to 19% develop respiratory insufficiency.[23-25] It is well known that certain characteristics, such as prematurity and chronic lung disease (CLD),[26-35] young age,[36 37] low birth weight,[38] cyanotic congenital heart disease (CHD),[39 40] and

immunodeficiencies[41] increase the risk for severe RSV disease. Other risk factors for severe RSV disease include parental smoking,[42] older siblings,[42] low socioeconomic status,[37] and day-care attendance.[43] Previously no attempts have been made to estimate individual RSV hospitalisation risks by constructing risk profiles for individual children, based on the presence or absence of several risk factors.

Fortunately, mortality rates associated with severe RSV disease are generally low (0.5% to 1.0%).[24 41] However, higher mortality rates have been reported in children with CLD (3.5% to 5%),[24 44] CHD (2.5% to 37%),[24 39 44 45] and immunodeficiencies (5% to 40%).[24 41]

Although reinfections occur frequently, disease severity decreases with increasing number of reinfections.[21 46] RSV infections occur worldwide. In temperate climates RSV infections follow a distinct seasonal pattern, with epidemics starting from October, peaking around December and ending before April. In tropical or subtropical climates with seasonal rainfall RSV epidemics are frequently associated with the rainy season.[47] The monthly incidence of RSV infections changes within the RSV season, with the highest risks observed during the peak of the epidemic. This makes RSV seasonality one of the factors influencing the individual RSV hospitalisation risk. Group A and B strains may occur alternately or concomitantly, with varying predominance of either group.[48-50] There is conflicting evidence about the relationship between severity of disease and infections caused by group A or B RSV.[25 50-53]

Genetic polymorphisms

Although there is an increasing interest in the influence of polymorphisms in host genes, encoding for proteins involved in specific and non-specific immune responses, on the occurrence and severity of infectious diseases, little is known about the influence of such polymorphisms on the severity of RSV disease.[54-59] RSV disease is a combined result of the direct cytopathogenic effect of RSV, and the concomitantly induced immune response.[60 61] T cell-mediated immunopathology, in particular a more pronounced T helper type 2 (Th2) response, may play an important role in the development of severe RSV disease. Genetic polymorphisms altering the quality or quantity of the immune response could influence the severity of RSV disease.

Clinical signs and treatment

RSV causes a spectrum of disease ranging from mild upper respiratory tract disease to severe lower respiratory tract disease or apnea. All infections initially start as an upper respiratory tract infection (rhinitis, pharyngitis or otitis media), characterised by

coryza, angina, cough, otitis, and sometimes low-grade fever. In some, the infection progresses to the lower airways, mostly causing bronchiolitis and rarely causing pneumonia, characterised by tachypnea, dyspnea, chest retractions, and wheezing.
To date, there is no causal treatment of RSV infections. Severely ill children require supportive treatment such as nasal washings, tube feeding, oxygen, and mechanical ventilation. Bronchodilators and steroids do not alter the course of disease. In some children bronchodilators improve wheezing. Antibiotics are only indicated in case of a secondary bacterial infection, which is only found in approximately 1.2% of cases.[62 63]
Approximately 45% of the infants that are hospitalised with severe RSV disease experience persisting periods of recurrent wheezing. The differences in the prevalence of recurrent wheezing between children that have been hospitalised with RSV and control children disappeared after five years of follow up.[64] The influence of RSV hospitalisation and the possible occurrence of recurrent wheezing on the health related quality of life (HRQoL) of hospitalised children is not known. Although estimates of parental costs associated with RSV hospitalisation in The Netherlands were studied before,[3] RSV hospitalisation costs have not been determined in large population based studies in The Netherlands.

Prevention

Vaccination

Early attempts to develop an effective and safe vaccine to prevent RSV infection failed. FI-RSV, a formalin-inactivated, alum-precipitated RSV vaccine, developed and tested in the 1960's, failed to protect vaccinated children against RSV infection. Moreover, the vaccine induced an exaggerated, altered clinical response to naturally occurring RSV infection among younger vaccinees, suggesting an altered host response. 80% of the children who received the RSV vaccine were hospitalised with severe RSV disease after naturally occurring RSV infection, whereas only 5% of the children in the control group were hospitalised. Unfortunately two infants that had received the RSV vaccine died as a result of severe RSV disease. Similar paradoxical vaccine effects were previously reported with rickettsial vaccine, trachoma vaccine, *Mycoplasma pneumoniae* vaccine and inactivated measles vaccine.[65-67]
The mechanisms responsible for this phenomenon, called enhanced pathology, are not yet completely understood. It is hypothesised that RSV vaccination results in insufficient production of serum neutralising antibodies and no induction of local immunity, leaving children susceptible to infection. Because FI-RSV did not prime for $CD8^+$ cytotoxic T-cell responses the virus was not cleared after infection and induced a direct cytopathic effect. The infection would also give rise to an unbalanced

Th1/Th2-immune response resulting in an influx of lymphocytes and eosinophils with release of additional mediators giving rise to inflammation and bronchoconstriction.[68] Since this early failure many different vaccination strategies have been explored. Several live attenuated virus vaccines, genetically engineered vaccines, vector delivery systems and subunit vaccines were evaluated.[68-71] Although tremendous progress has been made, none of the approaches has yet led to a safe and efficacious vaccine for infants and young children.

Several problems are encountered in the development of a RSV vaccine for young children. First, the peak incidence of RSV disease is observed at the age of two months, making early vaccination desirable. However, at this age children will still have significant quantities of maternally derived antibodies against RSV that may interfere with vaccination. Moreover, young children generally respond poorly to vaccination because of immaturity of the immune system. Furthermore, a RSV vaccine should protect against infections with the antigenically differing RSV groups A and B. Last, a RSV vaccine should not induce an exaggerated, altered clinical response to naturally occurring RSV infection as observed with FI-RSV. Evaluation of the safety and immunogenicity in healthy young adults will be the first step in the clinical evaluation of new candidate RSV vaccines.

Passive immunisation

In the absence of a safe and efficacious vaccine for infants and young children, passive immunisation against RSV is an alternative. Currently two compounds are available: RSV-IGIV (Respigam®) and palivizumab (Synagis®).

RSV-IGIV

RSV-IGIV is an intravenous polyclonal immune globulin, that is approximately sixfold enriched for neutralising antibodies against RSV.[72 73] A randomised, double blind, placebo controlled clinical trial was conducted among 510 children (250 RSV-IGIV, 260 placebo) with a gestational age of 35 weeks or less and an age below six months, or with BPD and an age below 24 months.[74] RSV-IGIV significantly reduced the incidence of RSV hospitalisation from 13.5% to 8.0% (41% reduction). In addition, RSV-IGIV significantly reduced the incidence of overall respiratory hospitalisations from 27% to 16 % (40% reduction). No reduction of the incidence of intensive care unit (ICU) admission, mechanical ventilation, or mortality was observed. RSV-IGIV had serious side effects in children with CHD, related to fluid overload. It should therefore not be used in these children.[75]

Palivizumab

Palivizumab is a recombinant humanised immunoglobulin G-1 monoclonal antibody that binds to a specific epitope of the F protein on the surface of RSV group A and B.[76] A randomised, double blind, placebo controlled clinical trial was conducted among 1,502 children (1,002 palivizumab, 500 placebo) with a gestational age of 35 weeks or less and an age below six months, or with BPD and an age below 24 months.[77] Palivizumab significantly reduced the overall incidence of RSV hospitalisation from 10.6% to 4.8% (55% reduction). A lower reduction of the incidence of RSV hospitalisation was found in children with BPD (39% reduction), and in children with a gestational age below 32 weeks (47% reduction). A higher reduction of the incidence of RSV hospitalisation was found in premature children without BPD (78% reduction), and in children with a gestational age of 32 through 35 weeks (80% reduction). Palivizumab also significantly reduced the number of ICU admissions. No reduction of the incidence of mechanical ventilation, or mortality was observed. No serious side effects were reported.

A Cochrane review combined four randomised, controlled trials with either RSV-IGIV or palivizumab in children with prematurity, BPD, or CHD.[1] A total of 2,598 children were included in these four studies. The reported pooled odds ratios were 0.48 (95% CI 0.37-0.64) for incidence of hospitalisation, 0.47 (0.29-0.77) for incidence of ICU admission, and 0.99 (0.48-2.07) for incidence of ventilation.

The first guidelines for the use of passive immunisation against RSV were issued by the American Academy of Pediatrics (AAP).[2] Other guidelines published afterwards in other countries, like those issued by the Dutch Society for Paediatrics, were based on these AAP guidelines in a more or less restrictive way.[78 79]

However, the costs of passive immunisation are considerable. The costs of immunisation with palivizumab for one child during a complete RSV season of five months approximate €4,700 (weight 6000 g, wastage of the drug assumed). These costs, combined with the moderate efficacy, and the generally low incidence of RSV hospitalisation, have led to a discussion concerning the cost-effectiveness of passive immunisation.[80-85] Several economic analyses of palivizumab for reduction of RSV hospitalisation have been performed.[27 29 30 86-94] Nine studies report cost increases,[27 29 30 86 90-94] of which five report incremental costs per hospitalisation averted ranging from $7,000 to $420,000.[27 86 90 92 94] Only three studies report cost savings.[87-89] Several methodological shortcomings were noted in these studies.[95-97] Since there is a geographic variability in the incidence and costs of RSV hospitalisation, development of guidelines should be based on the analysis of local hospitalisation risks and costs.[98]

A cost-effectiveness analysis of passive immunisation against RSV in The Netherlands has never been performed.

Study aims

The studies described in this thesis were designed to:

1. Develop a clinical prediction rule to estimate the individual monthly risk of hospitalisation for severe RSV disease in young children (**chapter 2.1**).
2. Determine the value of the average seasonality as a predictor for monthly hospitalisation risks, by comparing the performance of the prediction rule with and without seasonality, and evaluate the influence of variability of seasonality between RSV seasons on the performance of the prediction rule with and without seasonality (**chapter 2.2**).
3. Determine the RSV hospitalisation costs in infants and young children and to develop a prediction model that estimates anticipated mean RSV hospitalisation costs in children at-risk, based on several child characteristics (**chapter 3.1**).
4. Assess the incremental costs to prevent one RSV hospitalisation in high-risk children from a societal perspective, using a novel individualised monthly approach for decision making on passive immunisation (**chapter 3.2**).
5. Quantify the loss of HRQoL in children during the first months after RSV hospitalisation (**chapter 4**).
6. Study the association of severe RSV disease with a polymorphism located in the promoter region of the IL-4 gene and two polymorphism's located in the IL-4Rα gene (**chapter 5**).
7. Explore the safety, tolerability, and immunogenicity of BBG2Na, a recombinant RSV vaccine constructed from a G protein sequence, in healthy young adults (**chapter 6**).

2

Hospitalisation risk for RSV infection

2.1

Respiratory syncytial virus hospitalisation in young children: development of a clinical prediction rule

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Submitted

Abstract

Background

Passive immunisation against respiratory syncytial virus (RSV) is costly. Its use should therefore be restricted to well defined high-risk children. We aimed to develop a clinical prediction rule, which estimates the individual monthly risk of hospitalisation for RSV infection in young children.

Methods

A retrospective cohort study was conducted in the Southwest of the Netherlands. Children born between January 1, 1996 and December 31, 1998 and hospitalised with proven RSV infection were related to children at-risk born during the same period. The monthly risk was estimated with a logistic regression model including five clinical predictors (gestational age, presence of bronchopulmonary dysplasia (BPD), birth weight, gender and age) and the mean seasonal pattern of RSV infections. To assess the value of the prediction rule relative to the guidelines of the American Academy of Pediatrics (AAP) we applied specific thresholds of the monthly risk above which passive immunisation might be recommended.

Findings

Information was collected on 2,469 hospitalised children and 140,661 children who were 1,181,790 months at-risk. All predictors were statistically significant, with age and the seasonal RSV pattern showing the strongest effects. Thresholds for the monthly risk of 0.6% and 0.8% for children with a gestational age $\leq$28 weeks and 29-32 weeks respectively, led to a better distinction between high and low-risk children than the AAP guidelines and would have reduced the number of immunisations by 20%.

Interpretation

The prediction rule reliably estimates individual monthly RSV hospitalisation risks. It provides a better indication for passive immunisation, although further validation is required.

Introduction

Respiratory syncytial virus (RSV) is the major cause of viral lower respiratory tract infections in infants and young children. Almost all children are infected at least once before the age of two years. Approximately 0.5% to 2% of the infected infants need to be hospitalised because of severe RSV disease. It is well known that prematurely born children or children with bronchopulmonary dysplasia (BPD) are at an increased risk for the development of severe RSV disease. Seasonal hospitalisation risks up to 32% are reported for these children.[27-34] Other risk factors include low birth weight,[38] male gender,[99] young age,[37] cyanotic congenital heart disease (CHD),[40] immunodeficiencies,[41] and environmental factors.[42 43] RSV infections occur according to a distinctive seasonal pattern. In temperate climates a typical RSV season can start in October and last until May with a peak incidence in December or January.[100 101]
Passive immunisation for the prevention of severe RSV disease is available since several years.[74 77] Passive immunisation should be administered on a monthly basis during the period the child is at high-risk for RSV hospitalisation, and is safe and effective in specific groups of high-risk children.[1 74 77] However, the costs of passive immunisation are considerable.[86] Current guidelines of the American Academy of Pediatrics (AAP) define categories of high-risk children by gestational age, the presence of BPD and age. For these children passive immunisation against RSV is recommended for the complete RSV season.[2] However, RSV hospitalisation risks are also influenced by other risk factors like gender and birth weight. Moreover, within each season, the hospitalisation risk is influenced by the seasonal pattern of RSV infections. Considering more risk factors and the seasonal infection pattern might result in more accurate individual risk estimates.
The aim of this study was to develop a clinical prediction rule which may estimate the monthly risk of hospitalisation for severe RSV disease in young children. Such a rule should provide more accurate risk estimates than the AAP classification.

Methods

A retrospective cohort study was conducted in the Southwest of the Netherlands. This region has a population of approximately four million and an annual birth rate of approximately 47,000 children. 73% of the total population lived in urban areas, while 27% lived in rural areas. 88% of the population had a West European ethnicity.[102] All 29 hospitals in the region with a paediatric ward participated in this study. Two of these hospitals have paediatric intensive care facilities. We retrospectively related

children admitted to these 29 hospitals to the children at-risk in the region. The study was approved by the institutional review board of the Erasmus MC.

Children hospitalised with RSV

Routinely documented information was collected from medical records, in standardised forms. We included children hospitalised for severe RSV disease who met the following criteria:

- Hospitalisation in one of the 29 hospitals in the region.
- Hospitalisation during one of three RSV seasons 1996/97, 1997/98 and 1998/99 (passive immunisation against RSV was not in use during this period).
- Born between January 1, 1996 and December 31, 1998.
- Age less than one year at the beginning of the RSV season or less than two years for children with BPD.
- RSV infection confirmed by a positive direct immunofluorescent assay or viral culture of nasopharyngeal aspirates.

The decision to hospitalise for severe RSV disease was based on standard diagnostic criteria (feeding problems, dyspnoea or apnoea).[103] The registry of virological diagnostic assays was used to identify the children in each hospital. Children with nosocomial infections (defined by a positive RSV test more than five days after hospital admission for another cause than RSV disease) were excluded.
Child characteristics included residence, date of birth, gender, gestational age, birth weight, co-morbidity (e.g. BPD) and date of admission.

Children at-risk

Data from children born between January 1, 1996 and December 31, 1998 and residing in the study region were obtained from The Netherlands Perinatal Registry. This database contains individual data on all births and neonatal care nationwide. The information was prospectively collected by midwifes, obstetricians and paediatricians. Child characteristics included residence, week and year of birth, gender, gestational age, birth weight, perinatal and neonatal death and occurrence of neonatal morbidity (e.g. BPD). The data were corrected for incomplete registration.[104] Infants who died within the first four weeks of live (neonatal mortality) were excluded.

Predictors

Based on the literature six predictors were selected: gestational age,[27-34] presence of BPD,[27 29-34] birth weight,[38] gender,[99] age[37] and month of the season.[100 101] BPD was defined as the need for supplemental oxygen on day 28 after birth or at the

postconceptional age of 36 weeks, in the presence of typical abnormalities on the chest X-ray.[105 106]

Since nosocomial infections were not included in this study, the risk for RSV hospitalisation during the stay on a neonatal intensive care unit (NICU) after birth equals zero by definition. Therefore age (being the time the child is exposed to RSV in the community) of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks, as most of these children were not discharged before a postconceptional age of 38 weeks.

We defined the RSV season to start October 1 and end April 30. The peak of RSV infection in the observed RSV seasons 1996/97 and 1997/98 was in December. However, the peak of RSV infection in the season 1998/99 was earlier: in November. Since this strongly influenced the average pattern over the three seasons, we used Dutch nationwide data on RSV infections over eight seasons (Source: Dutch Infectious Diseases Bulletin 1991-1999, National Institute of Public Health and the Environment, Bilthoven, The Netherlands) to describe the average seasonal pattern, using sine and cosine functions.[107] For the prediction rule the seasonal effect was subsequently estimated with one parameter reflecting the amplitude of the fixed sine and cosine functions.

Missing values

29% (713 out of 2,469 children) of the hospitalised children had missing values for one or more predictors. The percentage of children with missing values was reduced to 19% (471 out of 2,469 children), by means of a questionnaire on gestational age and birth weight that was sent to the parents. Half (239 out of 471 children) of the remaining 19% had either missing values for gestational age or birth weight. These missing values could be reliably imputed using their strong intercorrelation and the correlation with gender as found in the complete population using regression analysis.[108] The remaining 9.4% (232 out of 2,469 children) of the children had either missing values for gestational age and birth weight (n=227), or BPD (n=5). If birth weight and gestational age were both missing, values were randomly drawn from the distributions as observed in the data that were collected with the questionnaires. We found that imputation of less values lower than 37 weeks for gestational age (0% of imputed values as premature) or more values lower than 37 weeks (10% of missing values as premature) led to similar results. Missing values on BPD were imputed as no BPD.

Of the children at-risk, 0.6% had missing values for one or more predictors. Missing values on gestational age or birth weight were imputed as described for the hospitalised children.[108]

If birth weight and gestational age were both missing, values were randomly drawn from the observed distributions in the at-risk population. In total, 4.7% of all predictor values for the hospitalised children and 0.1% of all predictor values for the children at-risk were imputed.

Statistical analysis

The number of hospitalised children in a month divided by the number of children at-risk in that same month equals the monthly risk of hospitalisation. To model the risk with logistic regression analysis, the birth data were expanded by creating separate records for the newborn children for each following month at risk during the RSV season with corresponding age in months. This dataset was then aggregated, resulting in a dataset with one record for each combination of predictor values. Each record contained a weight variable for the number of children at risk. The data set was merged with the data of the hospitalised children after matching on the predictor variables. We ignored potential dependency between infections in the same children, since we included only 21 children with multiple hospital admissions.

We examined non-linearity in the relationships of gestational age, birth weight and age with the monthly risk of severe RSV infection using restricted cubic spline (RCS) functions. A RCS is flexible and smooth, which makes it an adequate tool to fit curvatures.[109 110] The splines were subsequently approximated with simple transformations such as binomial terms, categories and linear terms.[109]

It is known that the risk of RSV hospitalisation in children is low in the first month of life compared to the second month of life. Furthermore, the risk gradually declines with increasing age after the first month of live.[28 36 99] To model this association we used a binomial term, that equals zero if age is zero (first month of life) and equals one if age is greater than zero, and a linear term for age larger than one.

The AAP guidelines suggest to administer passive immunisation against RSV until the age of six months or one year to premature children without BPD and until the age of two years to children with BPD.[2] This indicates that the association between age and hospitalisation risk may differ for children with and without BPD. Therefore, we studied this association separately for these two groups of children, by including a statistical interaction term between age and BPD. The relative strength of a predictor was calculated by comparing the deviance of the model with and without the predictor.

The performance of the prediction rule was studied with respect to discrimination and reliability (calibration). Discrimination refers to the ability of the prediction rule to distinguish hospitalised children from non-hospitalised children. We calculated the area under the receiver operating characteristics (ROC) curve for all children at-risk

and for children with gestational ages below 33 or 37 weeks.[111] Reliability indicates the agreement between the predicted risks of RSV hospitalisation and the observed frequency of RSV hospitalisations. An impression of the reliability was obtained by plotting the observed frequency of RSV hospitalisations against the predicted risks of RSV hospitalisation. In addition, reliability was tested by the Hosmer-Lemeshow goodness-of-fit test.[112] Internal validity was assessed with bootstrapping techniques.[113] Random samples were drawn with replacement to fit the model, which was tested in the original data.[109] The prediction rule is implemented in a spreadsheet that can be used to calculate individual monthly hospitalisation risks with 95% confidence intervals (http://www.eur.nl/fgg/mgz/software.html).

Comparison with guidelines

We compared the prediction rule with the AAP guidelines for the ability to distinguish between high and low-risk children among those with a gestational age of 28 weeks or less, or 29-32 weeks. For these children, the AAP guidelines recommend immunisation until the ages of 12 and 6 months respectively.[2] Since these ages corresponded to the lowest risks that indicate immunisation, the risks at these ages are the implicit AAP thresholds to recommend immunisation. The thresholds were quantified with a logistic regression model that included age as a predictor within each of the two categories. The thresholds were subsequently applied to the risks predicted by the prediction rule to identify patients requiring immunisation.

Role of the funding source

The Health Care Insurance Council of the Netherlands had no involvement in study design, data collection, analysis, and interpretation, writing of the report or decision to submit the paper for publication.

Results

The characteristics of the hospitalised children and the children at-risk are summarised in table 2.1.1. A total of 2,469 children were hospitalised for severe RSV disease. 95% of all children were hospitalised because of feeding problems, dyspnoea, or apnoea. 63% required tube feeding, intravenous fluids, supplemental oxygen, or mechanical ventilation. 93% were diagnosed with bronchiolitis or pneumonia, while most of the remaining hospitalised children were diagnosed with upper respiratory tract infection. 85% (2,087/2,469) of the hospitalised children were neither prematurely born nor suffered from BPD. The majority was younger than six months

Table 2.1.1. *Characteristics of hospitalised children and children at-risk.*

		Hospitalised children (*n*=2,469) N	Total months at-risk (*n*=1,181,790) N/1000	Monthly risk %
Gender	Male	1,463	603.5	0.24
	Female	1,006	574.8	0.17
Gestational age	≤ 28	35	2.6	1.35
(weeks)	29-30	30	2.7	1.11
	31-32	48	5.1	0.95
	33-34	105	14.3	0.74
	35-36	164	39.2	0.42
	≥ 37	2,087	1,117.9	0.19
Birth weight	≤ 2500	394	74.6	0.53
(grams)	2501-3000	443	190.7	0.23
	3001-3500	770	424.2	0.18
	3501-4000	609	355.4	0.17
	>4000	253	136.9	0.18
BPD	Yes	35	2.8	1.27
	No	2,434	1,179.0	0.21
Age*	0	120	65.4	0.18
(months)	1-3	1,120	223.4	0.50
	4-6	632	249.7	0.25
	>6	597	643.3	0.09
RSV season	1996/1997	815	345.9	0.24
	1997/1998	738	432.1	0.17
	1998/1999	916	403.8	0.23
Month of RSV season	October	97	139.0	0.07
	November	574	152.2	0.38
	December	824	163.1	0.51
	January	520	170.2	0.31
	February	263	177.5	0.15
	March	140	186.2	0.08
	April	51	193.6	0.03

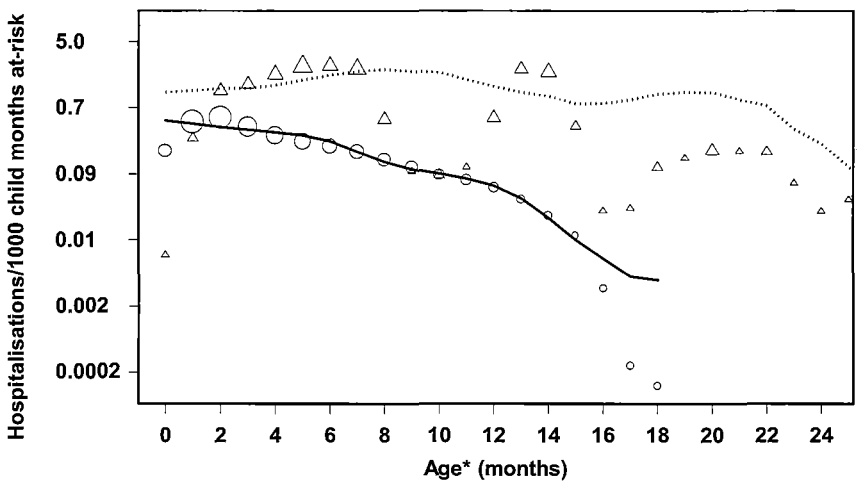
* Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

of age (76%, 1,872/2,469). Over half of the hospitalisations were in December and January (54%, 1,344/2,469).

The population at-risk consisted of 140,661 newborn children who were 1,181,790 months at risk. We found higher monthly hospitalisation risks for males and children suffering from BPD. The risk increased with decreasing gestational age and birth weight. It also varied within the RSV season, with a seven times as high risk in December compared to October. The risk decreased more steeply with increasing age for children without BPD compared to children with BPD (Figure 2.1.1). All hospitalised children with BPD and more than 95% of the children at-risk with BPD had a gestational age below 35 weeks (table 2.1.2).

The uni- and multivariable odds ratios (ORs) with 95% confidence intervals of the five clinical predictors are shown in table 2.1.3. The 95% confidence intervals of the multivariable ORs excluded 1, indicating statistically significant associations. Age and the seasonal RSV pattern were the strongest predictors, while gestational age, presence of BPD, birth weight and gender were less important. The final prediction rule included gender, gestational age (in 5 categories), birth weight (in 3 categories), two terms for age (with and without BPD, interaction between age and BPD was highly significant; $p<0.0001$) to model the differences in risk reduction over time,

Figure 2.1.1. *Relation between age* and hospitalisation risk for children with (dotted line) and without BPD (continuous line).*



* Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

○ Children without BPD, symbol size proportional to the number of RSV hospitalisations.

△ Children with BPD, symbol size proportional to the number of RSV hospitalisations.

Table 2.1.2. *Gestational age of hospitalised children and children at-risk with BPD.*

		Hospitalised children (*n*=35) N	Total months at-risk (*n*=2,752) N	Monthly risk %
Gestational age	≤ 28	24	1,496	1.60
(weeks)	29-30	5	647	0.77
	31-32	4	315	1.27
	33-34	2	171	1.17
	35-36	0	8	0
	≥ 37	0	115	0

Table 2.1.3. *Results of the uni- and multivariable regression analyses for the five clinical variables included in the prediction rule.*

		Univariable analysis		Multivariable analysis	
		OR	95% CI	OR	95% CI
Gender	Male	1.4	1.3 - 1.5	1.4	1.3 - 1.5
	Female†	1		1	
Gestational age	≤ 28	7.6	5.4 -11	3.2	2.1 - 4.8
(weeks)	29-32	5.3	4.3 - 6.7	2.8	2.1 - 3.8
	33-34	4.0	3.3 - 4.8	2.3	1.8 - 3.0
	35-36	2.2	1.9 - 2.6	1.6	1.3 - 1.9
	≥ 37†	1		1	
Birth weight	≤ 2500	3.0	2.7 - 3.3	1.7	1.5 - 2.0
(grams)	2501-3000	1.3	1.2 - 1.5	1.3	1.1 - 1.4
	> 3000†	1		1	
BPD‡	Yes	4.3		2.2	
	No†	1		1	
Age*	BPD absent	0.8	0.8 - 0.8	0.8	0.8 - 0.8
(months)	BPD present	1.0	1.0 - 1.0	0.9	0.9 - 1.0

* Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

† Reference category.

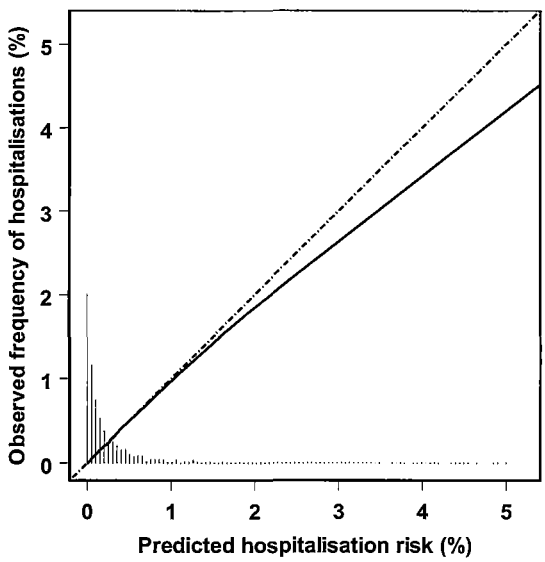
‡ At 6 months of age, confidence interval could not be estimated.

The mean seasonal pattern of RSV infections was also included in the uni- and multivariate analysis as a continuous term.

'age>0 months' (yes/no) to model the rise in hospitalisation risk from age=0 months to age=1 month, and a continuous term for season. The exact formula is included in appendix A.

The area under the ROC curve (AUC) of the prediction rule for all children at-risk was 0.80. For premature children with a gestational age below 33 or 37 weeks, the AUCs were 0.75 and 0.80 respectively. Figure 2.1.2 shows the agreement between the hospitalisation risks predicted by our prediction rule and the observed frequencies of hospitalisation in our cohort. The dotted line represents the calibration curve of the ideal situation that predicted RSV hospitalisation risks are identical to the real observed frequencies of hospitalisation (perfect calibration). The continuous line represents the calibration curve of our prediction rule, which deviates from the line representing perfect calibration for predicted risks over 1.5%. This inaccuracy is possibly related to the low percentage (5.6%) of months at-risk with such high

Figure 2.1.2. *Non-parametric calibration plot, showing the agreement between the hospitalisation risks predicted by our prediction rule and the real observed frequencies of hospitalisation. The dotted line represents the ideal situation of perfect agreement. The continuous line represents the calibration curve of our prediction rule. The distribution of the predicted risks is shown above the x-axis.*



predicted risks. The overall goodness of fit of the model was satisfactory, as indicated by a nonsignificant Hosmer-Lemeshow test (p=0.79). One hundred bootstraps indicated minor optimism in AUC (0.001), which was consistent with the large sample size that was used to develop the model

The implicit AAP thresholds for the monthly hospitalisation risk were 0.6% for children with a gestational age of 28 weeks or less, and 0.8% for children with a gestational age of 29-32 weeks. Children for whom the prediction rule estimated risks above the thresholds (passive immunisation recommended), had a mean observed monthly risk of 2.0% (65 hospitalisations in 3,179 months) compared to 1.6% for children immunised according to the AAP guidelines (62 hospitalisations in 3,996 months). Further, children for whom the prediction rule estimated risks below the thresholds (passive immunisation not recommended), had a mean observed monthly risk of 0.3% (15 hospitalisations in 4,721 months) compared to 0.5% for children immunised according to the AAP guidelines (18 hospitalisations in 3,904 months).

Some examples of predicted monthly and cumulative (seasonal) hospitalisation risks for children that would be immunised according to the AAP guidelines are shown in table 2.1.4. Grey cells represent months in which passive immunisation is recommended according to the prediction rule using the above defined thresholds. Compared to the AAP guidelines, the prediction rule limits the number of immunisations given to each child by excluding low-risk months. In our cohort the number of immunisations would theoretically have been reduced from 3,996 to 3,179 (-20%) for children with a gestational age of 32 weeks or less.

Discussion

We incorporated five important clinical risk factors (gestational age, presence of BPD, birth weight, gender and age) and the average seasonal pattern of the monthly incidence of RSV infections in a clinical prediction rule. This prediction rule estimated individual monthly risks for RSV hospitalisation and enabled a better targeting of passive immunisation than the AAP guidelines in children with a gestational age of 32 weeks or less, by excluding low-risk months. As a consequence, the number of immunisations could theoretically be reduced in this group. Since one immunisation with palivizumab costs about € 900, this could lead to a better balance between costs and effects than achieved with the AAP guidelines.

We are the first to consider individual monthly hospitalisation risks for severe RSV disease. Since all published studies report yearly or seasonal incidences of RSV

Table 2.1.4. *Examples of predicted monthly and cumulative (seasonal) hospitalisation risks (%) for children that would be immunised according to the AAP guidelines.*[2]

Child characteristics					Monthly risk							Cumulative risk
Gender *(M/F)*	Gestational age *(weeks)*	Birth weight *(grams)*	BPD *(Y/N)*	Age* *(months)*	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
F	28	1100	N	0	0.1	2.2	3.6	1.9	1.2	0.5	0.1	9.6
F	28	1100	N	6	0.2	0.6	1.0	0.5	0.3	0.1	0.0	2.9
F	28	1100	N	12	0.0	0.2	0.3	0.2	0.1	0.0	0.0	0.8
M	32	1800	N	1	0.6	2.5	4.1	2.2	1.4	0.6	0.1	11.5
M	32	1800	N	6	0.2	0.9	1.5	0.8	0.5	0.2	0.1	4.1

* Age at the beginning of the RSV season (October). Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks. Grey cells indicate that passive immunistation is recommended according to the prediction rule using thresholds of 0.6%, and 0.8% as described in the text.

hospitalisation we calculated the incidence of RSV hospitalisation in the first year of life in our cohort for comparison. We found higher hospitalisation risks for children born with a gestational age of 32 weeks or less compared to the total population (8.0% vs. 1.7%). Within the group of children born with a gestational age of 32 weeks or less, those with BPD had a higher risk compared to those without BPD (10.4% vs. 7.5%). These hospitalisation risks are within the range of other recently reported figures that vary from 9% up to 32% for premature children with BPD and from 0% up to 12% for premature children without BPD.[27 29-34] The higher hospitalisation risks for males, low birth weight children and young infants are also in accordance with the literature.[24 38 99]

The distribution of monthly hospitalisation risks varied strongly within a RSV season, making seasonality the second most important predictor. However, the use of a mean seasonal pattern has its limitations. It ignores the variability in the patterns of RSV infections that are observed year by year. The RSV season in The Netherlands generally starts in November and peaks in December or January. Occasionally it starts in October or peaks in February. However, during most seasons the highest risks are observed from November to February, as is the case with our mean seasonal pattern. In general, similar seasonal patterns are found in regions with temperate climates and we therefore do not expect the Dutch situation to differ considerably from other Northern European and American regions.[31 32 100] However, the prediction rule should be validated prospectively in our own and other settings before application is justified. Nonetheless, the use of more risk factors and especially seasonality is expected to determine individual RSV hospitalisation risks more accurately in any population.

To appreciate the results some aspects need to be addressed. For our analysis we assumed that almost all infants that were hospitalised in our region during the RSV season with a respiratory illness were tested for RSV. All hospitals in our region routinely perform RSV assays in children hospitalised with bronchiolitis to confirm the presence or absence of RSV to prevent nosocomial spread of RSV within the hospital. We therefore do not expect a major underestimation of the hospitalisation risk.

Second, we could not control for children migrating across the border of the study region. We assumed that the migration rate was low and balanced, and did not lead to an over- or underestimation of the hospitalisation risk.

Third, we could not include other potential predictors for RSV hospitalisation like CHD, immunodeficiencies and environmental factors in the prediction rule, since these predictors were not registered for the children at-risk. These characteristics may be important for final decision making in individual children.

Furthermore, we used thresholds to compare the prediction rule to the AAP guidelines. Ideally such thresholds should be based on a formal cost-effectiveness analysis, where the costs of passive immunisation are weighed against the benefits of preventing RSV related hospitalisations. However, currently available cost-effectiveness analyses of passive immunisation against RSV showed divergent results, which may be explained by differences in study methods and underlying assumptions.[95]

Finally, 85% of the hospitalised children were neither prematurely born nor suffered from BPD. These children are generally classified as being of low risk and will therefore not be eligible for passive immunisation. To prevent severe RSV disease in this vast majority of children, an inexpensive, save, and effective vaccine for active immunisation is needed.

We conclude that it is possible to reliably predict monthly RSV hospitalisation risks for individual children. These individual risks may be helpful to clinicians to allocate passive immunisation to the highest risk children at the most efficient time points within the RSV season, thus reducing the total number of immunisations given.

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2.2

The risk of hospitalisation in young children due to respiratory syncytial virus infection: the role of season in the validity of a prediction model

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Submitted

Abstract

We assessed the role of season in a prediction model for monthly risk of hospitalisation due to severe respiratory syncytial virus (RSV) infection. We studied children born in the Southwest of The Netherlands in 1996, 1997 or 1998 (1,181,790 children-months at-risk), who were admitted due to RSV infection (n=2,469) in one of the seasons 1996/97, 1997/98, or 1998/99. A logistic regression model was constructed with 5 clinical predictors and a variable for the average RSV seasonal pattern (seasonality). We compared this model to a model with the 5 clinical predictors only (clinical model). Model performance was assessed according to discrimination (area under the ROC curve, AUC) and calibration (graphically and with goodness of fit tests). The models, developed with the total data, were validated per RSV season leading to three validation samples. The model including seasonality (seasonal model) had on average significantly higher discriminative ability than the clinical model (AUC: 0.793 [95% CI: 0.792-0.794] vs. 0.738 [95% CI: 0.737-0.739]; $p<0.001$). Discrimination of the seasonal model in each of the validation samples was better than that of the clinical model. Calibration was poor however among the seasons for both the seasonal and clinical model. In conclusion, including the average seasonal RSV pattern in the model improved the discrimination, but differences in calibration among RSV seasons were found for both the seasonal and the clinical model. Incorporation of seasonality in a more dynamic way may improve the calibration.

Introduction

Respiratory syncytial virus (RSV) is the most frequent cause of severe viral respiratory infections in infants world-wide.[30 114] RSV epidemics result in significant morbidity and major health care expenditure.[115] Several groups of children are at high risk for severe RSV disease and hospitalisation, including prematurely born infants and infants with bronchopulmonary dysplasia (BPD).[114 116 117] Severe RSV infections may be prevented with passive immunisation.[2 74 77 118] However, because of the high costs, its use needs to be restricted to children at high risk for severe infections.[1 119]
We previously developed a logistic regression model to predict the monthly risk of hospitalisation due to severe RSV infection among children in the Southwest of The Netherlands. The model included five child characteristics (gender, presence of BPD, gestational age, birth weight, and age) and a variable describing the average seasonal pattern (seasonality). Seasonality was a strong predictor in the model. The average performance of the model was good, when evaluated in the same data as used for development of the model (apparent validation).
The pattern of RSV epidemics however differs substantially across seasons. RSV seasons show a marked heterogeneity with respect to the total number of cases, the peak month of the season, and the distribution of cases among the season months. Hence, the performance of the model in different RSV seasons can be seriously hampered. An alternative model might even be considered, excluding seasonality as a predictor.
We therefore aimed to determine the value of the average seasonality as a predictor, by comparing the performance of a prediction model including this predictor with a model excluding this predictor. Further, we aimed to determine the validity of the models per RSV season and to assess the impact of the seasonal variability.[120]

Patients and methods

Patients

We performed a prognostic study with data from a large Dutch population-based cohort. Cases were children born between January 1, 1996 and December 31, 1998, hospitalised in one of 29 hospitals in the Southwest of the Netherlands during one of the RSV seasons 1996/97, 1997/98 and 1998/99, and younger than 1 year (or younger than 2 years for children with BPD) at the beginning of the RSV season (defined from October 1 to April 30). RSV disease was confirmed by positive direct immunofluorescent assay or a positive viral culture. Data of 2,469 cases were derived

from individual patient records. Children at-risk were born between January 1, 1996 and December 31, 1998 and resided in the study region. At-risk data were obtained from The Netherlands Perinatal Registry. The total number of children at-risk was 140,661, together being 1,181,790 months at-risk.

Model

We developed logistic regression models to predict the risk of hospitalisation due to RSV infection. The predictors in the 'seasonal model' were 5 clinical characteristics and an external variable describing the average RSV seasonal pattern (seasonality). The clinical variables were gender, BPD, birth weight, gestational age, and age. For comparison, we developed a model with 5 clinical variables only (clinical model).
Birth weight and gestational age were included as categorical variables. Age (effective time of exposure to RSV, counted from 38 weeks of gestational age if the gestational age was lower than 38 weeks) was expressed in months and included linearly in the model. We included different age coefficients for children with BPD and without BPD, because of statistically significant interaction. The risk in the first month of age was modelled separately. Seasonality was expressed in months, from October to April, as the average of the Dutch national seasonal pattern of 8 RSV seasons (1991/92-1998/99), using sine and cosine functions.[31 107 121] The monthly risk of hospitalisation due to RSV infection can be calculated from the Appendix A.
We calculated the univariable and multivariable odds ratio (OR) of each variable included in the models. An univariable OR was calculated as the ratio between odds of being hospitalised for severe RSV infection and odds of not being hospitalised. The multivariable ORs were calculated for each RSV season separately (1996/97, 1997/98 and 1998/99) and for all the RSV seasons together.

Model performance

The performance of the seasonal and clinical models was studied with respect to discrimination and calibration. Discrimination refers to the ability to distinguish high risk subjects from low risk subjects. It is commonly quantified by the area under the receiver operating characteristic (ROC) curve (AUC). The AUC lies between 0.5 to 1 and is better if closer to 1.[109 122]
Calibration refers to whether the predicted risks agree with the observed risks. An impression of the calibration was obtained by plotting the observed frequencies (y axis) versus predicted risks (x axis). The resulted calibration plot can be described by an intercept (alpha) and calibration slope (beta).[122] To estimate the calibration slope, we fitted a logistic model with the linear predictor as a single covariable: observed hospitalisation = alpha + beta*(linear predictor). The observed hospitalisation was

dichotomous (yes/no) and the linear predictor was calculated as the linear combination of the regression coefficients with the values of the predictors for each patient in the validation data. To facilitate the interpretation of the intercept (alpha), it was reported with the calibration slope fixed at one (alpha | beta=1).[123]
Models which are adequately calibrated have a slope of 1. We tested the null hypothesis of good calibration with an unreliability statistic.[123] [124] This is a goodness of fit statistic which tests deviations of the intercept from zero and slope from 1, with 2 two degrees of freedom. We tested both the seasonal and clinical RSV model, assessing the model performance on the log likelihood scale.

Validation

Validation per season was performed by developing the prediction model with data of 3 seasons (1996/97, 1997/98 and 1998/99) and validating it in each one of the RSV seasons (e.g. 1996/97, 1997/98 or 1998/99). This procedure was considered appropriate because optimism was very limited given the large number of events.[122] [125] Alternatively, we performed two more validation procedures: 1. Cross-seasonal validation (i.e. the model was developed in 2 seasons and validated in the third season, for a total of three analyses) and 2. Split-sample validation (i.e. model was developed in season 1996/97 and tested in two seasons separately [1997/98 or 1998/99]). These additional validation procedures gave very similar results to the validation per season.
We tested whether the regression coefficients estimated in the validation seasons were similar to the average estimates by including interaction terms of variable*season. We used a significance level of $p<0.05$ and 95% Confidence Intervals (95% CI). The software used was SPSS 10.0 (SPSS Inc., Chicago IL, USA, 1999), SAS 6.12 (SAS Institute Inc., Cary, NC, USA, 1996) and S-PLUS 2000 (Insightful Inc, Seattle WA, USA).

Results

General Characteristics

Table 2.2.1 shows the distribution of cases and children-months at-risk for the variables included in the seasonal model. All 95% CIs excluded 1, indicating statistically significant associations. Boys with BPD, birth weight equal or less than 2500g, born with 28 weeks or less, aged 2 months in December had the highest risk to be hospitalised due to RSV infection (7.6%).

The observed number of cases per month in the three RSV seasons (1996/97, 1997/98 and 1998/99) and the average number of cases per month over 8 RSV seasons from The Netherlands (1991/92-1998/99) are shown in figure 2.2.1. RSV season 1996/97 follows a similar pattern as the average Dutch pattern, RSV season 1997/98 had less cases in the peak of the season than the average pattern and RSV season 1998/99 is considerably different from the other seasons: it began earlier, had a higher peak and finished earlier than the average pattern. Table 2.2.2 shows the peak months of the RSV seasons, the number of observed cases of each peak month and the relative weight when the months are compared to the average peak. It is demonstrated that the

Table 2.2.1. *Distribution of cases, children months at-risk and univariable Odds Ratios for the variables included in the logistic regression model.*

Variable	Definition	Cases (n=2,469)	Children months at-risk (n=1,181,790)	OR*	95% CI
Gender	Male	1,463	605,278	1.4	1.3 - 1.5
	Female‡	1,006	576,512	1	
Gestational age	<=28	35	2,584	7.5	5.4 - 10.4
(weeks)	29-32	78	7,775	5.3	4.2 - 6.7
	33-34	105	14,250	4.0	3.2 - 4.8
	35-36	164	39,246	2.2	1.9 - 2.6
	>=37‡	2,087	1,117,936	1	
Birth weight	<=2500	394	74,662	3.0	2.7 - 3.3
(grams)	2501-3000	443	190,670	1.3	1.2 - 1.4
	>3000‡	1,632	916,458	1	
BPD†	Yes	35	2,752	4.3	NA
	No‡	2,434	1,179,038	1	
Age	0 to 4	1,484	371,349	28	16 - 50
(months)	5 to 9	765	415,497	13	7.1 - 24
	10 to 14	209	317,357	4.6	2.5 - 8.5
	15 to 22‡	11	77,438	1	
RSV season	Oct	97	139,047	2.7	1.9 - 3.7
(months)	Nov	574	152,214	14	11 - 19
	Dec	824	163,092	19	15 - 25
	Jan	520	170,151	12	8.7 - 16
	Feb	263	177,473	5.6	4.2 - 7.6
	Mar	140	186,206	2.9	2.1 - 3.9
	Apr‡	51	193,607	1	

* OR: Univariate Odds Ratio.

† At 6 months of age, confidence interval not estimated (NA).

‡ Reference category.

Figure 2.2.1. *Seasonal pattern in three Southwest Region RSV seasons (continuous line) and mean pattern over 8 RSV seasons in the Netherlands (dotted line).*

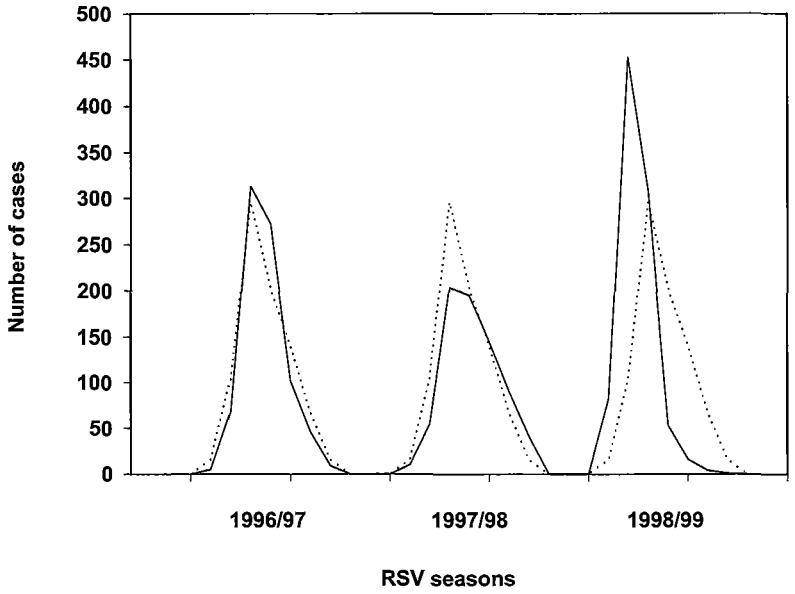


Table 2.2.2. *Peak months, observed cases and relative weight to the average peak month from 8 Dutch RSV seasons and 3 Southwest RSV seasons.*

RSV season	Peak		
	Month	Cases	Weight
The Netherlands			
1991/92	Dec	439	0.7
1992/93	Dec	481	0.8
1993/94	Jan-Feb	350	0.5
1994/95	Dec	1,385	2.2
1995/96	Feb	461	0.7
1996/97	Jan	615	1
1997/98	Jan	393	0.6
1998/99	Dec	1,030	1.6
Average		644	1
Southwest Region			
1996/97	Dec	313	1
1997/98	Dec-Jan	199	0.6
1998/99	Nov	452	1.4
Average		321	1

Table 2.2.3. *Number of cases and multivariable OR per RSV season and for all seasons. The chi-square valuesof the interactions variable*season are shown to indicate which ORs differ clearly by season.*

		1996/97 (n=815)		1997/98 (n=738)		1998/99 (n=916)		All seasons	Chi-square
Variable	Definition	Cases	OR	Cases	OR	Cases	OR	OR	Interaction
Gender	Male	490	1.5	417	1.3	556	1.5	1.4	2.3
BPD*	Yes	11	2.8	8	1.8	16	2.8	2.2	NA
Birth weight	<=2500	140	1.9	94	1.4	160	1.9	1.7	15.1
(grams)	2501-3000	146	1.3	125	1.1	172	1.4	1.3	
	>3000†	529	1	519	1	584	1	1	
Gestational age	<=28	9	3.6	9	2.9	18	3.0	3.2	4.8
(weeks)	29-32	27	3.5	20	3.0	30	2.2	2.9	
	33-34	39	2.5	21	1.9	45	2.4	2.3	
	35-36	63	1.9	40	1.4	61	1.4	1.6	
	>=37†	677	1	648	1	762	1	1	

Table 2.2.3. *Continued.*

		1996/97 (n=815)		1997/98 (n=738)		1998/99 (n=916)		All seasons	Chi-square
Variable	Definition	Cases	OR	Cases	OR	Cases	OR	OR	Interaction
RSV Season	Oct	5	1.6	10	0.9	82	0.8	1	102.6
by months‡	Nov	68		54		452			
	Dec	313		203		308			
	Jan	272		195		53			
	Feb	102		145		16			
	Mar	46		90		4			
	Apr	9		41		1			
Age with BPD			0.98		0.92		0.93	0.93	0.6
Age without BPD			0.82		0.84		0.78	0.81	26.0

* At 6 months of exposure age; confidence interval not estimated (NA).

† Reference category.

‡ Seasonality in each season was standardised to the average.

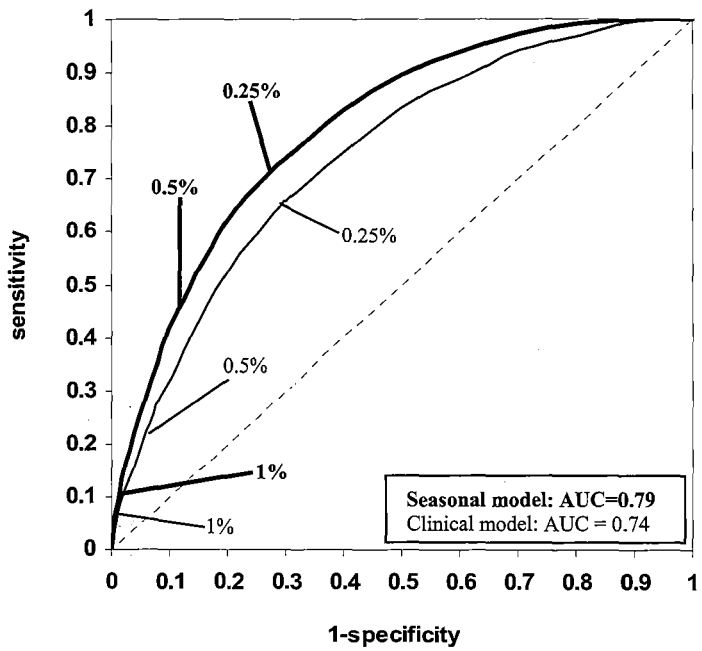
peak months of the RSV seasons differed substantially from the average peak and from each other.

Table 2.2.3 shows differences among the RSV seasons in the number of cases and in the multivariable ORs for the variables considered in the seasonal model. The interaction tests of seasonality and age without BPD were statistically different among the 3 RSV seasons. The differences in gender, birth weight, gestational age and age with BPD were not statistically significant. Also, the chi-squares of the interactions variable*season are shown. Seasonality varied the most among seasons. The RSV season 1996/97 had stronger effects than the seasons 1997/98 and 1998/99 in reference to BPD at 6 months, birth weight, gestational age and seasonality.

Overall performance

The overall discrimination of the seasonal model was good (AUC 0.793, 95% CI: 0.792-0.794). The discriminative ability of the clinical model was significantly lower (AUC 0.738, 95% CI: 0.737-0.739, p<0.001). Figure 2.2.2 shows the corresponding ROC curves with several cut-off points for the two models.

Figure 2.2.2. *Receiver Operating Characteristic (ROC) Curves of RSV seasonal model (thick line) and RSV clinical model (thin line). For each model three cut-off points of predicted risk are indicated in the graph (0.25%, 0.5% and 1%). The dotted line from (0,0) to (1,1) indicates no discrimination. AUC defines Area under the ROC curve.*



The predicted risks of the seasonal and clinical models were well calibrated for predicted risks up to 2%, but higher predicted risks were somewhat too high. For higher predicted risks, the deviation from the ideal line was larger for the clinical model than for the seasonal model.

Seasonal Validation

The discrimination of the seasonal model estimated in each validation was higher than the discrimination of the clinical model (table 2.2.4). The AUCs ranged from 0.77 to 0.82 in the seasonal model and from 0.71 to 0.78 in the clinical model. These results were in agreement with the results of the average performance.

The calibration slopes of nearly all validations showed values significantly different from 1 (figure 2.2.3A and 2.2.3B). The validation of the seasonal model in season 1996/97 and validation of the clinical model in the seasons 1996/97 and 1998/99 showed calibration slopes larger than 1. The other calibration slopes were less than 1. The calibration curves showed similar directions of miscalibration per validation season for both the seasonal and clinical model. For example, predictions in both models were too high in RSV season 1997/98. Below a predicted risk of 0.5%, the calibration was good for all RSV seasons and for both the seasonal and clinical models.

The tests for good calibration (Intercept=0 and calibration slope=1) showed statistically significant values for both models in nearly all seasons. Only the clinical model showed good calibration in the season 1996/97 (X^2=1.08, p=0.58). The sum of chi-squares over 3 seasons showed similar values (Total X^2=87 for the seasonal model and total X^2=79 for the clinical model). This indicates a slightly poorer calibration for the seasonal model.

Table 2.2.4. *Discrimination per validation season and average discrimination in both RSV seasonal and clinical model.*

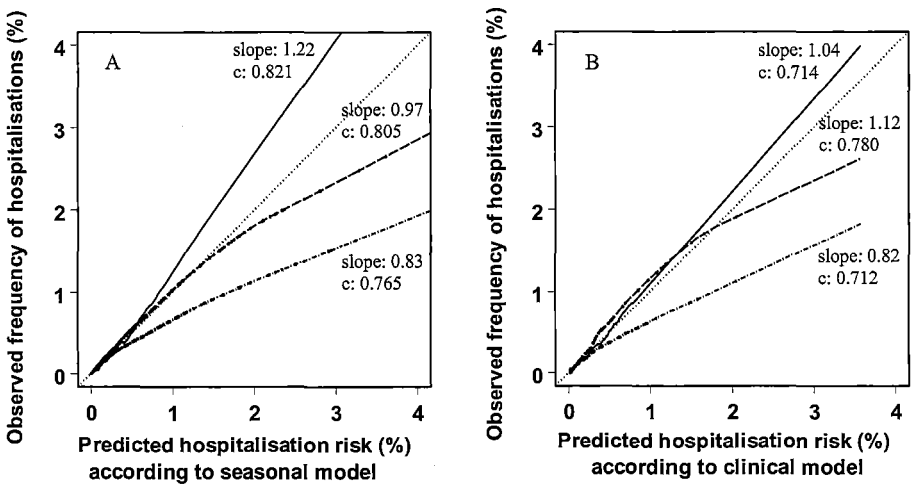
Validation season	c-statistic	
	Seasonal	Clinical
1996/97	0.82	0.71
1997/98	0.77	0.71
1998/99	0.81	0.78
Average	0.79	0.74

Discussion

This study showed that the inclusion of the average seasonal pattern (seasonality) led to a significant improvement of the discriminative ability of a model that predicts the risk of hospitalisation due to severe RSV infection in children. Hence, the prediction model should include seasonality. Further, the seasonal pattern of occurrence of RSV infections varies substantially among RSV seasons. We found that the performance measures across RSV seasons were substantially different, despite that the average model was not optimistic.

Calibration was poor in the validation seasons. This was slightly worse with the seasonal model than with the clinical model. These findings are related to the particular pattern of the RSV epidemics that differs substantially across seasons with respect to total number of cases, peak month of the season, and distribution of cases among season months. The poor calibration could only partly be explained by the heterogeneity of effects of the clinical predictors across seasons in comparison to the average model. Further, calibration was comparable among seasons and for both models below a predicted risk of 0.5%.

Figure 2.2.3A. and 2.2.3B. *Non-parametic calibration curves (continuous lines) of validation seasons in RSV seasonal model (3A) and RSV clinical model (3B). Calibration slope and AUC of each validation season are shown. The dotted line indicates perfect calibration.*



——— Season 1996/97.
—·—·— Season 1997/98.
– – – Season 1998/99.

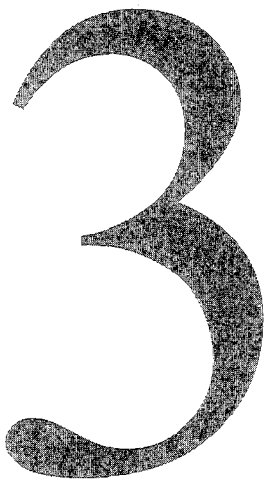
We noted that the discriminative ability in each of the validation seasons is not only affected by seasonal differences, but also by differences in the distributions of predictor variables ("case-mix"). The case-mix determines the distribution of the predicted risks. If many predictions are close to the overall risk, discrimination is poor. The difference in case-mix explains completely the different AUCs in the clinical model between the combined seasons and validation season 1996/97, because the regression coefficients of the models were very similar.

Some previous papers have evaluated the risk of hospitalisation and mortality due to severe RSV infection in children.[30 31 114 115 117 121] The majority of them did not consider seasonality as a major predictor. Three only described the associated clinical predictors, the risks and the related costs.[30 114 117] and one described a logistic model to predict prolonged hospital stay.[115] Two recent papers recognised the importance of seasonality in prediction models. In one of them seasonality was incorporated in a prediction model of mortality, using sine and cosine functions.[121] However, the incremental value of seasonality in the prediction of hospitalisation was not evaluated nor were validation procedures considered to evaluate the performance of the model including seasonality.

The heterogeneity of the seasonal patterns can possibly be accounted for in more detail by including seasonality as a dynamic variable in the model. For instance, when more cases than on average are identified before the average RSV peak, we might be able to adjust for this difference when making predictions for future months. For example, when fewer than average cases occur in November, our prediction for December should be lower than average. Bayesian methods might be useful to construct such a dynamic model.[126]

We expected small optimism for the validation procedures because we used a huge number of children at-risk and a large number of events per variable (EPV) in order to develop the model.[109 122] Indeed, we found that bootstrapping, a method used commonly to determine optimism, resulted in very small optimism estimates (data not shown).[122 125]

Our paper has several limitations. We only used three RSV seasons to develop the model and to assess the influence of variability among seasons. More RSV seasons would provide more reliable insights. Further, the model should be validated in other settings, even though we do not expect major differences in the seasonal pattern in countries with temperate climates. This will improve the assessment of external validity and will determine the importance of seasonality in other populations. [127]



Economic evaluation of passive immunisation against RSV

3.1

Costs of hospitalisation for respiratory syncytial virus infection in young children

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Submitted

Abstract

Background

Reliable estimates of hospitalisation costs for severe RSV infection are necessary to perform economic analyses of preventive strategies of severe RSV disease. We aimed to develop a model that predicts anticipated mean RSV hospitalisation costs of groups of young children at-risk for hospitalisation, but not hospitalised yet, based on readily available child characteristics.

Methods

We determined real direct medical costs of RSV hospitalisation from a societal perspective, using a bottom-up strategy, in 3,458 infants and young children hospitalised for severe RSV disease during the RSV seasons 1996-1997 to 1999-2000 in the Southwest of the Netherlands. We developed a linear regression model, that predicts anticipated mean RSV hospitalisation costs of groups of children at-risk, based on four child characteristics (age, gestational age, birth weight, and bronchopulmonary dysplasia (BPD))

Findings

The mean RSV hospitalisation costs of all patients were €3,110. The hospitalisation costs were strongly related to the duration of hospitalisation. Therefore RSV hospitalisation costs were higher for patients with lower gestational age (€5,555; gestational age ≤28 weeks), lower birth weight (€3,895; birth weight ≤2500 g), BPD (€5,785; with BPD), and young age (€4,730; first month of life). The linear regression model had an adjusted R^2 of 0.08. This indicates a low explanatory ability for hospitalisation costs of individual children. However, the model could accurately estimate the anticipated mean hospitalisation costs of groups of children with the same characteristics.

Interpretation

RSV hospitalisation costs were substantial, especially of specific high-risk groups, and were highly correlated with duration of hospitalisation. Although anticipated individual hospitalisation costs of children at-risk for RSV hospitalisation were hard to predict, anticipated mean hospitalisation costs of groups of children at-risk for RSV hospitalisation could well be estimated with four child characteristics (age, gestational age, birth weight, and BPD). These estimated costs can be used for economic analyses of preventive strategies for severe RSV disease.

Introduction

Respiratory syncytial virus (RSV) causes upper and lower respiratory tract infections in infants and young children. Of all infected infants 0.5%-2% are hospitalised because of severe disease. Children with prematurity or bronchopulmonary dysplasia (BPD) are at higher risk for hospitalisation. Passive immunisation against RSV can prevent severe RSV disease in these high-risk children. However, passive immunisation has a moderate efficacy (about 50%),[1] and is very costly (approximately €4,650 per child for a complete RSV season). Given the generally low incidence of RSV hospitalisation, concerns have been raised about the cost-effectiveness of passive immunisation.[80-85] Approximately 80% of all children hospitalised with a severe RSV infection are not considered to be at high-risk, and do not qualify for passive immunisation. For these children vaccines are being developed that could be available in the near future.[71]

Since health care budgets are limited, economic analyses are needed to optimise the allocation of resources in health care. Reliable estimates of costs of RSV hospitalisation are necessary to perform economic analyses of present and future preventive strategies of severe RSV disease. Current cost-effectiveness analyses of passive immunisation against RSV typically use mean RSV hospitalisation costs derived from broadly defined groups of high-risk children.[27 29 30 86-94 128-131] Some distinguish different subgroups, based on gestational age or presence of BPD. None of these studies has estimated specific costs of RSV hospitalisation of low-risk children. These costs are however highly relevant for economic analyses of future vaccination strategies. Furthermore, none has used a more individualised approach that accounts for a combination of factors that influence RSV hospitalisation costs. Such an approach would allow a more detailed estimation of RSV hospitalisation costs of children based on individual child characteristics.

We aimed to estimate anticipated RSV hospitalisation costs in children at-risk for hospitalisation, but not hospitalised yet. We developed a regression model that estimates anticipated mean RSV hospitalisation costs in groups of children at-risk, based on a limited set of readily available child characteristics.

Patients and methods

A population based cohort study was conducted in the Southwest of the Netherlands. All 29 hospitals in the region with a paediatric ward (one university hospital, five teaching hospitals, and 23 non-teaching hospitals) participated in the study. Two of

these hospitals have paediatric intensive care facilities. The study was approved by the institutional review board of the Erasmus MC.

Patients

We included patients who were hospitalised for severe RSV disease and met the following criteria:

- Hospitalisation in one of the 29 hospitals in the region.
- Hospitalisation during the RSV seasons 1996-1997 to 1999-2000 (RSV season starts October 1st and ends April 30th).
- Age less than one year at the beginning of the RSV season or less than two years for children with BPD.
- RSV infection confirmed by a positive direct immunofluorescent assay or viral culture of nasopharyngeal aspirates.

The registry of virological diagnostic assays was used to identify the children in each hospital. Children with nosocomial infections (defined by a positive RSV test more than five days after hospital admission without symptoms of RSV disease) were excluded.

Routinely documented patient characteristics were collected from medical records, in standardised forms, including gender, gestational age, birth weight, BPD, age, date of admission, type of hospital.

We defined BPD as the need for supplemental oxygen on day 28 after birth or at a postconceptional age of 36 weeks, in the presence of typical abnormalities on the chest X-ray. [105 106] We corrected age (being the time the child is exposed to RSV in the community) of children born prior to 38 weeks of gestation to a postconceptional age of 38 weeks.[132]

Consumption of health care

Detailed information on the consumption of health care (duration of hospitalisation, transfers between hospitals, level of care, diagnostics, and medical care) was collected from medical records and the hospital information system. Since at term patients were well represented in the study population, we chose to collect this extensive information only for at term patients hospitalised during one RSV season (1999-2000; n=806), but for all patients with prematurity and/or BPD (n=534), giving a total of 1,340 patients. For at term patients hospitalised during the RSV seasons 1996-1997 to 1998-1999 (n=2,118) we only collected information on duration of hospitalisation.

Missing values

Of the 3,487 patients studied, 29 had a missing value for duration of hospitalisation. These patients were excluded, leaving 3,458 patients for the statistical analyses. Gender and age were never missing. 308 (8.9%) patients had missing values for either gestational age or birth weight. Imputations of these missing values were based on the correlation between these variables.[132] 303 (8.8%) patients had missing values for gestational age and birth weight. Imputations of these missing values were randomly drawn from the observed distributions. If BPD was missing (n=11), we assumed that the patient did not have BPD.

Of the 1,340 patients considered for detailed consumption of health care, missing values were present in 70 (5.2%) for intensive care unit (ICU) admission (n=55; 4%), tube feeding (n=61; 5%), intravenous catheter (n=63; 5%), oral antibiotics (n=60; 4%), intravenous antibiotics (n=60; 4%), spray therapy (n=58; 4%), and extra oxygen (n=54; 4%). These missing data were imputed using the correlation with consumption of other items and the patient characteristics.

Costing methods

Real costs were estimated from a societal perspective using a bottom-up strategy.[133] We only considered direct medical costs of RSV hospitalisation. A detailed list of cost-items that covered the total costs of hospitalisation was formulated in discussion with physicians and nurses. Unit prices were collected for all these cost-items. All costs are reported in Euro's (€) as of the year 2000, rounded to five Euro (except costs below €5).

Unit prices

Since hospitalisation costs depend on the type of hospital, unit prices were determined in a university hospital and a non-teaching community hospital.[134] We estimated the unit prices in a teaching community hospital as the average of the unit prices in a university hospital and a non-teaching community hospital.

The unit price for the cost-item 'inpatient day' included accommodation, overhead, and non-disposable equipment. Costs of accommodation and overhead were estimated using detailed information from financial accounts and earlier cost studies (unpublished data).

For all other cost items a unit price was estimated consisting of costs of personnel and disposables. Time and equipment needed per unit were estimated in detail through interviews and questionnaires with caregivers involved, e.g. nurses and physicians. Data on wages were collected from the collective agreements for university hospitals

and general hospitals. Taxes, social security and holidays were all included. Prices of disposables were collected from the hospital administration.
The unit price of the cost-item 'diagnostics' was estimated using charges instead of real costs. Charges were obtained from the governmental body responsible for establishing charges in the Dutch health care sector (CTG Zorg).

Hospitalisation costs

Individual costs per cost-item were calculated for the 1,340 patients with prematurity, BPD, or born at term and hospitalised during the season 1999-2000, by multiplying the unit prices with the corresponding consumption of health care. Subsequently, individual total RSV hospitalisation costs were calculated as the sum of all costs per cost-item (table 3.1.1, step 1).
Data of these patients were used to develop a linear regression model (model I) with total RSV hospitalisation costs as outcome variable and three patient characteristics (gender, age, and BPD), duration of hospitalisation, and hospital type as predictor variables (table 3.1.1, step 2). Predictor variables that had a multivariable p-value <0.20 and clinically plausible effect were selected.[135] We examined non-linearity in the relationships of age and duration of hospitalisation with the total RSV hospitalisation costs. The relation of age with the total hospitalisation costs was approximated by a reciprocal transformation (1/(age+1)), in interaction with BPD. This perfectly described the relation of age with RSV hospitalisation costs in patients without BPD, but led to some overestimation for children with BPD younger than two months. This model was then used to estimate the total hospitalisation costs of at term patients hospitalised during the seasons 1996-1997 to 1998-1999 (n=2,118) (table 3.1.1, step 3).

Predictors of hospitalisation costs

Information on patient characteristics and hospitalisation costs of all patients (n=3,458) was used to construct a linear regression model (model II), that estimates anticipated mean hospitalisation costs of groups of children at-risk, based on four child characteristics (age, gestational age, birth weight, and BPD) (table 3.1.1, step 4). Predictor variables were selected with a backward stepwise selection procedure, removing predictors with p>0.20. In this model age was also included as a reciprocal transformation (1/(age+1)) in interaction with BPD. Duration of hospitalisation and hospital type were not included in this model, since these variables are not known before admission.

Table 3.1.1. *Patients and data used to determine calculated and estimated individual hospitalisation costs and develop linear regression models I and II.*

Step	Patients	Data used	Outcome
1	At term children hospitalised during 1999-2000 (n=809) All children with prematurity and/or BPD (n=525)	Detailed consumption of health care Duration of hospitalisation	Calculated individual hospitalisation costs
2	At term children hospitalised during 1999-2000 (n=809) All children with prematurity and/or BPD (n=525)	Duration of hospitalisation Patient characteristics Calculated indivdual hospitalisation costs	Linear regression model I†
3	At term children hospitalised during 1996-1997 to 1998-1999 (n=2,123)	Duration of hospitalisation Patient characteristics	Estimated individual hospitalisation costs*
4	At term children hospitalised during 1999-2000 (n=809) All children with prematurity and/or BPD (n=525)	Patient characteristics Calculated individual hospitalisation costs	Linear regression model II‡
	At term children hospitalised during 1996-1997 to 1998-1999 (n=2,123)	Patient characteristics Estimated individual hospitalisation costs	

† Linear regression model I predicts individual hospitalisation costs using the predictors gender, age, gestational age, birth weight, BPD, and duration of hospitalisation.

‡ Linear regression model II estimates mean hospitalisation costs using the predictors gender, age, gestational age, birth weight, and BPD.

* Using linear regression model I.

Results

Characteristics of the 3,458 patients hospitalised with a proven RSV infection in the Southwest of the Netherlands during the RSV seasons 1996-1997 to 1999-2000 are shown in table 3.1.2 Of all patients, 93% were hospitalised because of feeding problems, dyspnoea, or apnoea, and 94% were diagnosed with bronchiolitis or pneumonia.

Table 3.1.3 summarises the consumption of health care in at term patients hospitalised during the RSV season 1999-2000, and for all patients with prematurity and/or BPD (n=1,340). Patients with prematurity or BPD were significantly more often admitted to the ICU (7.5% versus 2.6%), transferred to another hospital (9.7% versus 4.0%). They also received significantly more often tube feeding (47.0% versus 38.2%), an intravenous catheter (19.5% versus 14.8%), and spray therapy (74.5% versus 65.0%). The mean duration of hospitalisation was significantly longer for children with prematurity or BPD (7.5 days versus 5.4 days). Furthermore, these patients had a longer duration of tube feeding (5.0 days versus 4.0 days), intravenous catheter (5.0 days versus 3.6 days), oral antibiotics (4.7 days versus 3.6 days), spray therapy (5.7 days versus 3.9 days), and extra oxygen (4.4 days versus 3.3 days). 34 patients of the 61 patients admitted to the ICU needed mechanical ventilation. Of the 127 transfers between hospitals, 87 (69%) were either to or from ICU. The mean duration of hospitalisation increased with lower gestational age (10.1 days for gestational age $\leq$28 weeks and 5.4 days for gestational age $\geq$37 weeks; $p<0.001$), lower birth weight (7.7 days for birth weight $\leq$2500g and 5.2 days for birth weight >4000 g; $p<0.001$), and younger age (8.4 days for age =0 months and 5.4 days for age >6 months; $p<0.001$).

The unit prices in a university hospital and a non-teaching community hospital are listed in table 3.1.4. One inpatient day on the ICU (€850) was more expensive than one inpatient day on low or medium care (university hospital €355, non-teaching hospital €295). Differences were found in costs of diagnostics (university hospital €385, non-teaching hospital €30) and tube feeding (university hospital €70, non-teaching hospital €25).

Table 3.1.5 shows the linear regression model that estimates the total hospitalisation costs of individual at term patients hospitalised during the seasons 1996-1997 to 1998-1999 (model I). Duration of hospitalisation was the strongest predictor. A model with duration of hospitalisation as only predictor had an adjusted R^2 of 0.83, and the complete model had an adjusted R^2 of 0.85. For example, estimated total individual hospitalisation costs of an at term boy (€0), without BPD (€0), and an age of three months ((1/(1+3)*526=€132), who had been hospitalised for five days

Table 3.1.2. *Characteristics of all children (n=3,458) hospitalised with a proven RSV infection in the Southwest of the Netherlands during the RSV seasons 1996-1997 to 1999-2000.*

		N	%
Gender	Male	2,031	58.7
	Female	1,427	41.3
Gestational age	≤ 28	50	1.4
(weeks)	29-30	39	1.1
	31-32	67	2.0
	33-34	139	4.0
	35-36	238	6.9
	≥ 37	2,924	84.6
Birth weight	≤ 2500	533	15.4
(grams)	2501-3000	625	18.1
	3001-3500	1,124	32.5
	3501-4000	825	23.9
	>4000	351	10.1
BPD	Yes	54	1.6
	No	3,404	98.4
Age*	0	165	4.8
(months)	1-3	1,569	45.4
	4-6	866	25.0
	>6	858	24.8
RSV season	1996/1997	851	24.6
	1997/1998	741	21.4
	1998/1999	929	26.9
	1999/2000	937	27.1
Hospital type	University	131	3.8
	Teaching	1,059	30.6
	Non-teaching	2,267	65.6

* Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

(5*497=€2,485) in a teaching community hospital (-€989) were €2,559 (€931 (intercept)+€0+€0+€132+€2,485-€989).

The mean hospitalisation costs of all patients were €3,110 (table 3.1.6). Hospitalisation costs were significantly higher for patients with lower gestational age (€5,555; gestational age ≤28 weeks), lower birth weight (€3,895; birth weight ≤2500 g), BPD (€5,785; with BPD), and young age (€4,730; first month of life).

The linear regression model that estimates anticipated mean hospitalisation costs of children at-risk for RSV hospitalisation (model II) is shown in table 3.1.7 and appendix B. This model had an adjusted R^2 of 0.08, which indicates a high variability of the costs at the individual level between children with the same characteristics. For 429 children with a gestational age ≥37 weeks, birth weight >3000 g, age of one month, without BPD, the observed mean hospitalisation costs were €3,320, with 95% of the costs lying between €1,050 and €8,490 (2.5 and 97.5 percentile). However, the model could accurately estimate mean hospitalisation costs of groups of children with these characteristics (estimated mean €3,370; 95% confidence interval €3,255 to €3,485).

Table 3.1.3. *Consumption of health care in at term children hospitalised during the season 1999-2000, and for all children with prematurity and/or BPD (n=1,340) (Number, %, and mean duration in days, standard deviation).*

	At term 1999-2000 (n = 806)				Premature and/or BPD (n = 534)			
	Number N (%)		Duration Mean (SD)		Number N (%)		Duration Mean (SD)	
Hospitalisation	806	(100)	5.4	(4.2)	534	(100)	7.5	(4.9)***
Intensive care unit	21	(2.6)	5.5	(4.9)	40	(7.5)***	6.8	(6.8)
Number of transfers								
0	774	(96.0)			482	(90.3)***		
1	20	(2.5)			25	(4.7)		
2	9	(1.1)			26	(4.9)		
3	3	(0.4)			1	(0.2)		
Tube feeding	308	(38.2)	4.0	(3.8)	251	(47.0)**	5.0	(5.7)*
Intravenous catheter	119	(14.8)	3.6	(3.8)	104	(19.5)*	5.0	(4.9)*
Antibiotics								
Oral	225	(27.9)	3.6	(2.2)	164	(30.7)	4.7	(2.8)***
Intravenous	71	(8.8)	4.0	(2.8)	65	(12.2)	5.0	(3.5)
Spray therapy	524	(65.0)	3.9	(3.6)	398	(74.5)***	5.7	(4.1)***
Extra oxygen	374	(46.4)	3.3	(2.9)	261	(48.9)	4.4	(3.1)***

* p<0.05 premature children compared to at term children.

** p<0.01 premature children compared to at term children.

*** p<0.001 premature children compared to at term children.

Table 3.1.4. *Unit prices (€) per admission, inpatient day, and event in a university hospital and a non-teaching hospital.*

		University hospital			Non-teaching hospital		
		Admission	Inpatient day	Event	Admission	Inpatient day	Event
Low/medium care							
Admission/discharge		90	-	-	110	-	-
Diagnostics*		385	-	-	30	-	-
Inpatient day		-	355	-	-	295	-
Feeding	Normal	-	85	-	-	95	-
	Tube	-	70	-	-	25	-
Intravenous catheter		40	3	-	60	10	-
Antibiotics	Oral	-	0	-	-	1	-
	Intravenous	-	20	-	-	20	-
Spray		2	1	10	4	3	20
Extra oxygen		20	-	-	20	-	-
Transfer		-	-	135	-	-	120
Intensive care							
Admission/discharge		185	-	-	-	-	-
Diagnostics*		300	-	-	-	-	-
Inpatient day		40	850	-	-	-	-

* Diagnostics include diagnostic viral assays and blood gas analyses.

Discussion

In a large population based cohort of RSV hospitalised children we observed mean RSV hospitalisation costs of €3,110, which varied widely however by patient characteristics. RSV hospitalisation costs were higher for patients with lower gestational age (€5,555; gestational age ≤28 weeks), lower birth weight (€3,895; birth weight ≤2500 g), BPD (€5,785), and younger age (€4,730; first month of life). These differences in RSV hospitalisation costs were mainly due to differences in duration of hospitalisation. Anticipated mean hospitalisation costs of groups of children at-risk for RSV hospitalisation could well be estimated with four child characteristics (age, gestational age, birth weight, and BPD). However, anticipated individual hospitalisation costs for children at-risk for RSV hospitalisation were hard to predict, as indicated by the low percentage of explained variation (8%). The strength of the regression model (model II) lies in the ability to estimate anticipated mean RSV hospitalisation costs of groups of children with different characteristics. For instance, according to the prediction model, the estimated mean RSV hospitalisation costs of high-risk children (age=3 months, gestational age ≤28 weeks, birth weight ≤2500 g, with BPD) were €7,675, compared to €2,585 of low-risk children (age=6 months, gestational age ≥28 weeks, birth weight ≥3001 g, without BPD).

Table 3.1.5. *Coefficients and p-values of the constant and predictor variables of linear regression model I that predicts individual hospitalisation costs of at term children hospitalised during 1996-1997 to 1998-1999 (based on n=1,340).*

		Coefficient (β)	Significance (*p*)
Constant		931	<0.001
Gender	Male†	0	
	Female	-155	<0.01
BPD	Yes	329	
	No†	0	
1/(Age*+1)	BPD absent	526	<0.0001
(months)	BPD present	3,424	
Duration of hospitalisation *(days)*		497	<0.001
Hospital type	University†	0	
	Teaching	-989	<0.001
	Non-teaching	-1,237	<0.001

*Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.
† Reference category.

Table 3.1.6. *Differences in mean hospitalisation costs (€) of several categories of hospitalised children (p-value of T-test or ANOVA for univariable differences of means as appropriate) (n=3,458).*

		Total hospitalisation costs (€)	Significance (*p*)
All		3,110	
Gender	Male	3,130	NS
	Female	3,085	
Gestational age	≤28	5,555	<0.001
(weeks)	29-30	4,310	
	31-32	3,830	
	33-34	3,975	
	35-36	3,290	
	≥37	2,980	
Birth weight	≤2500	3,895	<0.001
(grams)	2501-3000	3,120	
	3001-3500	2,910	
	3501-4000	2,970	
	>4000	2,885	
BPD	Yes	5,785	<0.001
	No	3,070	
Age*	0	4,730	<0.001
(months)	1-3	3,255	
	4-6	3,025	
	>6	2,625	

*Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

Table 3.1.7. *Coefficients and p-values of the constant and predictor variables of linear regression model II that estimates anticipated total hospitalisation costs of children at-risk for RSV hospitalisation (based on n=3,458).*

		Coefficient (β)	Significance (*p*)
Constant		2,274	<0.001
Gestational age	≤28	1,226	
(weeks)	29-34	313	<0.01
	≥35†	0	
Birth weight	≤2500	591	
(grams)	2501-3000	284	<0.001
	≥3001†	0	
1/(Age*+1)	BPD absent	2,192	<0.001
(months)	BPD present	14,344	<0.001

*Age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.
† Reference category.

Many other studies have estimated RSV hospitalisation costs. [3 27 29 30 86-94 117 129-131 136] Most of these estimates are restricted to high-risk children defined by prematurity or BPD. The Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) assessed the costs of RSV hospitalisation in children four years of age and younger.[136] The average costs per hospitalisation were $3,026 (mean duration of hospitalisation of 6.7 days) of tertiary-care patients and $1,512 (mean duration of hospitalisation of 3.7 days) of non-tertiary-care patients. Especially the costs of non-tertiary-care patients are lower than the lowest costs we found of low-risk children (€2,625 for children six months of age and older). Most of this difference does however disappear after correction for duration of hospitalisation (3.7 days versus 5.4 days) and inflation (they report costs in 1993 US dollars, we report costs in 2000 Euros). Unfortunately, hospitalisation costs were not differentiated by patient characteristics such as prematurity or BPD, making a direct comparison with our results impossible.

Miedema et al. estimated RSV hospitalisation costs in RSV hospitalised children in the Dutch region of Eindhoven to be $1,905, with a mean duration of hospitalisation of 5.8 days.[3] This estimate is less than the €3,110 found in our study. However, their patient group was relatively small (69 children), contained relatively few high-risk children (<5% were born premature, none had BPD), and children were excluded from the analysis once they were transported to an ICU.

Howard et al. determined charges instead of real costs in children four years of age and younger, hospitalised with RSV pneumonia. Mean charges were $9,655 of children without comorbidity, and more than $41,000 of children with cyanotic congenital heart disease or BPD. These higher charges in children with comorbidity were associated with a longer duration of hospitalisation, as was observed in our study.[117]

Estimates of RSV hospitalisation costs reported in current cost-effectiveness analysis range from $1,754 (£1,100; £1=$1.6) to $19,190.[27 29 30 86-94 129-131] Differences can be explained by differences in illness severity between the patient groups (reflected in differences in duration of hospitalisation and level of care), and the hospital type and country in which the studies were performed. Differences in the use of rather inexpensive medical interventions such as bronchodillators or antibiotics are not expected to influence the RSV hospitalisation costs dramatically. Like in our study, three studies report higher RSV hospitalisation costs for children with lower gestational ages or BPD.[27 30 86] In another study, Roeckl et al. report higher RSV hospitalisation costs with an increasing number of risk factors (male gender, BPD, presence of siblings visiting day-care attendance, and neonatal ICU discharge

between October and December).[94] O'Shea et al. however found no association between RSV hospitalisation costs and presence or severity of BPD.[129]

To appreciate our results some aspects need to be addressed. Differences in clinical management of RSV infections between hospitals in different countries and even within one country have been reported.[137 138] Duration of hospitalisation (the strongest predictor of total RSV hospitalisation costs in our study) was longer in continental Europe (8-9 days) compared to the United States, Australia, the United Kingdom or Finland (4 days). This will certainly influence the total RSV hospitalisation costs in different regions. Although differences in supportive therapies were also described, these are not expected to have a major influence on total RSV hospitalisation costs, because of their small contribution to the total RSV hospitalisation costs (<10% of the total hospitalisation costs in our study).

Although most unit costs were comparable, striking differences were found in costs of diagnostics (university hospital €385, non-teaching hospital €30) and tube feeding (university hospital €70, non-teaching hospital €25). Costs of diagnostics were much higher in the university hospital because both a direct immunofluorescent assay and viral culture of nasopharyngeal aspirates were performed, looking also for other respiratory viruses like influenza and parainfluenza. The differences in costs of tube feeding are caused by differences in time investment by nurses as a result of differences in feeding techniques between the two hospitals.

Our model (model II) only estimates direct medical costs of RSV hospitalisation. We did not consider indirect medical costs of RSV hospitalisation, such as costs related to persisting periods of recurrent wheezing after RSV infection. These costs are at the moment not relevant for cost-effectiveness analyses of passive immunisation, because passive immunisation has not been shown to influence the occurrence or severity of recurrent wheezing. Secondly, it will be difficult to predict the occurrence of recurrent wheezing in children at-risk for RSV hospitalisation, and thus the related costs. Direct non-medical costs were determined in detail by others.[3 139]

We conclude that mean RSV hospitalisation costs were substantial, and were highly correlated with duration of hospitalisation. As a result of a longer duration of hospitalisation, RSV hospitalisation costs were higher for patients with lower gestational age, lower birth weight, BPD, and younger age. Although anticipated individual hospitalisation costs of children at-risk for RSV hospitalisation were hard to predict, anticipated mean hospitalisation costs of groups of children at-risk for RSV hospitalisation could well be estimated with four child characteristics (age, gestational age, birth weight, and BPD). These estimated RSV hospitalisation costs can be used for economic analyses of preventive strategies for severe RSV disease.

Acknowledgment

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3.2

Passive immunisation against respiratory syncytial virus: a cost-effectiveness analysis

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Submitted

Abstract

Background

The introduction of passive immunisation against respiratory syncytial virus (RSV) led to a discussion concerning cost-effectiveness of this moderately effective and costly prophylactic intervention. We aimed to assess incremental costs to prevent one hospitalisation in high-risk children from a societal perspective, using a novel individualised monthly approach of passive immunisation.

Methods

We performed a cost-effectiveness analysis by combining estimates of individual hospitalisation costs and monthly hospitalisation risks, with immunisation costs, parental costs, and efficacy of passive immunisation. The reference case was a high-risk boy with a gestational age ≤28 weeks, birth weight ≤2500 g, BPD, and age of zero months at the beginning of the RSV season. We performed various sensitivity analyses and a cost-neutrality analysis.

Findings

Cost-effectiveness of passive immunisation varied widely by child characteristics and seasonal month. For the reference case it was most cost-effective in December at €15,520 per hospitalisation averted. Cost-effectiveness was most sensitive to changes in costs of passive immunisation. For the reference case cost-neutrality was reached in December, when acquisition costs of passive immunisation decreased from €930 to €335, monthly hospitalisation risk increased from 7.6% to 20%, or hospitalisation costs increased from €10,250 to €25,750 per hospitalisation. Even when passive immunisation would prevent all hospitalisations, costs per hospitalisation averted would still exceed €2,645.

Interpretation

Although cost-effectiveness of passive immunisation varied strongly by child characteristics and seasonal month, the incremental costs per hospitalisation averted were always high. We therefore recommend a restrictive immunisation policy. The costs of passive immunisation need to be reduced to reach acceptable levels of cost-effectiveness for high-risk children in high-risk months.

Introduction

Respiratory syncytial virus (RSV) is a major cause of respiratory morbidity in infants. 0.5% to 2% of all infected infants are hospitalised. Risk factors for RSV hospitalisation include prematurity, bronchopulmonary dysplasia (BPD), low birth weight, male gender and young age. Furthermore, the seasonal pattern of RSV infections highly influences the monthly hospitalisation risk.[132]

In the absence of a vaccine, passive immunisation against RSV is an alternative to prevent RSV hospitalisation. Palivizumab (Synagis®), a humanised monoclonal antibody to RSV, is safe and effective in preventing RSV hospitalisations in children with prematurity or BPD, when administered on a monthly basis during the RSV season.[77] A Cochrane review reports an odds ratio (OR) of 0.48 for incidence of hospitalisation, favouring passive immunisation.[1]

However, costs of palivizumab are considerable. Treatment of one child during a complete RSV season costs approximately €3,550 (mean weight 5 kg, 5 injections, no wastage).[86] These costs, combined with the moderate efficacy, and the generally low incidence of RSV hospitalisation, have led to a discussion concerning the cost-effectiveness of passive immunisation.[80-85] Several economic analyses of palivizumab for prevention of RSV hospitalisation have been published.[27 29 30 86-94] However, several methodological shortcomings were noted.[95-97] The aim of the present cost-effectiveness analysis was to assess the incremental costs to prevent one hospitalisation in high-risk children from a societal perspective, using a novel individualised monthly approach for decision making on passive immunisation.

Methods

To estimate RSV hospitalisation costs and hospitalisation risk we considered children hospitalised with a proven RSV infection in the Southwest of the Netherlands. This mixed urban-rural region has a population of approximately four million people and an annual birth rate of approximately 47,000 children.[102] All 29 hospitals in the region with a paediatric ward participated in this study. Two of these hospitals have paediatric intensive care facilities.

We defined BPD as the need for supplemental oxygen on day 28 after birth or at a postconceptional age of 36 weeks, in the presence of typical abnormalities on the chest X-ray.[105 106] We corrected age (being the time the child is exposed to RSV in the community) for children born prior to 38 weeks of gestation to a postconceptional age of 38 weeks.[132]

We report costs in Euro's (€) as of the year 2000, rounded to five Euro. The institutional review board of the Erasmus MC approved the study.

Medical costs

RSV hospitalisation costs

We calculated total costs per RSV hospitalisation by multiplying volumes of health care use with corresponding real costs.[140] We determined volumes of health care use in children hospitalised with RSV during the seasons 1996/1997-1999/2000 (n=3458). We performed a detailed measurement of investments of manpower, equipment, materials, housing, and overhead, by combining information obtained from structured interviews with nurses and physicians with information from financial accounts of the hospitals. Mean hospitalisation costs were €3,110 per hospitalisation at a mean duration of hospitalisation of six days. Hospitalisation costs were higher for children with a low gestational age (≤28 weeks; €5,550 per hospitalisation), low birth weight (≤2500 g; €3,900) and children with BPD (€5,800 per hospitalisation).

We constructed a linear regression model which estimates anticipated individual RSV hospitalisation costs for children at-risk, using four predictors: gestational age, presence of BPD, birth weight, and age (see appendix B).[140]

Costs of palivizumab

The costs of one vial of 50 mg or 100 mg of palivizumab were €560 or €930 respectively, according to the Dutch price system for pharmaceutical care. To calculate the dose per immunisation we used the mean weight of children hospitalised with RSV during the seasons 1996/1997-1999/2000 (6255 g). Given a dose of 15mg/kg, assuming drug wastage, a 100 mg vial would be required. We assumed that costs of administration of palivizumab equalled costs of a visit to the general practitioner, i.e. €20.

Non-medical costs

Parental costs

We estimated parental costs of hospitalisation to be 15.5% of the medical hospitalisation costs.[3] We further assumed that parents lost two working hours for each administration of passive immunisation.[86 90] With an average wage of €16 per hour, these parental costs were €32 per administration.

RSV hospitalisation risk

Children born between January 1, 1996 and December 31, 1998 and hospitalised with RSV during the seasons 1996/1997-1998/1999 (n=2,469) were related to children at-risk born during the same period (n=140,661; 1,181,790 months at-risk). We constructed a logistic regression model which estimates individual monthly RSV hospitalisation risks, using five clinical predictors (gestational age, presence of BPD, birth weight, gender and age) and the mean seasonal pattern of RSV infections (see appendix A).[132]

Efficacy of palivizumab

A Cochrane review reported an odds ratio (OR) of 0.48, 95% confidence interval (CI) 0.37-0.64, for the reduction in incidence of hospitalisation in all children. The OR was 0.27 (0.15-0.49) in premature children and 0.54 (0.37-0.80) in children with BPD.[1]

Cost-effectiveness analysis

We performed the primary cost-effectiveness analysis by programming prediction models for RSV hospitalisation costs and hospitalisation risk, with data on immunisation costs, parental costs, and efficacy of passive immunisation, in an Excel spreadsheet.[86 90] We calculated monthly costs and effects with and without prophylactic treatment. The primary outcome measure, the incremental costs to prevent one hospitalisation, was calculated for every month of the RSV season. The reference case was a child with the highest hospitalisation risk during the complete season (male, gestational age ≤28 weeks, birth weight ≤2,500 g, BPD, and age of zero months at the beginning of the season (October)).

Sensitivity analysis

To account for uncertainty in the estimates used in the cost-effectiveness analysis we performed univariate sensitivity analyses allowing changes of one variable at a time. These sensitivity analyses were performed for the reference case in the month passive immunisation was most cost-effective (December). We changed RSV hospitalisation risk, hospitalisation costs, immunisation costs, and efficacy of immunisation from half to double their values, and within the 95% CIs of the estimates. We also explored for which values the incremental costs per hospitalisation averted would be €0 (cost neutrality).

Results

Detailed results of the cost-effectiveness calculations across the RSV season for the reference case are shown in table 3.2.1. Costs per hospitalisation averted varied between €15,520 in December and €883,930 in October. For the most cost-effective month (December), the estimated monthly RSV hospitalisation risk without passive immunisation (7.6%) was reduced to 3.8% by passive immunisation (using an OR of 0.48 for efficacy of passive immunisation), giving a risk difference of 3.8% (7.6% minus 3.8%). The costs without passive immunisation were calculated by multiplying the estimated hospitalisation costs with the hospitalisation risk without passive immunisation (€10,250*7.6%=€780). To calculate the costs with passive immunisation, the estimated hospitalisation costs were multiplied with the hospitalisation risk with passive immunisation, and immunisation costs were added (€10,250*3.8%+€980=€1,370). The costs difference was calculated by subtracting the costs without passive immunisation from the costs with passive immunisation (€1,370-€780=€590). The incremental costs per hospitalisation averted could then be calculated by dividing the costs difference by the risk difference (€590/3.8%=€15,520). Every month costs per hospitalisation averted were higher for children without BPD and children with higher gestational ages (figure 3.2.1). Costs per hospitalisation averted were also higher for girls, and decreased with decreasing birth weight and age. Passive immunisation was always most cost-effective in December, but was never cost saving.

Sensitivity analysis

The estimated cost-effectiveness of passive immunisation for the reference case in December (€15,520 per hospitalisation averted) was most sensitive to changes in immunisation costs, followed by hospitalisation risk, efficacy of passive immunisation and hospitalisation costs (figure 3.2.2). Changes in monthly immunisation costs between €490 and €1,960 resulted in a linear increase of costs per hospitalisation averted from €2,635 to €41,290. For children with weights below 3,300 g, who could be immunised with the 50 mg vial at a cost of €560, costs per hospitalisation averted were €5,810. Acquisition costs of passive immunisation had to be decreased to €335 to reach cost neutrality.

Changes in monthly RSV hospitalisation risk between 3.8% and 15.2% resulted in an exponential decrease of costs per hospitalisation averted from €40,240 to €3,205. For

Table 3.2.1. *Calculation of incremental costs per hospitalisation averted (€) per seasonal month for the reference case (male infant, gestational age ≤ 28 weeks, birth weight ≤ 2,500 g, with BPD, and age* = 0 months at the start of the season).*

		Seasonal month						
		Oct	Nov	Dec	Jan	Feb	Mar	Apr
Age*	months	0	1	2	3	4	5	6
Hospitalisation risk without passive immunisation	%	0.2	4.0	7.6	4.5	3.2	1.6	0.4
Hospitalisation risk with passive immunisation†	%	0.1	2.0	3.8	2.2	1.6	0.8	0.2
Risk difference	%	0.1	2.0	3.8	2.3	1.7	0.8	0.2
Hodpitalisation costs‡	€	21,290	13,010	10,250	8,865	8,040	7,485	7,090
Immunisation costs§	€	980	980	980	980	980	980	980
Costs without passive immunisation	€	45	525	780	400	260	120	30
Costs with passive immunisation	€	1000	1235	1370	1175	1105	1035	995
Costs difference	€	955	710	590	775	845	920	965
Incremental costs per hospitalisation averted	€	883,930	34,540	15,520	34,030	51,260	112,535	420,015

* Age for children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

† Assuming OR for incidence of hospitalisation of 0.48 favouring passive immunisation.[1]

‡ Including 15.5% parental costs.[3]

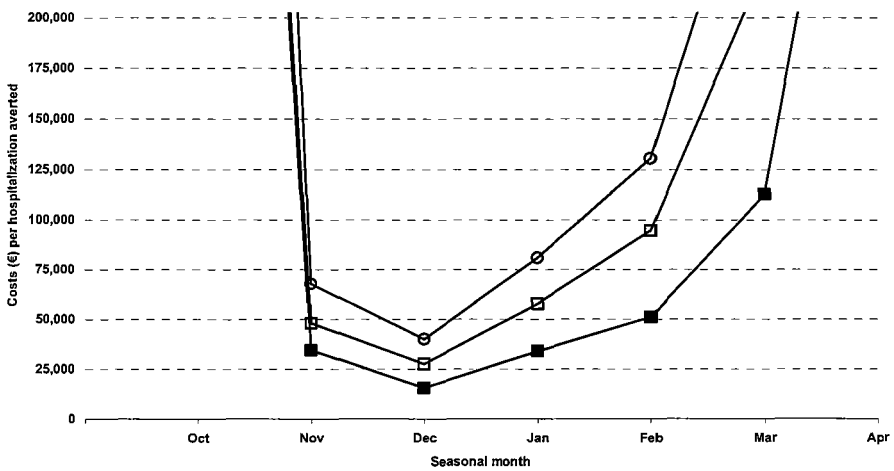
§ 100 mg vial of palivizumab (€930), administration costs (€20) and parental costs (€32).

the 95% CI of the risk estimate (5.1% to 11.3%), costs per hospitalisation averted ranged from €27,625 to €7,435. The monthly hospitalisation risk had to exceed 20% to reach cost neutrality.

Changes in efficacy of passive immunisation to RSV (OR 0.69 to 0.23) resulted in an exponential decrease of costs per hospitalisation averted from €33,695 to €6,810. For the 95% CI of the efficacy estimate (OR 0.64 to 0.37), costs per hospitalisation averted ranged from €27,445 to €10,835. Even when passive immunisation would prevent all hospitalisations (OR=0), costs per hospitalisation averted would still exceed €2,645.

Changes in RSV hospitalisation costs between €5,125 and €20,500 resulted in a linear decrease of costs per hospitalisation averted from €20,645 to €5,270. For the 95% CI of the hospitalisation costs estimate, ranging from €8,920 to €11,575, costs per hospitalisation averted ranged from €16,845 to €14,195. A hospitalisation had to cost over €25,750 to reach cost neutrality.

Figure 3.2.1. *Influence of variation in BPD and gestational age on costs per hospitalisation averted (€) per seasonal month (male gender, birth weight ≤ 2,500 g, and age* = 0 months at the start of the season).*



■ Gestational age 28 weeks, with BPD (refence case).

□ Gestational age 28 weeks, without BPD.

○ Gestational age 34 weeks, without BPD.

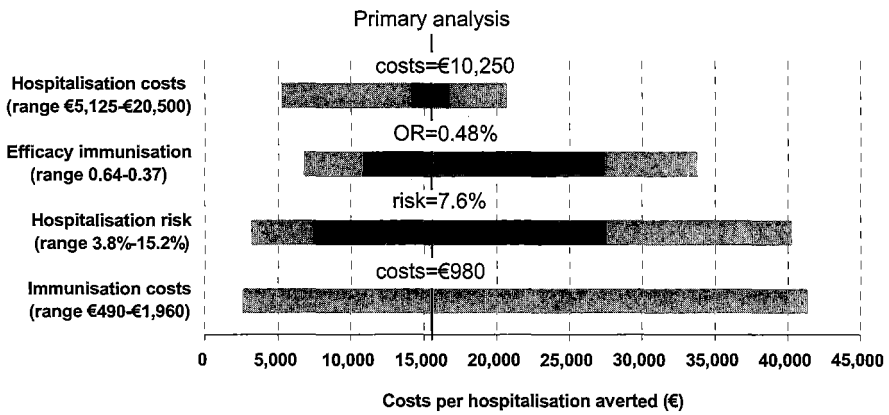
* Age for children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks.

Discussion

Cost-effectiveness of passive immunisation with palivizumab varied widely by child characteristics and seasonal month. This variation was caused by variation in hospitalisation costs and hospitalisation risk. In the reference case passive immunisation was most cost-effective in December, but even in this month the costs per hospitalisation averted were high (€15,520). The sensitivity analysis showed that cost-effectiveness could be improved most by lowering the acquisition costs of passive immunisation. However, for the reference case cost savings were only reached when acquisition costs were below €335. Changes in hospitalisation risk, efficacy of passive immunisation, and hospitalisation costs had less influence on the cost-effectiveness.

Several economic analyses of palivizumab have been published. However, we are the first to use a more individualised monthly approach. Nine studies report cost increases,[27 29 30 86 90-94] of which five report incremental costs per hospitalisation averted ranging from $7,000 to $420,000.[27 86 90 92 94] Three studies report cost savings.[87-89] However, these savings were only reached when high hospitalisation costs (over $15,000) were combined with high seasonal hospitalisation risks (28% or

Figure 3.2.2. *Sensitivity of costs per hospitalisation averted (€) to changes in estimates of RSV hospitalisation risk, hospitalisation costs, immunisation costs, and efficacy of immunisation, from half to double their values in the primary analysis and the 95% confidence intervals of the values used in the primary analysis for the reference case in December.*



▨ 0.5-2x estimate
■ 95% CI estimate

higher), high efficacy of passive immunisation (>80%), and low costs of immunisation ($2,500 per season),[88] or when high hospitalisation costs (>$50,000) and high seasonal hospitalisation risks (>20%) were assumed,[87] or when a very restrictive prophylaxis policy was followed.[89]

To appreciate our results some aspects need to be addressed. Instead of using mean hospitalisation costs we used a linear regression model to estimate anticipated individual hospitalisation costs.[140] This allowed for higher costs for children with BPD, low birth weight, and low age. However, cost-effectiveness was relatively insensitive to changes in hospitalisation costs between €5,125 and €20,500 (figure 3.2.2).

If the reference case had a weight below 3,300 g in December, a 50 mg vial of palivizumab would have been sufficient for immunisation, leading to a reduction of costs per hospitalisation averted from €15,520 to €5,810. However, only 9% (9/101) of the hospitalised infants with a gestational age below 35 weeks and birth weight below 3000 g had a weight on admission in December below 3,300 g.

Wastage of palivizumab is the most likely condition in actual practice, because the drug has a short shelf life and administration in The Netherlands is delegated to the general practitioner. A decrease of wastage by clustered administration would increase the cost-effectiveness of passive immunisation as a result of lower administration costs.

We used a logistic regression model to estimate individual monthly hospitalisation risks.[132] Every estimate is accompanied by a 95% CI. Should the actual risk for the reference case in December equal the upper boundary of this interval (11.3%), then costs per hospitalisation averted would be reduced by 52% (from €15,520 to €7,435). Cost neutrality would only be reached at very high monthly risks (>20%). However, the highest monthly risk estimated in our population was 8.1% (95% CI: 5.4%-12.2%). Monthly hospitalisation risks exceeding 3.8% (the lower boundary of our sensitivity analysis, corresponding to costs per hospitalisation averted of €40,240) were predicted for only 4% (422/10652) of all at-risk months of children with a gestational age below 35 weeks or BPD in our study.

We used the pooled OR reported in a Cochrane review to estimate the efficacy of palivizumab.[1] This OR is similar to the OR reported in the IMpact trial.[77] When we apply the subgroup specific ORs for premature children without BPD and children with BPD (0.27 and 0.54 respectively), passive immunisation is more cost-effective in premature children without BPD because of a higher efficacy (€19,020 versus €17,810 in December; reference case with and without BPD).

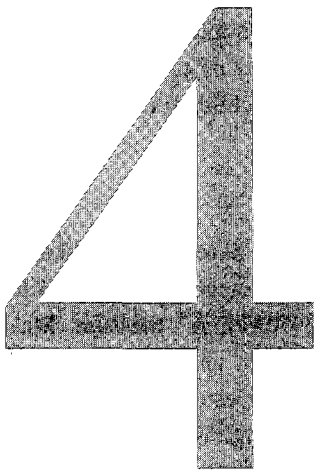
Only a small percentage of all children hospitalised with RSV eventually die as a result of the disease. No deaths were observed in our study. Passive immunisation has

not shown to have an effect on mortality caused by RSV. Therefore, in contrast to others,[86] we did not analyse costs per life year saved. Periods of recurrent wheezing following RSV hospitalisation have been reported.[64] Since there are no studies reporting any influence of passive immunisation on recurrent wheezing, we did not consider the related costs. Neither did we consider costs of adverse events, because no serious adverse events related to palivizumab were reported and the incidence of non-serious adverse events was low.[77]

RSV-IGIV(Respigam®), a polyclonal immune globulin enriched for neutralising antibody to RSV, is available for passive immunisation against RSV. However, palivizumab is dominantly used because it has several advantages above RSV-IGIV.[2] Therefore, we did not analyse cost-effectiveness of RSV-IGIV.

Since health care budgets are limited, economic analyses exploring the balance between costs and benefits of new medical interventions are essential to make choices about their reimbursement. However, there is no published threshold for cost-effectiveness expressed in costs per hospitalisation averted. This threshold should however be well below standards for costs per life-year saved (e.g. $50,000).[141] Robbins et al. estimated willingness to pay by presenting facts about RSV lower respiratory tract infection (LRTI) and passive immunisation with RSV-IGIV to a small sample of physicians and nurses, asking them what they would be willing to pay for a treatment that could prevent a high-risk child from being admitted to hospital with RSV LRTI.[128] They estimated that prevention of RSV hospitalisation in a high-risk child may cost between $1,325 and $8,700 (mean $5,787). This is far below the cost-effectiveness ratios found in our study. There are no data on the loss of quality of life of hospitalised children and their parents, caused by RSV hospitalisation. Therefore we did not value this loss.

We conclude that although the cost-effectiveness of passive immunisation with palivizumab varied strongly by child characteristics and seasonal month, the costs per hospitalisation averted were always high. We therefore recommend a restrictive immunisation policy. Lower acquisition costs of passive immunisation are required to reach acceptable levels of cost-effectiveness for high-risk children in high-risk months.



Quality of life after hospitalisation for a respiratory syncytial virus infection

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Submitted

Abstract

Background

To determine which children should receive passive immunisation against respiratory syncytial virus (RSV), the impact of RSV hospitalisation on physical, mental, and social functioning of children and their parents should be taken into account, together with costs and clinical parameters of effectiveness. The aim of this study was to quantify the loss of health related quality of life (HRQoL) in children during the first months after RSV hospitalisation.

Methods

A few months after children were hospitalised with RSV, parents were asked to complete a questionnaire to explore the health of the participating child, and the generic TNO-AZL Preschool Children Quality of Life (TAPQoL) measure. Mean TAPQoL scale scores were compared between this group of children and healthy children. Within the group of RSV hospitalised children we investigated the influence of the severity of RSV disease during hospitalisation, persisting respiratory problems, and possible confounding factors with univariate and multivariate analyses.

Findings

Parents of 50 children completed the questionnaires four months (median) after discharge. Compared to healthy children, children who had been hospitalised with severe RSV disease had significantly lower average scores on most TAPQoL scales, with moderate to large negative effects on the TAPQoL scales lungs and motor functioning. Children with persisting respiratory problems had significantly lower scores on the TAPQoL scales sleeping, appetite, and lungs, compared to children without persisting respiratory problems. Differences in disease severity during hospitalisation did not influence the HRQoL.

Interpretation

Several months after RSV hospitalisation, children had a lower HRQoL compared to healthy children. Negative effects on the TAPQoL scales sleeping, appetite, and lungs were primarily caused by persisting episodes of dyspnoea and/or wheezing after discharge.

Introduction

Respiratory syncytial virus (RSV) is the most important cause of viral lower respiratory tract infections in infants and young children. Almost all children have been infected at least once before the age of two years. 0.5% to 2% of all infected infants need to be hospitalised for severe RSV disease. Approximately 45% of the infants that are hospitalised with severe RSV disease experience recurrent wheezing in the following five years.[64]

Recently, palivizumab (Synagis®), a humanised monoclonal antibody to RSV, has become available for passive immunisation against RSV in premature children and children with BPD. Passive immunisation combines a moderate efficacy with high costs. Several economic analyses of passive immunisation have been published, mainly reporting incremental costs per hospitalisation averted or incremental costs per child at-risk.[27 29 30 86-94] Most of these studies report high costs relative to the benefits realised.[27 29 30 86 90-94] Based on these findings a restrictive use of passive immunisation seems recommendable. However, choices about reimbursement of passive immunisation should not be based on costs and clinical effectiveness parameters alone. The impact of RSV hospitalisation on the physical, mental, and social functioning of children and their parents should also be considered. This impact can be measured using health related quality of life (HRQoL). Ideally, measurements of HRQoL should be from the child's own perspective. However, in young children use of proxies is unavoidable (parents as surrogate responders).[142]

HRQoL can be measured with generic and disease specific questionnaires. In contrast to disease specific questionnaires, generic questionnaires can measure quality of life both in the general population and in a group of children with specific health problems.[143]

The aim of this study was to quantify the loss of HRQoL in children during the first months after RSV hospitalisation.

Methods

Patients

We assessed HRQoL in children hospitalised for severe RSV disease, a few months after discharge from the paediatric ward of a university teaching hospital or a community teaching hospital.

Children were eligible if they were hospitalised between October 2001 and May 2002, with a proven RSV infection (positive direct immunofluorescent assay or viral

culture), that was not nosocomially acquired. Their parents had to have sufficient knowledge of the Dutch language.
The medical records of the children were reviewed to obtain routinely documented information on duration of hospitalisation, level of care, and treatment, using standardised forms.

Questionnaire

The parents of the children completed a questionnaire containing selected items from the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study questionnaire to explore the incidence and severity of respiratory problems in children after RSV infection,[144] and general items, exploring the socio-economic background of the family and health of the participating child. They also completed the TNO-AZL Preschool Children Quality of Life (TAPQoL) measure to assess the HRQoL of the children.[145] This generic measure has been designed for children up to the age of five years. For the purpose of this study we reduced the recall period from three months to four weeks. The four domains in the TAPQoL measure are physical development, and social, cognitive, and emotional functioning. These domains are organised into 12 separate scales: sleeping, appetite, lungs problems, stomach problems, skin problems, motor functioning, social functioning, problem behaviour, communication, anxiety, positive emotion, and liveliness. The TAPQoL scales motor functioning, social functioning, and communication were only appropriate for children of 18 months or older. Parents could score the HRQoL of their children by answering 46 items with 'never', 'occasionally', or 'often'. If questions relating to the TAPQoL scales sleeping, appetite, lung problems, stomach problems, skin problems, motor functioning, and communication were answered with 'occasionally' or 'often', the parents had to indicate if their child felt 'fine', 'not so good', 'quite bad' or 'bad' under these circumstances. With this information, the TAPQoL ascertains problems in every day functioning, measured by the amount of negative emotions a child has with these problems.

Definitions

BPD was defined as the need for supplemental oxygen on day 28 after birth or at the postconceptional age of 36 weeks, in the presence of typical abnormalities on the chest X-ray.[105 106] Dyspnoea and wheezing were defined as at least one reported episode of dyspnoea or wheezing in the last four weeks. The definitions of bronchiolitis, pneumonia, and upper respiratory tract infection (URTI) were based on clinical characteristics.[25]

Statistical analysis

To investigate the impact of RSV hospitalisation on the HRQoL in children a few months after discharge from hospital, the mean TAPQoL scale scores of the study group were compared to the mean TAPQoL scale scores of a healthy general population.[145] We investigated whether differences in the severity of RSV disease had an influence on the HRQoL, by investigating the correlation between TAPQoL scale scores and duration of hospitalisation, and investigating differences in median TAPQoL scale scores between children who did or did not need supplemental oxygen, intensive care unit (ICU) admission, or mechanical ventilation. To investigate the influence of persisting respiratory problems (dyspnoea and/or wheezing) after RSV hospitalisation on the HRQoL a few months after discharge from hospital, we investigated differences in median TAPQoL scale scores between children with persisting respiratory symptoms and children without such problems. We investigated whether other factors than RSV hospitalisation or persisting respiratory problems (child's age and gender, time between hospitalisation and completion of the questionnaire, responder's gender and educational level) influenced the different TAPQoL scale scores within our group of children. Finally, we performed multivariate linear regression analyses with the characteristics that were significantly associated with different TAPQoL scale scores within the group of RVS hospitalised children in the univariate analyses (i.e. gender, age, persisting respiratory problems, and duration of hospitalisation) as independent variables, and TAPQoL scale scores as dependent variables.

We used the Student's *t*-test to investigate differences between the means of two groups, and the Mann-Whitney *U* Test to investigate differences between the medians of two groups. The Kruskal-Wallis test was used to investigate differences between the medians of more than two groups. In case of two or more ordered groups, we used the Jonckheere-Terpstra test to investigate differences between the medians. We investigated correlation between two variables with Spearman's rank correlation coefficient (r_s). *P*-values below 0.05 were considered statistically significant. Statistical analyses were performed with SPSS.

Differences between groups are reflected by the relative difference between the means in relation to the biggest standard deviation (SD), called Cohen's effect size (*d*). Effect sizes between 0.2 and 0.5 indicate a small effect. Effect sizes between 0.5 and 0.8 indicate a moderate effect. Effect sizes of 0.8 or larger indicate a large effect.[146]

Ethics

The local ethics committees of the Erasmus MC-Sophia Children's Hospital in Rotterdam and the Juliana Children's Hospital in The Hague approved the study. Informed consent was obtained from the parents of all children prior to participation.

Results

During the RSV season 2001-2002 108 children were hospitalised with a proven RSV infection in both hospitals. 76 of these fulfilled the inclusion criteria. Parents of 50 children agreed to participate in the study and returned a fully completed

Table 4.1. *Base line characteristics of hospitalised children (n=50) participating in the study (%, unless stated otherwise).*

General characteristics			
Gender	Female	46	
	Male	54	
Gestational age *(weeks)*	≤32	4	
	33-36	10	
	≥37	86	
Hospitalisation characteristics			
Age at admission *(months)**		2	(1-8)
Duration of hospitalisation *(days)**		5	(4-8)
Pre-existent morbidity		10	
Highest level of care	MCU	80	
	ICU	20	
Therapy	Mechanical ventilation	14	
	Extra oxygen	44	
	Tube feeding	64	
	Venous catheter	38	
	Other	32	
Diagnosis	Bronchiolitis	72	
	Pneumonia	2	
	URTI	14	
	Other	12	
Characteristics at inclusion			
Age at inclusion *(months)**		7.5	(5-13)
Time past since hospitalisation *(months)**		4	(3-5)
Persisting respiratory problems	None	40	
	Dyspnoea and/or wheezing	60	

BPD = Bronchopulmonary dysplasia; MCU = Medium care unit; ICU = Intensive care unit
URTI = Upper respiratory tract infection.
* Median (interquartile range).

questionnaire (response rate 66%). Base line characteristics are summarised in table 4.1. Fourteen percent of the children were born prematurely. 70% of the children were six months of age or less at admission. Although 10% had an underlying disease (metabolic disease 4%, neurologic disease 4%, multiple congenital malformations 2%), none had BPD. 52% needed supportive care (mechanical ventilation, extra oxygen, tube feeding or intravenous therapy) during admission. At inclusion 17 children (34%) were 12 months or older. 60% of the children had experienced at least one episode of dyspnoea and/or wheezing. None of the children received passive immunisation against RSV. Most of the questionnaires were completed by the mothers (80%). The median age of the responding parents was 31 years (interquartile range 27-35). 6% had had no education at all, 14% had a low educational level, 33% an intermediate, and 47% a high educational level.

We found significant differences between the group of RSV hospitalised children and a group of healthy children[145] in average TAPQoL scale scores regarding sleeping, appetite, lungs, stomach, skin, motor functioning, problem behaviour, positive mood, and liveliness (table 4.2). For these significantly differing TAPQoL scale scores

Table 4.2. *Mean TAPQoL scale scores for children who have been hospitalised for RSV compared to the mean TAPQoL scale scores for healthy children,[145] standard deviation (SD), p-value of T-test for difference of means, and effect size (d).*

Scale	Children after RSV hospitalisation† (n=50)		Healthy children† (n=251)		Significance (*p*)	Effect size‡ (*d*)
Sleeping	72.6	(24.8)	83.1	(17.0)	<0.001	-0.42*
Appetite	80.4	(25.9)	85.9	(12.0)	<0.05	-0.21*
Lungs	80.8	(20.7)	97.2	(9.0)	<0.001	-0.79**
Stomach	81.5	(22.7)	92.6	(13.0)	<0.001	-0.49*
Skin	88.7	(21.2)	92.8	(10.0)	<0.05	-0.19
Motor functioning	92.2	(10.4)	98.8	(4.0)	<0.001	-0.63**
Social functioning	80.9	(20.2)	91.4	(15.0)	NS	-0.52**
Problem behaviour	82.8	(17.6)	67.7	(15.0)	<0.001	0.86***
Communication	87.5	(13.0)	91.7	(10.0)	NS	-0.32*
Anxiety	80.2	(23.2)	79.2	(17.0)	NS	0.04
Positive mood	90.6	(18.5)	98.9	(6.0)	<0.001	-0.45*
Liveliness	86.1	(24.6)	98.1	(8.0)	<0.001	-0.49*

† Higher scores indicate better HRQoL.

‡ Difference of means divided by largest SD; positive effect sizes indicate better scores in children after RSV hospitalisation, negative effect sizes indicate better scores in healthy children.

* $0.2 \leq d < 0.5$: small effect; ** $0.5 \leq d < 0.8$: moderate effect; *** $d \geq 0.8$: large effect.

NS=not significant.

moderate to large negative effects were found for the scales lungs and motor functioning, indicating lower mean scores in the group of RSV hospitalised children. A large positive effect was found for the scale problem behaviour, indicating a higher mean score in the group of RSV hospitalised children.

Considering the severity of RSV disease within the group of RSV hospitalised children, we only found a week significant positive correlation between duration of hospitalisation and the score on the TAPQoL scale sleeping (r_s=0.29, p<0.05). We found no differences on the TAPQoL scale scores between children that did or did not need supplemental oxygen therapy, ICU admission, or mechanical ventilation during hospitalisation.

Children with persisting respiratory problems (dyspnoea and/or wheezing) after RSV hospitalisation had significantly lower average scores on the TAPQoL scales sleeping, appetite, and lungs, compared to children without persisting respiratory problems (figure 4.1). Especially for the TAPQoL scale lungs, a large negative effect size of 1.3 was calculated.

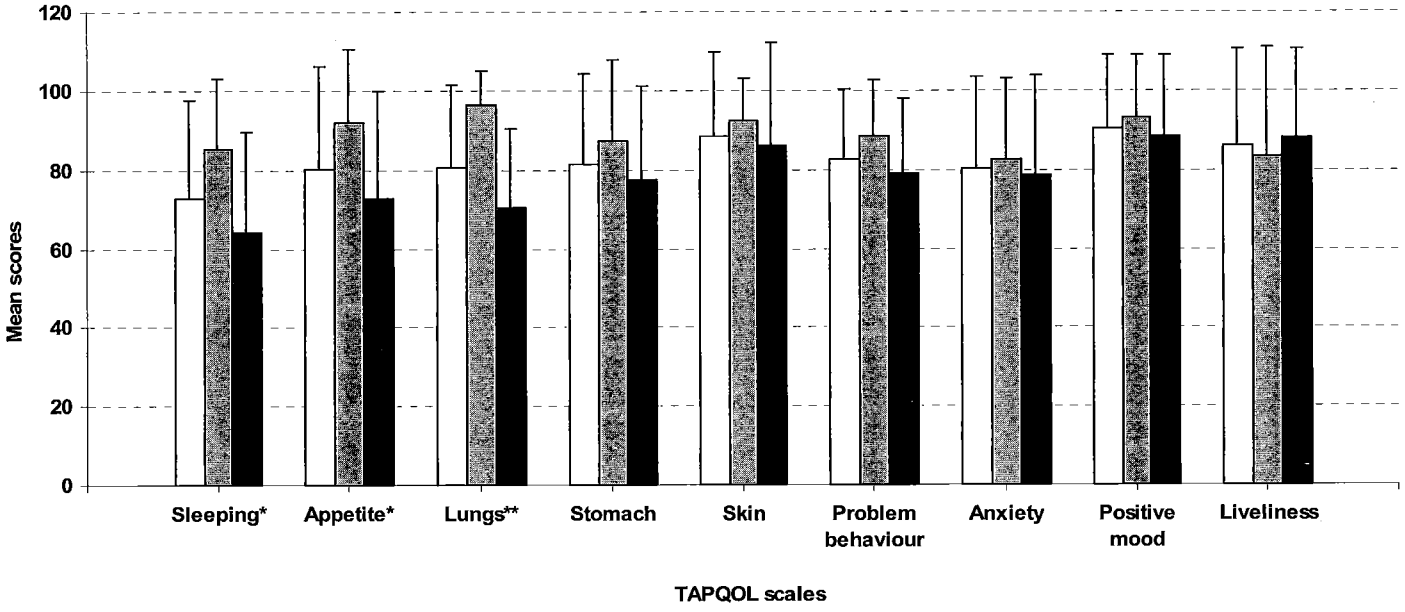
We found significant negative correlations between age at inclusion in the study of all RSV hospitalised children and the TAPQoL scale scores sleeping, appetite, lungs, problem behaviour and anxiety (table 4.3). Boys had significant lower scores on the TAPQoL scale problem behaviour compared to girls (mean 76.6 versus 87.9, p<0.05, effect size -0.41). The time between hospitalisation and completion of the questionnaire, gender of the responder, and the educational level of the responder had no influence on the TAPQoL scale scores.

We performed a multivariate analysis to investigate the associations between gender, age, persisting respiratory problems, and duration of hospitalisation and the different

Table 4.3. *Correlation between age of the child at inclusion in the study and the TAPQoL scale scores, Spearman's rho (r_s) and p-value.*

Scale	Correlation (r_s)	Significance (p)
Sleeping	-0.30	<0.05
Appetite	-0.45	<0.01
Lungs	-0.46	<0.01
Stomach	-0.16	NS
Skin	0.17	NS
Problem behaviour	-0.32	<0.05
Anxiety	-0.39	<0.01
Positive mood	-0.03	NS
Liveliness	0.01	NS

Figure 4.1. *HRQoL profiles of all children after RSV hospitalisation (n=50), and children after RSV hospitalisation without (n=20) or with (n=30) persisting respiratory problems (dyspnoea and/or wheezing), as measured with the TAPQoL questionnaire. Columns represent mean scores, error bars represent standard deviations.*



□ All RSV hospitalised children.

▨ RSV hospitalised children without respiratory problems.

■ RSV hospitalised children with respiratory problems.

* $p<0.01$, children without persisting respiratory problems compared with children with persisting respiratory problems.

** $p<0.001$, children without persisting respiratory problems compared with children with persisting respiratory problems.

TAPQoL scale scores within the group of RSV hospitalised children (table 4.4). Significantly lower scores were found on the TAPQoL scale problem behaviour for boys compared to girls (coefficient -10.4, $p<0.05$), and on the TAPQoL scale anxiety for older children compared to younger children (coefficient -0.04, $p<0.01$). Children with persisting respiratory problems had significantly lower scores on the TAPQoL scales sleeping (coefficient -23.5, $p<0.01$), appetite (coefficient -23.0, $p<0.01$), and lungs (coefficient -23.6, $p<0.001$) compared to children without persisting respiratory problems. Children with a longer duration of hospitalisation had significantly higher scores on the TAPQoL scales sleeping (coefficient 3.4, $p<0.01$), appetite (coefficient 3.2, $p<0.01$), and stomach (coefficient 2.7, $p<0.05$) compared to children with a shorter duration of hospitalisation.

Discussion

This is the first study that describes the quality of life in RSV hospitalised children, measured several months after discharge. Compared to healthy children, children who had been hospitalised with severe RSV disease had a lower HRQoL, as indicated by significantly lower average scores on most TAPQoL scales. Moderate to large negative effects were found for the TAPQoL scales lungs and motor functioning. Negative effects on the scores of the TAPQoL scales sleeping, appetite and lungs were most apparent in children with persisting episodes of dyspnoea and/or wheezing after hospitalisation.

Surprisingly, children who had been hospitalised with severe RSV disease had significantly higher scores on the TAPQoL scale problem behaviour than healthy children. Although girls had higher scores on the TAPQoL scale problem behaviour than boys, this could not explain the difference, since the percentages of girls in both

Table 4.4. *Significant associations between gender, age at inclusion, persisting respiratory problems, and duration of hospitalisation and scores on the TAPQoL scales sleeping, appetite, lungs, stomach, problem behaviour, and anxiety in the multivariate analysis (p-values, empty cells indicate non-significant associations).*

	Gender	Age at inclusion	Persisting respiratory problems	Duration of hospitalisation
Sleeping			<0.01	<0.01
Appetite			<0.01	<0.01
Lungs			<0.001	
Stomach				<0.05
Problem behaviour	<0.05			
Anxiety		<0.01		

groups were equal (46% and 44%; respectively). Age differences between both groups might have caused the difference (66% of all RSV hospitalised children were younger than one year, 66% of all healthy children were older than two years).Unfortunately, age specific reference values of the TAPQoL scores in infants are not available. These are needed to more accurately compare the HRQoL of ill infants with healthy infants.

The negative effects on the TAPQoL scales sleeping, appetite and lungs were most apparent in the group of children with persisting respiratory problems. Since these persisting respiratory problems might just as well be found after RSV disease in children that are not hospitalised, a control group of non-hospitalised RSV infected children might have been more appropriate.

Differences in disease severity, measured by need for supplemental oxygen, ICU admission or mechanical ventilation, did not influence the HRQoL measured shortly after discharge. However, longer hospital stay was associated with higher scores on the TAPQoL scales sleeping, appetite and stomach, indicating increasing HRQoL with increasing duration of hospitalisation. When a longer duration of hospitalisation indicates more severe RSV disease, one would expect a lower HRQoL. Apparently, duration of hospitalisation is not a good marker of disease severity, since we found a positive, rather than a negative association.

Although age correlated negatively with scores on the TAPQoL scales sleeping, appetite and lungs in the univariate analysis, these associations were not found in the multivariate analysis. This could be explained by the fact that children with persisting respiratory problems, who have lower scores on several TAPQoL scales, are significantly older ($p<0.01$) than children without persisting respiratory problems. So, the lower scores were not caused by older age, but by persisting respiratory problems. However, age was associated with lower scores on the TAPQoL scale anxiety in the univariate and multivariate analyses. This could indicate that younger children express less anxiety than older children or could be caused by a lower sensitivity of the TAPQoL for anxiety in young children.

To appreciate our results some aspects need to be addressed. Differences between groups and correlations were tested for every TAPQoL scale separately. These multiple comparisons lead to a high probability of finding a significant difference just by change (Type I error).[147] To overcome this problem statistical significance can be considered at a lower *P*-value (e.g. $p<0.01$). Although this would eliminate some of the differences found, the final conclusion would still be valid.

From the reference group only the size, the means and the SD of the different TAPQoL scales were known. Therefore a Student's *t*-test had to be used to evaluate the differences between both groups, although a non-parametric test would have been

more appropriate because most values on the TAPQoL scales had a skewed distribution.

We conclude that several months after hospitalisation with severe RSV disease, children have a lower HRQoL, compared to healthy children. Negative effects on the TAPQoL scales sleeping, appetite, and lungs are primarily caused by persisting episodes of dyspnoea and/or wheezing after discharge.

Acknowledgment

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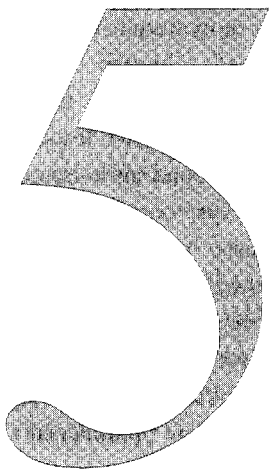

Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor α polymorphisms

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Abstract

The association of variants of genes encoding interleukin (IL)-4 and the IL-4 receptor α chain (IL-4Rα) with respiratory syncytial virus (RSV) bronchiolitis was examined in hospitalised infants. Polymorphisms in IL-4 (C-590T) and IL-4Rα (I50V and Q551R) were genotyped by restriction fragment-length polymorphism analysis. Control subjects included parents of the hospitalised children (for the transmission/disequilibrium test), and a random population sample (for the case-control study). Results were also analysed in a combination of these two tests, using Fisher's method (combination test). The IL-4 590T allele was found more frequently among children hospitalised with RSV than expected in the case-control (odds ratio [OR], 1.43; P=0.04) and combination (OR, 1.41; P=0.02) tests. Among children who were >6 months old when they were hospitalised, compared with the control group or with the <6 months old who were hospitalised for RSV infection, higher frequencies of both the IL-4 590T allele and the IL-4Rα R551 allele were found. These results indicate that gain-of-function variants of T helper type 2 cytokine genes may play a role in increasing the severity of RSV disease, which appears more pronounced after the first half year of life.

Introduction

Each year during the winter season, respiratory syncytial virus (RSV) causes severe respiratory infections in infants and young children. Almost all children are infected with RSV in their first or second year of life. Reinfection may occur throughout life, but the first infection often is the most severe and may result in bronchiolitis and pneumonia.[14] The severity of disease in these young children varies from no symptoms at all or a mild upper respiratory tract infection to a more or less severe lower respiratory tract infection (found in 40% of infected children). This may lead to respiratory failure, which is the reason for hospitalisation in 0.5% to 2% of the infected children, and occasionally to death (in 1% of hospitalised children).[100 148] The reasons for this variation in disease severity are not completely clear. Some children (e.g., children born prematurely, children with congenital heart or lung disease, and children with immune deficiencies) are at higher risk of developing more serious symptoms of the disease.[26 39 41 44] We hypothesised that genetic heterogeneity in the immune response of infected children explains some of these differences. To test our hypothesis, we investigated the role of immune response gene variants (polymorphisms) in an association study in which both the classic case-control design (comparing individuals with the severe form of RSV infection and a control group) and a transmission/disequilibrium test (TDT; comparing alleles that were transmitted from the parents to the affected child and alleles that were not transmitted).

Several authors have suggested that T cell-mediated immunopathology, in particular, a more pronounced T helper type 2 (Th2) response, may play an important role in the development of severe RSV disease.[149 150] This has been suggested because children vaccinated with a formalin-inactivated vaccine developed a more severe course of disease when natural infection occurred.[66] Later, it was shown in mice that this enhanced form of disease was accompanied by a shift in the immune response toward a Th2 response.[151-153] However, the significance of Th2 responses in natural RSV infections in humans remained unclear. Some studies demonstrated Th2 cytokine responses when natural infection occurred in children who went on to develop severe RSV disease,[150 154 155] whereas, in other studies, such a shift toward a Th2 response was not found.[156 157] It should be mentioned that the induction of a T helper type 1 (Th1) response does not necessarily exclude the presence of a concomitant Th2 response and its associated pathologic effects, because Th2 cytokines and Th1 cytokines can be detected simultaneously.[158 159] In addition, Aberle et al. and Bont et al. showed that children with severe RSV disease had a lower level of expression of the Th1 cytokine interferon (IFN)-γ in peripheral blood mononuclear cells than did

children with mild forms of the disease,[160 161] which suggests that Th1 cytokines may diminish the severity of RSV disease.

Another reason to study genetic predisposition to Th2 responses in RSV disease is the suggested relationship between the occurrence of severe RSV disease and the development of asthma later in life. In the Tucson cohort study, it was shown that patients who experienced RSV bronchiolitis had recurrent wheezing that lasted up to 11 years, but RSV bronchiolitis did not increase the risk of allergy sensitisation.[162] In contrast, Sigurs et al. presented evidence that RSV infections may increase allergy.[163] Although RSV might affect the development of asthma in several ways, such findings could suggest that individuals with a genetic predisposition toward strong Th2 responses are at risk for development of more-severe RSV disease as well as asthma. RSV might affect the development of asthma by allergic processes or by nonallergic processes, such as bronchial hyperresponsiveness, wheezing, and bronchus obstruction.[164 165] These findings prompted us to study the association between severe RSV disease and gain-of-function mutations in Th2 cytokine genes.

The Th2 cytokine interleukin (IL)-4 is one of the cytokines that may play a role in the pathogenesis of asthma. The gene is located in the Th2 cytokine cluster on human chromosome 5q31 and encodes for IL-3, -4, -5, and -13, among others.[166] In genetic studies, this chromosomal area is linked with total IgE concentration, asthma, and bronchial hyperreactivity.[167-169] IL-4 plays an important role in the stimulation and differentiation of Th2 cells. It promotes the proliferation and differentiation of activated B cells, promotes the recruitment of circulating cells by up-regulation of vascular cell adhesion molecule 1 on endothelial cells, and suppresses IFN-γ production by Th1 cells. That last quality makes the study of IL-4 in the context of severe RSV disease particularly interesting, because results from studies in both human and mice indicate that IFN-γ protects against severe RSV disease.[160 161 170] IL-4 mediates its biological effects by binding to the IL-4 receptor, which is located on almost all hematopoietic cells and a large number of nonhematopoietic cells. After binding of IL-4 to its receptor, the kinases Jak-1 and Jak-3 become phosphorylated, and in turn phosphorylate the receptor, the insulin receptor-like substrates, and the transcription factor STAT6.[171 172] The IL-4 receptor consists of 2 chains: the IL-4 receptor α chain (IL-4Rα) and the common cytokine receptor γ chain. The latter chain is present in a large number of cytokine receptors (e.g., IL-2, -7, -9, and -15),[173-176] whereas IL-4Rα is also a part of the IL-13 receptor.[177] The IL-4Rα gene is located on chromosome 16p21.[178] We studied the genetic association of severe RSV disease with one polymorphism located in the promoter region of the IL-4 gene and two polymorphisms located in the IL-4Rα gene.

Subject, materials and methods

Study design

Children included in the study were hospitalised because of RSV bronchiolitis during the period 1992-2000. RSV infection was confirmed by direct immunofluorescent assay of nasopharyngeal cells. Blood samples or buccal swabs were collected from 200 children and from both of the parents of each child for DNA isolation. In 7 cases, samples were obtained from the child and the mother only. All parents completed a questionnaire that gathered medical data and information about pregnancy and ethnic origin. As far as possible, the medical data were verified against clinical records. A subset of the children (110 children) from the Wilhelmina Children's Hospital took part in a follow-up study that examined recurrent wheezing that occurred as a result of RSV infection.[179 180] An unselected control population of 447 persons born in The Netherlands (37% of whom were women) was randomly taken from the REGENBOOG study, a large Dutch population health examination survey.[181] In this survey, a random sample of the Dutch population was interviewed, and 30% of those individuals participated in an additional health examination at a municipal health centre. There were no major differences, with respect to many background and health-related variables, between participants interviewed at home and those who underwent physical examination.

The mean age of the children at the time of hospitalisation was 115 days (SD, 111 days). Of the 207 children included in the study, 148 were native Dutch (their parents and grandparents were born in The Netherlands and consider themselves to be native Dutch); the mean age of this group at the time of hospitalisation was 113 days (SD, 96 days). At the time of inclusion in the present study, the mean age for all children was 3.4 years (SD, 1.1 year).

Informed consent was obtained from the parents of each study subject in accordance with the guidelines of the medical ethical committees of both the Wilhelmina Children's Hospital and the Sophia Children's Hospital, and these committees approved the study. For the control population, informed consent was obtained in accordance with the guidelines of the medical ethical committee of Netherlands Organisation for Applied Scientific Research (TNO) Leiden.

DNA isolation

DNA was isolated from blood samples or, when blood was not available, from buccal swabs, using the QIAamp DNA Blood Mini or Midi Kit (Qiagen). DNA from blood samples from parents was obtained using an isolation kit for mammalian blood

(Roche). For the control population (REGENBOOG samples), genomic DNA was extracted from buffy coats by digestion with proteinase K, followed by salting out with potassium acetate and chloroform/isoamyl alcohol extraction.[182] The DNA concentration was determined using PicoGreen (Molecular Probes).

Polymerase chain reaction (PCR) amplification and genotyping

All hospitalised children, parents, and adult control subjects were genotyped for polymorphisms in the IL-4 gene (C-590T) and the IL-4Rα gene (I50V and Q551R) by PCR-restriction fragment-length polymorphism analysis. Primers and experimental conditions are listed in table 5.1. All PCRs were performed on a GeneAmp PCR system 9700 (Applied Biosystems), with an initial denaturation step of 6 min at 95°C and then 35 cycles of the specific program. The program ended with an additional 10 min at 72°C. PCR products were digested, electrophoresed in 3% agarose gel with ethidium bromide, and visualised by UV transillumination. Two different investigators independently evaluated the gels.

Statistical analysis

The data were analysed for the total group of children, for children with different severities of RSV bronchiolitis (children receiving and those not receiving mechanical ventilatory support), children with known risk factors (children born prematurely, children who had RSV infection at age <6 months, and children with cardiac or lung disease), and allergic children (children with eczema or food allergy).

To analyse the role of different alleles at specific loci, two different but complementary tests were used: (1) The genotyping results for all hospitalised children and parents were used for analysis in the TDT.[183] This test determines whether the "risk" allele is transmitted at greater frequency than the "normal" allele from a heterozygous parent to an affected offspring. All parents can be included in this analysis, regardless of their ethnic origins; differences in allele distribution in different ethnic populations are independent of the transmission of the risk allele. (2) A case-control approach to analysis of data from native Dutch children was used (to prevent bias by population admixture). In this analysis, allele frequencies (frequency with which a particular allele is found 0, 1, or 2 times in different groups) among Dutch case patients and Dutch population controls were compared as risk factors for RSV infection. Results from case and control samples were compared using a Mantel-Haenszel trend test for the number of susceptibility alleles of interest (0, 1, or 2), essentially assuming a codominant penetrance model. That is, the more copies of the candidate gene are present, the higher the risk of disease. Because both tests make use

Table 5.1. *Polymerase chain reaction (PCR) conditions, primers, and restriction enzymes used to determine genetic variations at polymorphic sites in the interleukin (IL)-4 and IL-4 receptor α (IL-4Rα) genes.*

Polymorphic site, polymorphism	Primers* (5'-3')	Base change	PCR conditions	Restriction enzyme and fragment		Reference
				Polymorphic	Wild type	
IL-4 gene promoter region, C-590T	Forward, TAAACTTGGGAGAACATgGT; Reverse, TGGGGAAAGATAGAGTAATA	C→T	30 sec 95°C, 30 sec 50°C, 45 sec 72°C	195 bp	*Ava*II → 177+ 19 bp	(primers)[189]
IL-4R α gene, I50V	Forward, GGCAGGTGTGAGGAGCATCC; Reverse, GCCTCCGTTGTTCTCAGGtA	A→G	30 sec 95°C, 30 sec 60°C, 45 sec 72°C	*Rsa*I → 254+19 bp	273 bp	(primers)[4]
Q551R	Forward, sGCCCCCACCAGTGGCTcTC; Reverse, CTGGCAAGCAGGCTTGAGAAG	A→G	30 sec 95°C, 30 sec 59°C, 45 sec 72°C	128 bp	*Dde*I → 111+20 bp	(sequence)[5]

*Lowercase letters represent changed nucleotides that create a restriction site to distinguish the polymorphic sites.

of data from the same children (case patients), results from these 2 tests are not statistically independent.

Whenever one of these tests resulted in a (borderline) significant association, we combined all available information, making use of the fact that a case-control comparison of parents of case patients with control subjects is statistically independent of the TDT (N.J.D.N., T.G.K., and B.H., unpublished data).[184 185] This comparison could be used to explore the same hypothesis, that an association exists between disease and a putative risk allele: if affected children are selected for the presence of specific alleles, then, by implication, their parents are also selected for these alleles (although less so). We thus combined evidence from the TDT with a Mantel-Haenszel trend test, comparing allele frequencies at the loci of interest between Dutch parents and control subjects (combination test). Combining evidence from the two tests increases the overall power to detect an association between severe disease and the putative risk allele. For this combination of tests, we used the generally applicable Fisher's method for combining multiple independent tests of the same hypothesis.[186] Under the null hypothesis, the 1-sided *P* values from the two tests are both uniformly (0 and 1) distributed. Thus, we can test the null hypothesis, that no association exists between disease and a candidate allele, by comparing $-2\{ln\ (p_{TDT}) + ln\ (p_{pc})\}$ with a χ^2 statistic with 4 degrees of freedom, where p_{TDT} is the (1-sided) *P* value of the TDT and p_{pc} is the same, for the comparison of parents and control subjects under the codominant penetrance model. Genetic interaction between the different polymorphisms was tested using logistic regression.

Results

IL-4 C-590T polymorphism

The results of the TDT and the case-control analysis are given in table 5.2. In the case-control study, the T allele was found in a significantly higher frequency among children hospitalised with RSV bronchiolitis than in the control group (odds ratio [OR], 1.43; *P*=0.04). Although the result was not statistically significant, the TDT analysis also showed that the level of transmission of the T allele was higher than the expected 50% (OR, 1.33; *P*=0.13). When all available information was used in the combination test, the association was still significant, which confirmed that a genetic association exists between the occurrence of the T allele and RSV bronchiolitis (OR, 1.41; *P*=0.02). When specific features among the children were examined (e.g., known risk factors or other clinical manifestations), different levels of association were found in both the TDT and the case-control analysis. In the TDT, the T allele had been transmitted significantly more frequently among children who were

not receiving mechanical ventilatory support (OR, 1.68; P=0.02) and among children who became infected at an age >6 months (OR, 2.22; P=0.04). A significant association was also found for these two groups of children in the case-control study; the T allele was found at a higher frequency in both groups than it was in the control group (OR, 1.63; P=0.01, and OR, 1.83; P=0.04, respectively). In addition, in the case-control study, a significant association was found for children who had no recurrent wheezing (OR, 1.95; P=0.01), for those who had no cardiac or lung disease (OR, 1.49; P=0.03), and for those who did not have eczema (OR, 1.48; P=0.04). In the combination test, all of these associations remained significant or became more significant, and in all cases, the T allele was found more frequently than expected by chance. Although significant associations were not found for prematurely born children and children without food allergy in the TDT and case-control study, significant results were found for these groups in the combination test (P=0.02 and P=0.05, respectively). This discrepancy is probably explained by the higher power of the combination test.

IL-4Rα I50V and Q551R polymorphisms

The results of the TDT and case-control studies of the IL-4Rα polymorphisms I50V and Q551R are given in tables 5.3 and 5.4. No significant association between the I50V polymorphism and RSV bronchiolitis was found in any of the tests. Remarkably, no homozygous II genotype was found among children with cardiac or lung disease (P=0.06 for genotype distribution). Although only 13 children with such diseases were included in our study, we would expect, on basis of the findings for the control group (in which 29% of subjects were homozygous for this allele), 3 to 4 of these children to be homozygous for this allele. Transmission of the R551 allele was significantly more frequent among children who were hospitalised for RSV infection at an age >6 months, (OR, 2.75; P=0.01; TDT). Although no significant association with this allele was found in the case-control study (P=0.26), the results of the combination test were significant (OR, 1.66; P=0.03).

Hardy-Weinberg equilibrium and genetic interactions

For all three polymorphisms studied, the genotype distributions in the Dutch population (both RSV-infected children and control subjects) were in Hardy-Weinberg equilibrium. We could not demonstrate genetic interaction between the different polymorphisms (data not shown).

Table 5.2. *Results of the transmission/disequillibrium test, case-control study, and combination test for the interleukin-4 C-590T polymorphism among children hospitalised for respiratory syncytial virus (RSV) infection.*

Subject group	Transmission/disequilibrium test													Combination test, P*	
	Total no. of children	No. of informative probands†	Allele transmitted to children			Total no. of subjects	Genotype				Allele			Parents vs. control subjects	All information
			C, %	T, %	P		CC, %	CT, %	TT, %	P‡	C, %	T, %	P§		
Controls						447	77	21	3		87	13			
All children hospitalised for RSV infection	207	114	43	57	0.13	148	68	29	3	0.10	82	18	**0.04**	**0.05**	**0.02**
Receiving ventilatory support															
Yes	50	31	58	42	0.37	42	76	21	2	1.00	87	13	0.98		
No	155	83	37	63	**0.02**	105	64	32	4	**0.03**	80	20	**0.01**	**0.04**	**0.004**
Recurrent wheezing															
Yes	53	29	62	38	0.58	45	76	22	2	0.98	87	13	0.93		
No	57	33	36	64	0.12	43	58	37	5	**0.03**	77	23	**0.01**	**0.04**	**0.02**
Premature birth															
Yes	59	39	41	59	0.26	42	64	31	5	0.20	80	20	0.07	**0.02**	**0.02**
No	148	75	44	56	0.26	106	69	28	3	0.25	83	17	0.14		

Table 5.2. *Continued.*

Subject group	Transmission/disequilibrium test													Combination test, P*	
	Total no. of children	No. of informative probands†	Allele transmitted to children			Total no. of subjects	Genotype				Allele			Parents vs. control subjects	All information
			C, %	T, %	P		CC, %	CT, %	TT, %	P‡	C, %	T, %	P§		
Age at RSV infection															
<6 months	161	85	47	53	0.59	114	70	26	4	0.37	83	17	0.16		
>6 months	46	29	31	69	**0.04**	34	59	38	3	0.06	78	22	**0.04**	0.10	**0.02**
Cardiac or lung disease															
Yes	14	8	50	50	1.00	13	77	23	0	0.84	89	12	0.83		
No	193	106	43	58	0.12	135	67	30	4	0.07	82	19	**0.03**	**0.04**	**0.02**
Eczema															
Yes	52	24	33	67	0.10	34	71	27	3	0.74	84	16	0.47		
No	155	90	46	54	0.40	114	67	30	4	0.10	82	18	**0.04**	**0.02**	**0.05**
Food allergy															
Yes	23	14	36	64	0.29	18	61	39	0	0.17	81	19	0.27		
No	184	100	44	56	0.23	130	69	28	4	0.17	82	18	0.06	**0.04**	**0.05**

$P<0.05$ was considered to be significant; all such values are shown in boldface type.

* Fisher's combination test. P values are 1-sided.

† Informative probands are heterozygous parents.

‡ χ^2 test.

§ Mantel-Haenszel trend test.

Table 5.3. *Results of the transmission/disequillibrium test, case-control study, and combination test for the interleukin-4 receptor α I50V polymorphism among children hospitalised for respiratory syncytial virus (RSV) infection.*

Subject group	Transmission/disequilibrium test													Combination test, P*	
	Total no. of children	No. of informative probands†	Allele transmitted to children			Total no. of subjects	Genotype				Allele			Parents vs. control subjects	All information
			I, %	V, %	P		II, %	IV, %	VV, %	P‡	I, %	V, %	P§		
Controls						447	29	49	22		54	46			
All children hospitalised for RSV infection	207	205	52	48	0.62	148	32	45	22	0.70	55	45	0.71		
Receiving ventilatory support															
Yes	50	49	47	53	0.67	42	21	55	24	0.56	49	51	0.38		
No	155	155	54	46	0.38	105	37	41	22	0.24	58	42	0.33		
Recurrent wheezing															
Yes	53	51	59	41	0.21	45	31	47	22	0.95	54	46	0.91		
No	57	57	51	49	0.89	43	35	44	21	0.74	57	43	0.58		
Premature birth															
Yes	59	55	49	51	0.89	42	29	48	24	0.95	52	48	0.80		
No	148	150	53	47	0.52	106	34	44	22	0.56	56	44	0.55		

Table 5.3. *Continued.*

Subject group	Transmission/disequilibrium test													Combination test, P*	
	Total no. of children	No. of informative probands†	Allele transmitted to children			Total no. of subjects	Genotype				Allele			Parents vs. control subjects	All information
			C, %	T, %	P		CC, %	CT, %	TT, %	P‡	C, %	T, %	P§		
Age at RSV infection															
<6 months	161	164	50	50	1.00	114	33	43	24	0.51	55	45	0.79		
>6 months	46	41	59	42	0.27	34	29	53	18	0.84	56	44	0.74		
Cardiac or lung disease															
Yes	14	7	43	57	0.71	13	0	77	23	0.06	39	62	0.12	**0.05**	0.09
No	193	198	52	48	0.57	135	36	42	22	0.31	57	43	0.42		
Eczema															
Yes	52	48	46	54	0.56	34	29	38	32	0.31	49	52	0.41		
No	155	157	54	47	0.38	114	33	47	19	0.68	57	43	0.39		
Food allergy															
Yes	23	23	57	44	0.53	18	33	39	28	0.69	53	47	0.90		
No	184	182	51	49	0.77	130	32	46	22	0.79	55	45	0.66		

P<0.05 was considered to be significant; all such values are shown in boldface type.

* Fisher's combination test. P values are 1-sided.

† Informative probands are heterozygous parents.

‡ χ^2 test.

§ Mantel-Haenszel trend test.

Table 5.4. *Results of the transmission/disequilibrium test, case-control study, and Fisher's combination test for the interleukin-4 receptor α Q551R polymorphism among children hospitalised for respiratory syncytial virus (RSV) infection.*

Subject group	Transmission/disequilibrium test													Combination test, P*	
	Total no. of children	No. of informative probands†	Allele transmitted to children			Total no. of subjects	Genotype				Allele			Parents vs. control subjects	All information
			Q, %	R, %	P		QQ, %	QR, %	RR, %	P‡	Q, %	R, %	P§		
Controls						447	60	36	4		78	22			
All children hospitalised for RSV infection	207	126	49	51	0.85	148	62	35	3	0.88	79	21	0.66		
Receiving ventilatory support															
Yes	50	27	56	44	0.56	42	62	36	2	0.84	80	20	0.68		
No	155	98	48	52	0.69	105	61	35	4	0.97	79	21	0.82		
Recurrent wheezing															
Yes	53	32	53	47	0.72	45	60	36	4	1.00	78	22	0.99		
No	57	31	55	45	0.59	43	70	30	0	0.25	85	15	0.12		
Premature birth															
Yes	59	34	41	59	0.30	42	62	36	2	0.84	80	20	0.68		
No	148	92	52	48	0.68	106	61	35	4	0.95	79	21	0.77		

Table 5.4. *Continued.*

Subject group	Transmission/disequilibrium test													Combination test, P*	
	Total no. of children	No. of informative probands†	Allele transmitted to children			Total no. of subjects	Genotype				Allele			Parents vs. control subjects	All information
			Q, %	R, %	P		QQ, %	QR, %	RR, %	P‡	Q, %	R, %	P§		
Age at RSV infection															
<6 months	161	94	53	44	0.22	114	64	34	2	0.40	81	19	0.27		
>6 months	46	32	28	72	**0.01**	34	53	38	9	0.42	72	28	0.26	0.63	**0.03**
Cardiac or lung disease															
Yes	14	10	20	80	0.06	13	46	54	0	0.35	78	22	0.55		
No	193	116	52	48	0.71	135	63	33	4	0.82	80	20	0.53		
Eczema															
Yes	52	32	47	53	0.72	34	53	47	0	0.25	77	24	0.79		
No	155	94	50	50	1.00	114	64	32	4	0.70	80	20	0.51		
Food allergy															
Yes	23	18	44	56	0.64	18	50	50	0	0.37	75	25	0.68		
No	184	108	50	50	1.00	130	63	33	4	0.81	80	20	0.54		

P<0.05 was considered to be significant; all such values are shown in boldface type.

* Fisher's combination test. P values are 1-sided.

† Informative probands are heterozygous parents.

‡ χ^2 test.

§ Mantel-Haenszel trend test.

Discussion

Some indications for the involvement of genetic heterogeneity in RSV-induced bronchiolitis were obtained in two recent studies: Hull and colleagues showed genetic association with the chemokine IL-8 gene region,[54 55] which is involved in the neutrophil response, and Löfgren et al. found an association with the surfactant protein A locus,[57] which is involved in the innate immune response. We focused our study on genetic heterogeneity in the Th2 cytokines, in particular IL-4 and the IL-4 receptor, expecting that the frequency of gain-of-function variants of these Th2 cytokine genes would be higher among children with severe RSV bronchiolitis. If so, this would provide further evidence that the Th2 pathway plays a role in natural RSV disease.

A number of polymorphisms have been described in the promoter area of the IL-4 gene, of which the C-590T polymorphism is best studied. In vitro experiments have shown that the T allele has a higher level of promoter activity, probably as a result of the introduction of a site resembling the recognition site for the nuclear factor of activated T cell family of transcription factors.[187 188] In association studies, the T allele was positively associated with asthma,[189-192] but in other studies, this association could not be confirmed.[193 194]

Other polymorphisms described in the IL-4 gene are a C-34T polymorphism,[195] a C+33T polymorphism,[196] a 70-bp repeat in intron 3,[197] and a dinucleotide repeat in intron 2.[167] Although the five polymorphisms have never been tested together for linkage disequilibrium, strong linkage disequilibrium exists between different individual polymorphisms, at least in Japanese,[195 198 199] Australian,[200] and French populations.[201]

In our study, we found a genetic association between the IL-4 locus and RSV bronchiolitis: the -590T allele was more frequently found among children hospitalised for RSV bronchiolitis than in the control group (P=0.04; case-control study) and was more often transmitted from the parents of RSV infected children than would be expected to occur by chance (although this difference was not significant). Combination of all available information in the combination test resulted in a P value of 0.02 and an OR of 1.41.

We found no association with the IL-4 locus in the group of children with most severe disease (those who required mechanical ventilatory support). This could mean that IL-4 does not play an important role in disease in these children and that other biological processes probably are involved in the development of respiratory failure. However, this group of children might have been too small for detection of a significant association.

Very interesting is our finding of a significant association between the IL-4 locus and severe RSV disease among children who were hospitalised because of RSV bronchiolitis at age <6 months or age >6 months (OR, 2.09; P=0.02; combination test), whereas no association could be found among children <6 months old. This fits quite well with the current understanding of maturation of the immune system in infants.[202] Thus, it is easily conceivable that gain-of-function variation in IL-4 and the IL-4 receptor may be more relevant at age 6 months and after. In addition to maturation of the immune system, the loss of maternal antibodies could be relevant.

A subset of the children took part in a follow-up study in which post-RSV infection recurrent wheezing was examined. In children who did not experience recurrent wheezing during or after hospitalisation, a higher frequency of the IL-4-590T allele was observed (OR, 1.89; P=0.02; combination test), in contrast to the control group and to children with recurrent wheezing. Van Schaik et al. suggested that the release of leukotrienes might be involved in the acute (wheezing) phase of RSV-induced bronchiolitis, probably as a result of enhanced IFN-γ production.[203] In this context, it would be very interesting to study the genetic association between RSV disease, recurrent wheezing, and IFN-γ gene variants.

The IL-4Rα locus is characterised by a large number of polymorphic sites (almost 30). Ten polymorphic sites result in an amino acid substitution, and 9 of these are located in the intracellular domain.[204] Using 7 of these amino acid substitutions, 11 different putative haplotypes were calculated, of which 4 have a cumulative frequency of 90%.[205]

In our study, we investigated the association of severe RSV disease with two polymorphic sites of IL-4Rα: the I50V and the Q551R polymorphisms. The I50V polymorphism is located in the extracellular domain of the IL-4 receptor. Different approaches have been used to demonstrate that the 50I variant has the ability to up-regulate the receptor response to IL-4 than does the 50V variant.[172 206 207] The I allele was associated with atopic asthma in 2 Japanese association studies,[206 208] but the association was not found in a third Japanese study.[4] The I50V polymorphism is not in linkage disequilibrium with the Q551R polymorphism, as was shown in the present study and elsewhere.[209 210]

The Q551R polymorphism is located in the intracellular domain of the receptor in a region known to play an important role in IL-4-induced activation of STAT6 DNA-binding activity.[211] Hershey et al. found that the level of expression of CD23 is higher after stimulation with IL-4 in peripheral blood mononuclear cells from R551 homozygotes and R551 heterozygotes, which suggests that the signalling ability of the R551 allele is greater.[212] The level of binding of STAT6 was similar for both alleles. The same group reported a genetic association of the R551 allele with severe atopic

eczema and severe asthma.[212 213] Mitsuyasu et al. found no association between this allele and atopic asthma.[207] Finally, Kruse et al. found lower IgE levels in individuals bearing the R allele.[214]

In our study, we did not find any indication of a genetic association between the I50V polymorphism and RSV bronchiolitis. In contrast, we found a significant association between the R551 allele and severe RSV bronchiolitis in children who were hospitalised at an age >6 months (OR, 1.66; P=0.03; combination test). This further supports the idea that a Th2-mediated cell response is more important after the first half year of life. Because both the IL-4 receptor and the IL-13 receptor contain IL-4Rα, we cannot determine to what extent the positive association may result from binding of IL-4, IL-13, or both to their receptors. Interestingly, IL-13 polymorphisms were found to be associated with asthma and atopy.[215 216] Moreover, a significant gene-gene interaction between the S478P polymorphism in IL-4Rα and the -1111 promoter variation in IL-13 was associated with a risk of developing asthma that was 5 times greater among individuals who carry these risk alleles.[217] These findings and the observation that IL-13 induces airway hyperreactivity during RSV infection of mice make IL-13 an important candidate gene for future studies on RSV disease in children.[218-220]

In conclusion, our results demonstrate positive associations of gain-of-function mutations in two genes involved in Th2-type immune response with severe RSV bronchiolitis. Our results support the idea that Th2 responses may contribute to severe RSV disease. Our study also supports the idea that genetic differences may, to a certain extent, explain the differences in disease severity among RSV-infected children. In theory, positive associations do not prove causality. Another locus in close linkage disequilibrium with the examined one may be responsible (i.e., confounding). On the other hand, we have chosen markers with proven functional significance, which supports the suggestion that they play a causative role in the RSV disease process. Our results reveal interesting aspects of the pathogenesis of RSV disease. Because there were discrepant results for children younger than and children older than 6 months of age, it appears that Th2-mediated pathology is relatively more important at older ages, after the loss of maternal antibodies. Further studies should elucidate the relative role of these and other cytokine polymorphisms. In particular, whether children with a Th2 genotype have higher levels of Th2 cytokines in their lungs should be examined. In addition, it would be interesting to examine the extent to which genetic predisposition to severe RSV disease contributes to the development of asthma.

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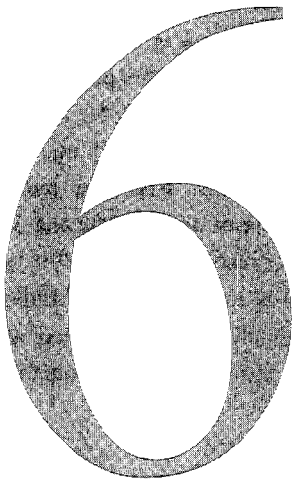

Safety and immunogenicity of a novel recombinant subunit respiratory syncytial virus vaccine (BBG2Na) in healthy young adults

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Abstract

A novel recombinant respiratory syncytial virus (RSV) subunit vaccine, designated BBG2Na, was administered to 108 healthy adults randomly assigned to receive 10, 100, or 300 μg of BBG2Na in aluminum phosphate or saline placebo. Each subject received 1, 2, or 3 intramuscular injections of the assigned dose at monthly intervals. Local and systemic reactions were mild, and no evidence of harmful properties of BBG2Na was reported. The highest ELISA and virus-neutralising (VN) antibody responses were evident in the 100 and 300 μg groups; second or third injections provided no significant boosts against RSV-derived antigens. BBG2Na induced ≥2-fold and ≥4-fold increases in G2Na-specific ELISA units in up to 100% and 57% of subjects, respectively; corresponding RSV-A-specific responses were 89% and 67%. Furthermore, up to 71% of subjects had ≥2-fold VN titre increases. Antibody responses to two murine lung protective epitopes were also highly boosted after vaccination. Therefore, BBG2Na is safe, well tolerated, and highly immunogenic in RSV-seropositive adults.

Introduction

Respiratory syncytial virus (RSV) is the leading cause of severe lower respiratory tract illness in newborns and young infants.[148 221] It is increasingly recognised to be an important pathogen in elderly and immunocompromised patients.[222] No effective treatment is available for RSV-induced disease, and the cost-effectiveness of promising prophylactic antibodies is under investigation. Thus, the development of a safe and effective vaccine remains an important strategy in the fight against RSV-associated diseases. However, in the 1960s, vaccination of young infants with formalin-inactivated alum-adsorbed RSV (FI-RSV) failed to protect them against infection and resulted in exacerbated disease when RSV infection occurred.[66] This event had a dramatic negative impact on the development of subunit vaccines. Although use of a live, attenuated RSV vaccine is not associated with enhanced disease, an appropriate balance between immunogenicity and innocuity remains elusive.[68]

In a novel strategy, we developed an RSV subunit vaccine based on a recombinant prokaryote-expressed protein, designated BBG2Na.[223] BBG2Na induces protective immune responses against RSV subgroups A and B, without evidence of FI-RSV-like enhanced immunopathology, in appropriate animal models.[223 224] Furthermore, BBG2Na induces protective immune responses in one week old mice, even in the presence of high titres of anti-RSV-A maternal antibodies.[225] In this study, we assessed the safety and immunogenicity of BBG2Na in healthy young adult volunteers.

Materials and methods

Vaccine

BBG2Na was purified (.99%) from *Escherichia coli* cell lysates. Preclinical toxicologic safety studies using the clinical formulation of BBG2Na were in compliance with current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Step 4 guidelines and US Food and Drug Administration guidelines (data not shown).[226 227] The vaccine was supplied in 2 separate vials that held BBG2Na freeze-dried with mannitol and aluminum phosphostate (Adjuphos; Superfos) suspended in water. The formulation was reconstituted just before use and was injected into the deltoid muscle. Vaccinees received BBG2Na in doses of 10, 100, or 300 μg, each containing 560 μg of aluminum, or received placebo (saline).

Study population and design

In all, 108 healthy subjects aged 18 to 45 years were enrolled, vaccinated, and followed initially for 8 weeks after vaccination. Volunteers were assigned to vaccine dosage groups (n = 9; 1, 2, or 3 doses of 10, 100, or 300 μg of BBG2Na at 4-week intervals) or placebo groups (n = 9; 1, 2, or 3 doses of placebo) in a randomised, single-blind fashion. Blood samples were obtained before each injection and 4 and 8 weeks after the last injection. Participants were monitored for 4 h after vaccination on site and by telephone at 24 h to determine immediate reactions. Local and systemic reactions were recorded on vaccination report cards by each volunteer for postvaccination days 0 to 6. Subjects were also followed for evidence of RSV-related upper respiratory tract infections (URTIs). The present article focuses on perivaccination safety, tolerance, immunogenicity, and occurrence of URTIs.

All volunteers enrolled in the trial gave written informed consent. The trial was approved by the Medical Ethical Committee, Erasmus University, and followed good clinical practice.

Immunologic assays

Antigen-specific serum IgG levels were determined by ELISA, using RSV-A (long)- or -B (B1 wild type; ATCC VR1400)-infected HEp-2 lysates; BBG2Na; G2Na; BB; or keyhole limpet hemocyanin (KLH)-coupled peptides $G_{144\text{-}159}$, $G_{164\text{-}176}$, $G_{172\text{-}184}$, or $G_{190\text{-}204}$ as coating antigens at previously optimised protein concentrations. The preparation and characterisation of KLH-peptide conjugates are described elsewhere.[228] Single serum dilutions specifically determined for each antigen by use of reference human serum samples were incubated in duplicate with the coating antigens. Reference serum samples were also included on each plate. Bound IgG was detected with horseradish peroxidase (HRP)-conjugated anti-human IgG (Biosource International). IgG1 isotype titres were determined similarly, except that murine anti-human IgG1 antibody (Chemicon) was added as a secondary antibody, whereas bound IgG1 was revealed by addition of goat anti-mouse IgG HRP-conjugated antibodies (Dako). Total IgG levels against viral antigens in test serum samples were expressed as arbitrary units (AU) based on standard curves generated with reference serum samples following absorbance reading at OD_{450}. Otherwise, antibody levels were expressed as optical density units (ODU) following reading of extinction at OD_{450}. Geometric means (GMs) and the percentage of persons in each group with ≥2- and ≥4-fold increases in AU or ODU between pre- and postvaccination serum samples were determined for each antigen.

Antigen-specific IgG2, IgG3, IgG4, and IgE serum antibody titres were determined in capture ELISA by using isotype-specific antibody-coated microtiter plates (CLB).

Serum samples were diluted 1 : 100, and 100 µL was deposited in each well of a 96-well microtiter plate. Antigen-specific antibody isotypes were detected by addition of HRP-conjugated antigens (RSV-A, BBG2Na, G2Na, and BB). Isotype titres were expressed as ODU, as described above. We incubated 96-well plates coated with anti-human IgA antibodies (Meddens Diagnostics) with patient serum. Bound IgA was detected with HRP-conjugated purified RSV nucleoprotein.

For virus-neutralisation (VN) assay, serial 2-fold dilutions of heat-inactivated (56°C for 30 min) test serum were prepared in triplicate in Dulbecco's MEM with 1% fetal bovine serum (BioWhittaker) in 96-well flat-bottomed plates (Greiner; 50 µL/well). Pre and postvaccination serum samples from each subject were always tested together in a single assay, along with the appropriate reference serum samples. Approximately 100 $TCID_{50}$ of RSV-A or -B in 50 µL of medium was added to each well, and plates were incubated at 37°C for 1 h. After incubation, 10^4 and 5 x 10^3 HEp-2 cells were added to each well for RSV-A and -B VN assays, respectively. After incubation for 6 to 8 days at 37°C, cytopathic effect was scored for each well. We calculated 50% VN titers by the Reed and Muench method.[229] Two or four reference human serum samples with known VN titres were treated similarly and used to calculate VN AU titres against RSV-A and -B, respectively, for each test serum sample by the following formula: (VN titre of test serum/mean VN titre of reference serum) x 100. GMs and the percentage of subjects demonstrating 2- and 4-fold increases in AU between pre- and postvaccination serum samples were determined for each RSV subtype.

Detection of RSV infection

RSV infections were detected in nasopharyngeal aspirates by direct immunofluorescence, using fluorescein isothiocyanate-conjugated RSV-specific monoclonal antibodies (Dako), as described elsewhere.[230]

Data-analyses

Statistical analysis was done with SAS software (version 6.12; SAS Institute). In view of the limited number of subjects in each group, safety and immunogenicity analyses were exploratory and descriptive.

Results

Vaccine tolerance and safety

All vaccine doses were well tolerated, and no serious adverse events were recorded (table 6.1; data not shown). Immediate vaccine-associated reactions consisted of mild

Table 6.1. *Local and systemic reactions in study volunteers within seven days of vaccination with BBG2Na, a respiratory syncytial virus vaccine candidate.*

Reaction	Injection number	BBG2Na	Placebo
Local			
Pain at injection site	1	27/81 (33.3%)	1/27 (3.7%)
	2	35/54 (64.8%)	1/18 (5.6%)
	3	18/27 (66.7%)	NR
Redness	1	1/80 (1.25%)†	1/27 (3.7%)
	2	2/54 (3.7%)	NR
	3	1/27 (3.7%)	NR
Induration	1	NR	NR
(hard bump)	2	NR	NR
	3	NR	NR
Swelling	1	NR	NR
	2	2/54 (3.7%)	NR
	3	1/27 (3.7%)	NR
Systemic			
Oral temperature	1	2/80 (2.5%)†	NR
≥37.5°C	2	1/54 (1.85%)	2/17 (11.8%)†
	3	2/26 (7.7%)	NR
Chills	1	5/81 (6.2%)	NR
	2	3/54 (5.6%)	NR
	3	NR	NR
Cough	1	9/81 (11.1%)	3/27 (11.1%)
	2	5/54 (9.3%)	2/18 (11.1%)
	3	5/27 (18.5%)	2/9 (22.2%)
Muscle ache	1	25/81 (30.9%)	NR
	2	9/54 (16.7%)	1/18 (5.6%)
	3	2/27 (7.4%)	NR
Decreased activity	1	8/81 (9.9%)	4/27 (14.8%)
	2	7/54 (13.0%)	2/18 (11.1%)
	3	4/27 (14.8%)	NR

Data are number of reactions/number of subjects injected (%).

NR, none reported.

† Vaccination report card was not fully completed by one individual.

to moderate pain at the injection site, especially after second and third injections. Rare vaccine-associated oedema was also reported. No induration was evident. Vaccine-associated systemic reactions included occasional myalgia and chills. Overall, no evidence of any harmful properties of BBG2Na was reported.

Respiratory infections

During the vaccination period, one URTI was reported and confirmed to have been caused by RSV, using positive antigen detection in nasal lavage. The subject was in

Table 6.2.A. *Humoral immune responses in healthy volunteers after injection with various doses of BBG2Na, an RSV vaccine candidate, or with placebo.*

Dose, Injection number	N‡	Serum IgG ELISA responses to RSV-A, AU (GM) T0	T4	≥2-Fold increase†	VN responses to RSV-A, AU (GM) T0	T4	≥2-Fold increase†
10 μg							
1	27	145	228	10	265	318	6
2	18	141	221	9	245	300	4
3	9	129	238	4	203	241	2
100 μg							
1	27	167	445	18	174	310	10
2	17	162	402	9	157	312	9
3	7	106	483	6	125	412	5
300 μg							
1	25	186	616	18	248	432	10
2	16	179	606	14	249	386	7
3	9	152	607	8	187	293	3
Placebo							
1	27	189	164	1	239	228	2
2	18	211	176	3	213	165	0
3	9	184	138	1	250	233	0

AU, arbitrary units (calculated as described in Materials and Methods); GM, geometric mean; RSV, respiratory syncytial virus; T0, prevaccination blood samples; T4, blood samples taken four weeks after indicated vaccine injection; VN, virus-neutralising.

† Data are number of subjects.

‡ Subjects with evidence of intercurrent infection were eliminated from the analysis.

the group given three 100-µg injections and had upper respiratory signs after the second injection. Three other subjects had serologic evidence of intercurrent RSV infection. Postinfection serologic samples from these subjects were excluded from the immunogenicity analyses to avoid bias associated with a natural infection.

Immunogenicity

Humoral IgG responses to RSV-A, G2Na, BBG2Na, and BB were consistent with a vaccine dose effect (tables 6.2.A, 6.2.B, and 6.2.C). In general, 300- and 100-µg doses induced high IgG responses, and 10-µg doses did not. Interestingly, especially with regard to RSV-A and G2Na antigens, a single dose was usually sufficient to induce

Table 6.2.B. *Humoral immune responses in healthy volunteers after injection with various doses of BBG2Na, an RSV vaccine candidate, or with placebo.*

		Serum IgG ELISA responses (GM) to indicated antigen								
		G2Na, OD_{450}			BBG2Na, OD_{450}			BB, OD_{450}		
Dose, Injection number	N‡	T0	T4	≥2-Fold increase†	T0	T4	≥2-Fold increase†	T0	T4	≥2-Fold increase†
10 µg										
1	27	0.17	0.63	20	0.38	0.96	18	0.09	0.30	17
2	18	0.18	0.72	15	0.40	1.02	13	0.10	0.39	12
3	9	0.20	0.68	7	0.50	1.27	6	0.13	0.63	7
100 µg										
1	27	0.26	1.11	26	0.46	1.46	20	0.14	0.67	18
2	17	0.27	1.18	15	0.46	1.93	14	0.17	0.84	13
3	7	0.30	1.17	7	0.50	2.04	7	0.14	1.10	6
300 µg										
1	25	0.29	1.26	22	0.55	1.50	16	0.17	0.61	18
2	16	0.38	1.26	10	0.66	1.71	8	0.22	0.88	10
3	9	0.42	1.37	7	1.55	2.76	2	0.80	1.87	6
Placebo										
1	27	0.27	0.27	0	0.59	0.62	2	0.24	0.23	0
2	18	0.33	0.30	0	0.74	0.78	1	0.32	0.32	0
3	9	0.38	0.35	0	0.99	1.02	1	0.42	0.40	0

GM, geometric mean; T0, prevaccination blood samples; T4, blood samples taken four weeks after indicated vaccine injection.

† Data are number of subjects.

‡ Subjects with evidence of intercurrent infection were eliminated from the analysis.

maximum antibody responses, which remained stable during the 8-week follow-up period (data not shown). IgG1 was the most abundant isotype detected after vaccination; a slight G2Na-specific IgG2 response was also observed. No significant IgG3, IgG4, or IgE responses were evident (data not shown). Furthermore, no significant RSV-B-specific antibody responses were detected.

Within the high-response groups (recipients of 100- and 300-μg doses), 53%-89% and 26%-67% of subjects demonstrated ≥2- and ≥4-fold increases, respectively, in RSV-A-specific antibodies, compared with prevaccination titres (table 6.2.A; data not shown). Furthermore, 63%-100%, 22%-100%, and 63%-86% of subjects from all 3 vaccine groups had ≥2-fold IgG antibody rises to G2Na, BBG2Na, and BB,

Table 6.2.C. *Humoral immune responses in healthy volunteers after injection with various doses of BBG2Na, an RSV vaccine candidate, or with placebo.*

		Serum IgG ELISA responses (GM) to indicated antigen					
		KLH-$G_{172-187}$, OD_{450}			KLH-$G_{144-159}$, OD_{450}		
Dose, Injection number	N‡	T0	T4	≥2-Fold increase†	T0	T4	≥2-Fold increase†
10 μg							
1	27	0.13	0.27	13	0.07	0.15	12
2	18	0.15	0.42	12	0.07	0.14	4
3	9	0.16	0.50	7	0.08	0.14	3
100 μg							
1	27	0.12	0.46	22	0.09	0.32	18
2	17	0.13	0.46	13	0.10	0.27	9
3	7	0.14	0.62	6	0.12	0.27	4
300 μg							
1	25	0.15	0.77	21	0.08	0.35	18
2	16	0.17	0.81	13	0.07	0.18	7
3	9	0.16	0.71	7	0.07	0.16	3
Placebo							
1	27	0.14	0.14	0	0.07	0.08	1
2	18	0.14	0.15	1	0.07	0.07	0
3	9	0.15	0.13	0	0.07	0.07	1

GM, geometric mean; KLH, keyhole limpet hemocyanin; T0, prevaccination blood samples; T4, blood samples taken four weeks after indicated vaccine injection.

† Data are number of subjects.

‡ Subjects with evidence of intercurrent infection were eliminated from the analysis.

respectively, whereas placebo recipients had no significant responses (table 6.2.B). As expected, our data suggest an inverse correlation between antibody titre rises and prevaccination titres (data not shown).

We reported elsewhere the induction of low to moderate VN titres in seronegative mice immunised with BBG2Na.[223] Therefore, we were surprised to observe significant increases in VN titres after vaccination of subjects (table 6.2.A). Indeed, 33%-71% of subjects had ≥2-fold increases in VN titre after 100- or 300-μg doses, and up to 22% of persons who received 10-μg doses showed similar increases. This contrasts with the results for the placebo groups, in which only 7% of subjects demonstrated a ≥2-fold increase in VN titre. With regard to ELISA GMs, a ≥2-fold increase in VN titre was inversely correlated with prevaccination VN titres. In addition, no significant RSV-B VN responses were evident after BBG2Na vaccination (data not shown).

Peptides $G_{144-159}$, $G_{164-176}$, $G_{172-187}$, and $G_{190-204}$ are independent B cell murine lung protective epitopes (protectopes) that cross-react with human serum samples obtained during the convalescent phase of RSV infection.[228] We used these peptides to determine whether BBG2Na induced antibody responses to these protectopes in humans. Strong responses against KLH-$G_{144-159}$ and KLH-$G_{172-187}$ were evident (table 6.2.C). Higher anti-KLH-$G_{172-187}$ antibody GMs were induced as a function of increasing vaccine dose, although all doses were highly immunogenic compared with placebo. Indeed,.>80% of subjects vaccinated once with 100- or 300-μg of BBG2Na demonstrated ≥2- fold increases in anti-KLH-$G_{172-187}$ titres, and 48%-60% had ≥4-fold increases (data not shown). Increases in anti-KLH-$G_{144-159}$ GM were similar in the recipients of 100- and 300-μg doses after a single injection, whereas the 10-μg dose induced considerably lower anti-peptide responses. Importantly, ≥2- fold increases were observed in 67%-72% of persons after 1 dose of 100 or 300 μg of BBG2Na. In contrast, no significant responses to KLH-$G_{164-176}$ or KLH-$G_{190-204}$ were evident after BBG2Na vaccination (data not shown).

Discussion

Here we describe the first administration to humans of BBG2Na, a promising RSV subunit vaccine, in terms of safety, tolerability, and immunogenicity. Our findings demonstrate that BBG2Na formulated in Adjuphos is safe, well tolerated, and highly immunogenic in healthy young adults. The local reactions reported after vaccination (e.g., pain) are considered to be normal responses to the administration of Adjuphos. The infrequent vaccine-associated systemic reactions (e.g., myalgia and chills) are also consistent with flu-like syndromes that have been described after administration

of commercial vaccines.[231] Therefore, no harmful effects attributable to BBG2Na were evident.

The components of protective immunity against RSV disease in humans remain enigmatic. In clinical studies, polyclonal human serum with high RSV VN activity (RespiGam; Med-Immune) and an RSV-F protein-specific monoclonal antibody (Synagis; MedImmune) had positive effects on RSV disease burden after prophylactic administration to high-risk children.[74 232] Therefore, antibodies constitute at least one component in protection against RSV-mediated lower respiratory tract disease in humans, as observed in animal models.[223 233]

The capacity of BBG2Na to induce strong humoral responses in healthy adults is consistent with its immunogenicity in animals.[223] In particular, the anti-RSV-A antibody responses confirm that the intrinsically nonglycosylated, prokaryote-derived BBG2Na is capable of inducing relevant RSV-specific immune responses in humans. Because the native G protein is poorly immunogenic in infants, possibly due to its heavy glycosylation, and BBG2Na immunogenicity in neonatal mice is unaffected by maternal antibodies,[225] the nonglycosylated nature of BBG2Na may be a positive attribute in this population. It is also encouraging that antibody responses to two murine B cell lung protectopes were substantially increased after BBG2Na vaccination.

Although titration techniques differ, our data compare well with other reports involving purified F protein preparations (PFP-1 and -2).[68 234] We were also encouraged by increases in VN titres, because BBG2Na induces only low to moderate VN titers in seronegative mice. The absence of detectable RSV-B responses may reflect poor RSV-B-specific immunogenicity or masking by preexisting antibodies in adults. Interestingly, four subjects had evidence of intercurrent RSV infection, one with URTI symptoms. However, this is consistent with data from cotton rats, in which no upper respiratory tract protection was induced after intramuscular administration of BBG2Na.[223] More important, no serious adverse clinical events were evident after RSV infection, suggesting that BBG2Na vaccination of adults does not cause exacerbated disease. In conclusion, BBG2Na, a novel recombinant subunit vaccine candidate for use against RSV, is safe, well tolerated, and capable of inducing RSV-specific lower respiratory tract protection-associated humoral immune responses in healthy young adults.

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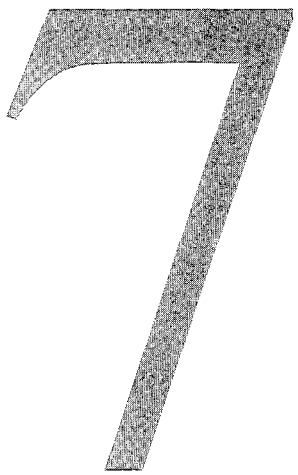


Summary and general discussion

7.1

Summary

Introduction

Respiratory syncytial virus is the main cause of lower respiratory tract infections in infants and young children. Although almost all children are infected before the age of two years, less than 2% develop severe disease necessitating hospitalisation. Risk factors for severe RSV disease include prematurity, chronic lung disease (CLD), young age, low birth weight, congenital heart disease (CHD), and immunodeficiencies.

The aims of the studies described in this thesis were to develop a clinical prediction rule to estimate the individual monthly risk of hospitalisation for severe RSV disease in young children (**chapter 2.1**), and to assess the role of the average seasonal RSV pattern in the performance of this prediction rule (**chapter 2.2**). Furthermore, we aimed to perform a cost-effectiveness analysis of passive immunisation against RSV. Therefore we determined RSV hospitalisation costs in infants and young children and developed a prediction model that estimated anticipated mean RSV hospitalisation costs in children at-risk, based on several child characteristics (**chapter 3.1**). Then we assessed the incremental costs to prevent one RSV hospitalisation in high-risk children from a societal perspective, using a novel individualised monthly approach for decision making on passive immunisation (**chapter 3.2**). In addition we aimed to quantify the loss of health related quality of life (HRQoL) in children during the first months after RSV hospitalisation (**chapter 4**), study the association of severe RSV disease with polymorphisms located in the promoter region of the IL-4 gene and in the IL-4Rα gene (**chapter 5**), and explore the safety, tolerability, and immunogenicity of BBG2Na, a recombinant RSV vaccine constructed from a G protein sequence, in healthy young adults (**chapter 6**).

A clinical prediction rule for RSV hospitalisation in young children

Monthly administration of passive immunisation against RSV during the RVS season can reduce severe RSV disease. Because of the high costs associated with passive immunisation, its use should be restricted to well defined high-risk children. Current guidelines of the American Academy of Pediatrics (AAP) define categories of high-risk children by gestational age (less than 35 weeks), and the presence of BPD and age (younger than one or two years). For these children passive immunisation against RSV is recommended for the complete RSV season.

We conducted a retrospective cohort study in the Southwest of the Netherlands, to develop a clinical prediction rule which included five child characteristics (gestational age, presence of bronchopulmonary dysplasia (BPD), birth weight, gender and age) and the average seasonal pattern of RSV infections as predictors and estimated the individual monthly risk of RSV hospitalisation in young children (**chapter 2.1**). We collected information on 140,661 children born from 1996 to 1998, who were 1,181,790 months at-risk during the RSV seasons 1996-1997 to 1998-1999. During these seasons, 2,469 of these children were hospitalised with severe RSV disease.

We found higher monthly hospitalisation risks for males and children suffering from BPD. The hospitalisation risk increased with decreasing gestational age and birth weight, and varied within the RSV season, with a seven times as high risk in December compared to October. The hospitalisation risk decreased more steeply with increasing age for children without BPD compared to children with BPD. All six predictors had statistically significant associations with the monthly RSV hospitalisation risk. Age and the seasonal RSV pattern were the strongest predictors, while gestational age, presence of BPD, birth weight and gender were less important.

The performance of the prediction rule was studied with respect to discrimination and reliability (calibration). Discrimination refers to the ability to distinguish hospitalised children from non-hospitalised children and is commonly quantified by the area under the receiver operating characteristics (ROC) curve (AUC). The AUC of the prediction rule for all children at-risk was 0.80. For premature children with a gestational age below 33 or 37 weeks, the AUCs were 0.75 and 0.80 respectively. Reliability indicates the agreement between the predicted risks of RSV hospitalisation and the observed frequency of RSV hospitalisations. For predicted risks over 1.5%, the hospitalisation risks predicted by our prediction rule were somewhat higher than the observed frequencies of hospitalisation in our cohort. This inaccuracy was possibly related to the low percentage (5.6%) of months at-risk with such high predicted risks. In addition, the overall goodness of fit of the model was satisfactory, as indicated by a nonsignificant Hosmer-Lemeshow test (p=0.79). Internal validity was assessed with bootstrapping techniques. One hundred bootstraps indicated minor optimism in AUC (0.001), which was consistent with the large sample size that was used to develop the model.

We compared the prediction rule with the AAP guidelines for the ability to distinguish between high and low-risk children among those with a gestational age of 32 weeks or less, using implicit AAP thresholds for the monthly hospitalisation risk. The prediction rule could better distinct between high and low-risk children than the AAP guidelines and would have reduced the number of immunisations from 3996 to 3179 (-20%) for children with a gestational age of 32 weeks or less. Compared to the

AAP guidelines, the prediction rule limited the number of immunisations given to each child by excluding low-risk months.
We concluded that it is possible to reliably predict monthly RSV hospitalisation risks for individual children. These individual risks may be helpful to clinicians to allocate passive immunisation to the highest risk children at the most efficient time points within the RSV season, thus reducing the total number of immunisations.

Influence of RSV season on the validity of a clinical prediction rule for RSV hospitalisation risk in young children

The pattern of RSV epidemics differs across seasons. Marked differences are found regarding the total number of cases, the peak month of the season, and the distribution of cases among the different months. Hence, the performance of the clinical prediction rule for RSV hospitalisation risk in young children in different RSV seasons can be seriously hampered. An alternative model might even be considered, excluding seasonality as a predictor.
The prediction model with 5 clinical predictors and a variable for the average RSV seasonal pattern (seasonal model) was compared to a prediction model with the 5 clinical predictors only (clinical model) (**chapter 2.2**). Model performance was assessed according to discrimination and calibration. Furthermore, we determined the validity of the models per RSV season and assessed the impact of the seasonal variability. The models, developed with the total data, were validated per RSV season leading to three validation samples. The seasonal model had on average significantly higher discriminative ability than the clinical model. Discrimination of the seasonal model in each of the validation samples was better than that of the clinical model. Calibration was poor however among the seasons for both the seasonal and clinical model.
We concluded that including the average seasonal RSV pattern in the model improved the discrimination, but differences in calibration among RSV seasons were found for both the seasonal and the clinical model. Incorporation of seasonality in a more dynamic way may improve the calibration.

Costs of hospitalisation for respiratory syncytial virus in young children

Since health care budgets are limited, economic analyses are needed to optimise the allocation of resources in health care. Reliable estimates of costs of RSV hospitalisation are necessary to perform economic analyses of preventive strategies of severe RSV disease. We performed a population based cohort study in the Southwest of the Netherlands, to develop a model that predicts anticipated mean RSV hospitalisation costs of groups of young children at-risk for RSV hospitalisation, but not hospitalised yet, based on four child characteristics (age, gestational age, birth weight, and BPD) (**chapter 3.1**).

Real direct medical costs of RSV hospitalisation of all children hospitalised with severe RSV disease during the seasons 1996-1997 to 1999-2000 (n=3,458) were determined from a societal perspective, using a bottom-up strategy. The mean hospitalisation costs of all patients hospitalised for severe RSV disease were €3,110. The hospitalisation costs were strongly related to the duration of hospitalisation. Therefore RSV hospitalisation costs were higher for patients with lower gestational age (€5,555; gestational age ≤28 weeks), lower birth weight (€3,895; birth weight ≤2500 g), BPD (€5,785; with BPD), and young age (€4,730; first month of life).

Information on the characteristics and hospitalisation costs of all patients was used to construct a linear regression model, that estimated anticipated mean hospitalisation costs of groups of children at-risk, based on four child characteristics (age, gestational age, birth weight, and BPD). This model had an adjusted R^2 of 0.08, which indicates a low explanatory ability for hospitalisation costs of individual children. However, the model could accurately estimate the anticipated mean hospitalisation costs of groups of children with the same characteristics.

We concluded that RSV hospitalisation costs were substantial, especially of specific high-risk groups, and were highly correlated with duration of hospitalisation. Although anticipated individual hospitalisation costs of children at-risk for RSV hospitalisation were hard to predict, anticipated mean hospitalisation costs of groups of children at-risk for RSV hospitalisation could well be estimated with four child characteristics (age, gestational age, birth weight, and BPD). These estimated costs can be used for economic analyses of preventive strategies for severe RSV disease.

Cost-effectiveness of passive immunisation against RSV in young children

The costs of passive immunisation against RSV (palivizumab) are considerable. Treatment of one child during a complete RSV season costs approximately €3,550 (mean weight 5 kg, 5 injections, no wastage). These costs, combined with the moderate efficacy (odds ratio (OR) of 0.48 for effectiveness of passive immunisation), and the generally low incidence of RSV hospitalisation, have led to a discussion concerning the cost-effectiveness of passive immunisation.

We performed a cost-effectiveness analysis of passive immunisation against RSV by programming prediction models for monthly hospitalisation risks (**chapter 2.1**) and monthly RSV hospitalisation costs (**chapter 3.1**), with data on immunisation costs, parental costs, and efficacy of passive immunisation, in an Excel spreadsheet (**chapter 3.2**). We calculated monthly costs and effects with and without prophylactic treatment. The primary outcome measure, the incremental costs to prevent one hospitalisation, was calculated for every month of the RSV season. The reference case was a high-risk boy with a gestational age ≤28 weeks, birth weight ≤2500 g, BPD, and age of zero months at the beginning of the RSV season.

Cost-effectiveness of passive immunisation varied across the RSV season between €15,520 in December and €883,930 in October for the reference case. Every month costs per hospitalisation averted were higher for children without BPD and children with higher gestational ages. Costs per hospitalisation averted were also higher for girls, and decreased with decreasing birth weight and age. Passive immunisation was most cost-effective in December, but was never cost saving.

The estimated cost-effectiveness of passive immunisation against RSV for the reference case in December (€15,520 per hospitalisation averted) was most sensitive to changes in immunisation costs, followed by hospitalisation risk, efficacy of passive immunisation and hospitalisation costs. For the reference case cost-neutrality was reached in December, when acquisition costs of passive immunisation decreased from €930 to €335, monthly hospitalisation risk increased from 7.6% to 20%, or hospitalisation costs increased from €10,250 to €25,750 per hospitalisation. Even when passive immunisation would prevent all hospitalisations, costs per hospitalisation averted would still exceed €2,645.

We concluded that although cost-effectiveness of passive immunisation varied strongly by child characteristics and seasonal month, the incremental costs per hospitalisation averted were always high. We therefore recommended a restrictive

immunisation policy. The acquisition costs of passive immunisation need to be reduced to reach acceptable levels of cost-effectiveness for high-risk children in high-risk months.

Quality of life after hospitalisation for respiratory syncytial virus infection

Most economic analyses of passive immunisation against RSV report high costs relative to the benefits realised. Based on these findings a restrictive use of passive immunisation seems recommendable. However, choices about reimbursement of passive immunisation should not be based on costs and clinical effectiveness parameters alone. The impact of RSV hospitalisation on the physical, mental, and social functioning of children and their parents should also be considered. This impact can be measured using health related quality of life (HRQoL).

We used the generic TNO-AZL Preschool Children Quality of Life (TAPQoL) measure to quantify the loss of HRQoL in 50 children, four months (median) after RSV hospitalisation during the RSV season 2001-2002 (**chapter 4**). Mean TAPQoL scale scores were compared between RSV hospitalised children and healthy children. Compared to healthy children, children who had been hospitalised with severe RSV disease had significantly lower average scores on most TAPQoL scales. Moderate to large negative effects (Cohen's effect size: the relative difference between the means of two groups in relation to the biggest standard deviation of the two groups) were found on the TAPQoL scales lungs and motor functioning, indicating lower HRQoL in the group of RSV hospitalised children. A large positive effect was found for the scale problem behaviour, indicating a higher mean score in the group of RSV hospitalised children.

Differences in disease severity during hospitalisation (measured by duration of hospitalisation, the need for supplemental oxygen therapy, ICU admission, or mechanical ventilation during hospitalisation) did not influence the HRQoL. Children with persisting respiratory problems (dyspnea and/or wheezing) after RSV hospitalisation had significantly lower average scores on the TAPQoL scales sleeping, appetite, and lungs, compared to children without persisting respiratory problems, with a large negative effect size for the TAPQoL scale lungs.

We found significant negative correlations between age at inclusion in the study of all RSV hospitalised children and the TAPQoL scale scores sleeping, appetite, lungs, problem behaviour and anxiety. Boys had significant lower scores on the TAPQoL scale problem behaviour compared to girls. The time between hospitalisation and

completion of the questionnaire, gender of the responder, and the educational level of the responder had no influence on the TAPQoL scale scores.
We performed a multivariate analysis to investigate the associations between gender, age, persisting respiratory problems, and duration of hospitalisation and the different TAPQoL scale scores within the group of RSV hospitalised children. Significantly lower scores were found on the TAPQoL scale problem behavior for boys compared to girls, and on the TAPQoL scale anxiety for older children compared to younger children. Children with persisting respiratory problems had significantly lower scores on the TAPQoL scales sleeping, appetite, and lungs compared to children without persisting respiratory problems. Children with a longer duration of hospitalization had significantly higher scores on the TAPQoL scales sleeping, appetite, and stomach compared to children with a shorter duration of hospitalization.
We concluded that several months after RSV hospitalisation, children had a lower HRQoL compared to healthy children. Negative effects on the TAPQoL scales sleeping, appetite, and lungs were primarily caused by persisting episodes of dyspnea and/or wheezing after discharge.

Association of severe RSV infection with IL-4 and IL4 receptor α polymorphisms

The severity of RSV disease in infants and young children ranges from mild upper respiratory tract disease to severe lower respiratory tract disease with respiratory insufficiency and even death. Several factors are associated with more severe RSV disease. However, the reasons for variation in disease severity are not completely clear. Some of this variation might be explained by genetic heterogeneity in the immune response of infected children. T cell-mediated immunopathology, in particular a more pronounced T helper type 2 (Th2) response, may play an important role in the development of severe RSV disease. It is also suggested that there is a relationship between the occurrence of severe RSV disease and the development of asthma later in life.
We studied the association between severe RSV disease and gain-of-function mutations in the Th2 cytokine genes encoding interleukin (IL)-4 (C-590T) and the IL-4 receptor α chain (IL-4Rα) (I50V and Q551R) (**chapter 5**). The results of 207 infants hospitalised with severe RSV disease were compared with the results of two control groups. One group consisted of the parents of the hospitalised children (for the transmission/disequilibrium test), and the other group (n=447) consisted of a random

population sample (for the case-control study). Results were also analysed in a combination of these two tests.
The IL-4 590T allele was found more frequently among children hospitalised with RSV than expected in the case-control and combination tests. Among children who were more than six months old when they were hospitalised, compared with the control group or with the children less than six months old who were hospitalised for RSV infection, higher frequencies of both the IL-4 590T allele and the IL-4Rα R551 allele were found. These results indicated that gain-of-function variants of Th2 cytokine genes may play a role in increasing the severity of RSV disease, which appears more pronounced after the age of six months.

Safety and immunogenicity of a novel subunit RSV vaccine in healthy young adults

Since severe RSV disease causes significant morbidity in infants and young children, prevention of severe RSV disease is highly needed. We assessed the safety and immunogenicity of BBG2Na, a novel recombinant RSV subunit vaccine, in a randomised, single-blind, placebo controlled trial in 108 healthy young adult volunteers (**chapter 6**). Volunteers were randomly assigned to receive 1, 2, or 3 intramuscular injections of 10, 100, or 300 μg of BBG2Na in aluminium phosphate or saline placebo at monthly intervals. Blood samples were obtained before each injection and four and eight weeks after the last injection. Local and systemic reactions were recorded until six days after vaccination. Subjects were also followed for evidence of RSV-related upper respiratory tract infections (URTIs).
We only observed mild local and systemic reactions. Humoral IgG responses to RSV-A, G2Na, BBG2Na, and BB were highest in the 100 μg and 300 μg groups. In all subjects receiving 100 μg or 300 μg of BBG2Na, 2-fold or higher and 4-fold or higher increases in G2Na-specific ELISA units were induced in up to 100% and 57% of subjects, respectively; corresponding RSV-A-specific responses were 89% and 67%. Interestingly, a single dose was usually sufficient to induce maximum antibody responses, which remained stable during the eight-week follow-up period. No significant RSV-B-specific antibody responses were detected. Significant increases in virus-neutralising (VN) titres after vaccination were evident in the 100 μg and 300 μg groups. Up to 71% of subjects had 2-fold or higher VN titre increases. No significant RSV-B VN responses were evident after vaccination. Antibody responses to two murine lung protective epitopes were also highly boosted after vaccination. Four subjects had evidence of intercurrent RSV infection, of which one was accompanied by clinically evident URTI symptoms.

We concluded that BBG2Na was safe, well tolerated, and highly immunogenic in healthy young RSV-seropositive adults.

7.2

Implications for clinical practice and future perspectives

Implications for clinical practice and future perspectives

The development of a safe and efficacious vaccine against RSV is one of the priorities of the Global Programme for Vaccines of the World Health Association.[69] However, despite extensive research, no vaccine for infants and young children is available yet.[68 71]

BBG2Na, a novel recombinant respiratory syncytial virus (RSV) subunit vaccine has been shown to be safe, well tolerated, and highly immunogenic in RSV-seropositive adults. Whether this leads to long lasting protection against serious RSV disease caused by RSV groups A and B needs to be examined. Furthermore, data on the induction of cell-mediated immunity are lacking. Further studies are necessary to assess the safety and immunogenicity of BBG2Na in RSV-naive infants and young children. However, there are major concerns about the development of enhanced disease upon natural RSV infection following vaccination, as was observed in the FI-RSV trial. BBG2Na is a chimeric protein, containing a fraction of RSV group A G protein (G2Na). The RSV G protein has been associated with the development of a T helper 2 type cell-mediated immune response, which is associated with enhanced disease.[60 235] Other approaches, especially live attenuated RSV vaccines, might be more appropriate for RSV naive infants and young children. These vaccines, when given intranasally, induce both local and systemic immune responses, that closely resemble the response to natural RSV infection, and thus do not induce enhanced disease.[68] However, several problems concerning over- or under-attenuation, and genetic instability need to be solved. Maternal vaccination against RSV to induce high levels of maternal antibody in the newborn infant has been proposed.[236] Studies with administration of polyclonal and monoclonal antibodies have shown that high levels of circulating antibody can reduce severe RSV disease.[74 77] However, high-risk premature children, especially those with a gestational age of 28 weeks or less, are unlikely to benefit from maternal immunisation, because transfer of maternal antibody is minimal in these children.

Passive immunisation to reduce severe RSV disease in high-risk children is available since a few years.[74 77] Most cost-effectiveness analyses of passive immunisation show high incremental costs to prevent one hospitalisation and no cost-savings.[27 29 30 86 90-94] To minimise the incremental costs of passive immunisation, it should be reserved for the highest-risk children at the most efficient moments during the RSV season.

It is possible to reliably predict individual monthly RSV hospitalisation risks for infants and young children with a clinical prediction rule which uses five child characteristics (gestational age, presence of bronchopulmonary dysplasia (BPD), birth

weight, gender and age) and the average seasonal pattern of RSV infections as predictors. The distribution of monthly hospitalisation risks varied strongly within a RSV season, making seasonality the second most important predictor. However, the use of a mean seasonal pattern has its limitations. It ignores the variability in the patterns of RSV infections that are observed year by year. Including seasonality in the prediction rule improved the discrimination, but large differences in calibration among RSV seasons were found. In the future, this might be improved by the incorporation of a dynamic variable for season, which uses data on historical RSV seasonal patterns and the current incidence of RSV infections to predict the short term future RSV incidence. However, this will have important practical implications. To incorporate a dynamic variable for season, the model needs to function in real time and should continuously adapt its predictions, based on the current incidence of RSV infections. Recommendations on the administration of passive immunisation can be given no longer than approximately one month in advance. Such a system necessitates a database of potential candidates for passive immunisation, and a network to advise the treating paediatricians.

We are currently collecting data for external validation of the prediction rule in the Southwest of the Netherlands. This, and external validation in other settings is needed. Other potential predictors for RSV hospitalisation like CHD, immunodeficiencies and environmental factors could not be included in the prediction rule, since these predictors were not registered for the children at-risk. Furthermore, there is no information on the safety and efficacy of passive immunisation in these children yet. However, these characteristics may be important for final decision making in individual children.

Anticipated mean hospitalisation costs of groups of children at-risk for RSV hospitalisation could well be estimated with four child characteristics (age, gestational age, birth weight, and BPD). However, differences in clinical management of RSV infections between hospitals in different countries and even within one country may influence the total RSV hospitalisation costs in different regions.[137 138]

Since passive immunisation has not been shown to influence the occurrence or severity of recurrent wheezing, these costs are at the moment not relevant for cost-effectiveness analyses of passive immunisation, and were therefore not considered. Direct non-medical costs were determined in detail by others.[3 139]

The cost-effectiveness of passive immunisation against RSV in The Netherlands varied strongly by child characteristics and seasonal month. The incremental costs per hospitalisation averted were always high (€15,520 or more), which is in accordance with most other cost-effectiveness analyses performed.[27 29 30 86-94]

When cost-savings would be the criterion for reimbursement of passive immunisation, it would not be indicated for any child in The Netherlands. However, the prevention of morbidity caused by severe RSV disease and the accompanying stress to children and there parents may impose some costs upon society. Unfortunately, there is no published threshold for cost-effectiveness expressed in costs per hospitalisation averted. This threshold should however be well below standards for costs per life-year saved (e.g. $50,000).[141] Robbins et al. estimated that prevention of RSV hospitalisation in a high-risk child may cost between $1,325 and $8,700 (mean $5,787), which is far below the cost-effectiveness ratios found in our study.[128] If one assumes that the incremental costs to prevent one RSV hospitalisation should not exceed several thousand Euros, passive immunisation would not be indicated for any child in The Netherlands.

Passive immunisation against RSV can however be considered for premature infants with BPD. These infants have the highest hospitalisation risk, and as a consequence, the lowest incremental costs to prevent one RSV hospitalisation. Furthermore, these infants have more severe RSV disease, characterised by a longer duration of hospitalisation, and higher incidence of oxygen therapy, ICU admission, and mechanical ventilation.[26] Passive immunisation in these children, during the three highest risk months of the RSV season (November, December, and January) leads to minimal incremental costs to prevent one RSV hospitalisation of €27.500. In The Netherlands, about 350 premature infants with BPD are diagnosed every year. Supposing three vaccinations per infant, the total yearly costs of passive immunisation for these infants in The Netherlands are approximately one million Euros (350 x 3 x €980). With an average cumulative hospitalisation risk from November to January of 10%, about 15 of 35 expected hospitalisations could potentially be prevented in these infants (OR = 0.48 for effectiveness of passive immunisation).

The costs of passive immunisation need to be reduced to reach acceptable levels of cost-effectiveness for high-risk children in high-risk months. Passive immunisation can become cost saving in premature infants with BPD when the current costs of passive immunisation are reduced by two thirds. Such a reduction can be obtained by lowering the acquisition costs of passive immunisation, reduction of wastage by clustered administration of passive immunisation, and/or marketing of a 25 mg vial. The results of this cost-effectiveness analysis of passive immunisation against RSV will be discussed with Dutch paediatricians. Since our cost-effectiveness model includes data on all children (premature and at term), it can well be used for cost-effectiveness analyses of future vaccine strategies against RSV.

Several months after RSV hospitalisation, children had a lower HRQoL compared to healthy children, with negative effects on the TAPQoL scales sleeping, appetite, and

lungs, that were primarily caused by persisting episodes of dyspnea and/or wheezing after discharge. It will be interesting to study the persistence of these effects on HRQoL over time and the loss of HRQoL during admission. Currently one is working on a method to convert the different TABQoL scale scores per child into one utility score per child. This would enable cost-utility analyses of passive immunisation against RSV.

Besides known risk factors for severe RSV disease, host genetic polymorphisms in genes encoding for proteins involved in the immune response against RSV are of additional interest. We showed that gain-of-function variants of Th2 cytokine genes may play a role in increasing the severity of RSV disease, especially after the first half year of life. This age effect could be a result of maturation of the immune system. Furthermore, the loss of maternal antibodies could be relevant. More studies are needed to unravel the influence of host genetic polymorphisms in genes encoding for relevant proteins on the severity of RSV disease. It would be interesting to examine whether the presence of a Th2 genotype is associated with higher levels of Th2 cytokines in lungs of children with a RSV lower respiratory infection. In addition, the extent to which genetic predisposition to severe RSV disease contributes to the development of recurrent wheezing needs to be examined.

7.3

Samenvatting

Inleiding

Het respiratoir syncytieel virus (RSV) is de belangrijkste verwekker van virale luchtweginfecties bij zuigelingen en jonge kinderen. Infecties met RSV komen in Nederland voor in jaarlijkse epidemieën tussen oktober en april. Vrijwel alle kinderen hebben voor de leeftijd van twee jaar een infectie met RSV doorgemaakt. Het merendeel van deze infecties verloopt mild. Echter, 0,5% tot 2,0% van alle geïnfecteerde zuigelingen ontwikkelt een ernstige lagere luchtweginfectie waarvoor ziekenhuisopname noodzakelijk is. Te vroeg geboren kinderen (ex-prematuren), kinderen met restschade aan de longen na beademing of zuurstofgebruik (bronchopulmonale dysplasie (BPD)), lopen een beduidend hoger risico op het krijgen van een ernstige RSV-infectie.

Tot op heden is geen veilig en effectief vaccin tegen RSV beschikbaar. Wel is het sinds enkele jaren mogelijk om het opnamerisico voor een RSV-infectie voor ex-prematuren en kinderen met BPD te verlagen middels passieve immunisatie. Echter, de hoge kosten van passieve immunisatie (€4.500 per kind per seizoen), gecombineerd met de matige werkzaamheid (55% reductie van ziekenhuisopnames), bij een overwegend lage incidentie van RSV hospitalisatie, hebben geleid tot vragen over de kosteneffectiviteit.

Omdat specifieke Nederlandse gegevens over het hospitalisatierisico voor RSV-infecties bij jonge kinderen ontbreken, ontwikkelden wij een besliskundig model dat het maandelijkse risico op ziekenhuisopname voor RSV-infecties schat bij jonge kinderen in het algemeen, en ex-prematuren en kinderen met BPD in het bijzonder (**hoofdstuk 2.1**). Vervolgens hebben wij gekeken wat de waarde was van het gemiddelde seizoensbeloop van RSV-infecties bij het schatten van het maandelijkse opnamerisico door het besliskundig model (**hoofdstuk 2.2**). Gegevens over de kosten van alleen symptomatische behandeling van RSV-infecties in Nederland zijn niet beschikbaar. Daarom bepaalden wij de kosten van ziekenhuisopname voor RSV bij jonge kinderen en ontwikkelden wij een besliskundig model waarmee deze kosten kunnen worden voorspeld bij kinderen die risico lopen om te worden opgenomen, maar nog niet opgenomen zijn (**hoofdstuk 3.1**). De gegevens over risico op ziekenhuisopname en kosten van ziekenhuisopname werden, samen met gegevens over de kosten van passieve immunisatie en effectiviteit van passieve immunisatie, gebruikt om de kosteneffectiviteit (de extra kosten per voorkomen ziekenhuisopname) van passieve immunisatie versus géén passieve immunisatie tegen RSV te analyseren bij jonge kinderen in Nederland (**hoofdstuk 3.2**). Het is nog onbekend wat de invloed is van het doormaken van een ernstige RSV-infectie op de kwaliteit van leven van

kinderen. Daarom bepaalden wij de kwaliteit van leven van kinderen in de eerste maanden na ziekenhuisopname voor een RSV-infectie (**chapter 4**). Omdat de ziekteverschijnselen bij een RSV-infectie voor een belangrijk deel worden veroorzaakt door de afweerreactie van het lichaam op RSV, hebben wij gekeken of specifieke kleine variaties in de erfelijke codes van een aantal eiwitten die betrokken zijn bij de afweer van het lichaam tegen RSV, gerelateerd waren aan de ernst van de RSV-infectie (**hoofdstuk 5**). Tenslotte hebben wij de veiligheid en werkzaamheid bepaald van een nieuw kandidaat vaccin tegen RSV (BBG2Na) bij jong volwassen vrijwilligers (**hoofdstuk 6**).

Bepalen van het risico op ziekenhuisopname voor RSV bij jonge kinderen in Nederland

Wij voerden een retrospectief cohortonderzoek uit in de regio Zuidwest Nederland (**hoofdstuk 2.1**). Uit de Landelijke Verloskundige Registratie (LVR) en de Landelijke Neonatale Registratie (LNR) werd informatie verkregen van alle kinderen die in de jaren 1996 t/m 1998 waren geboren in deze regio (n=140.661). Deze kinderen liepen tezamen 1.181.790 maanden risico om met een RSV infectie te worden opgenomen tijdens de RSV-seizoenen 1996-1997 t/m 1998-1999. Gedurende deze seizoenen werden 2.469 van deze kinderen opgenomen met RSV.

Het maandelijks risico op ziekenhuisopname voor RSV werd geschat door het aantal kinderen dat in het ziekenhuis werd opgenomen te delen door het aantal kinderen dat werd geboren, en dus risico liep om met RSV te worden opgenomen ('at-risk'). Hogere maandelijkse hospitalisatierisico's werden gevonden bij jongens en kinderen met BPD. Het hospitalisatierisico nam toe met een kortere zwangerschapsduur en een lager geboortegewicht. Het hospitalisatierisico vertoonde sterke variatie binnen het RSV-seizoen, met een zeven maal hoger risico in december dan in oktober. Het risico nam sterker af met toenemende leeftijd bij kinderen zonder BPD.

Vervolgens ontwikkelden wij een besliskundig model dat op basis van vijf kenmerken van het kind (geslacht, zwangerschapsduur, geboortegewicht, aan- of afwezigheid van BPD, en kalenderleeftijd) en het gemiddelde seizoensbeloop van RSV-infecties in Nederland, het maandelijkse risico op ziekenhuisopname voor RSV schat. Alle zes voorspellers hadden een significante associatie met het maandelijkse risico op ziekenhuisopname. Leeftijd en het seizoenspatroon van RSV infecties waren de sterkste voorspellers. De prestatie van het model (in termen van discriminatie, calibratie en interne validiteit) was goed.

Wij concludeerden dat het mogelijk was om voor jonge kinderen op een betrouwbare wijze maandelijkse risico's op ziekenhuisopname voor RSV te voorspellen met

behulp van een besliskundig model met de voorspellers geslacht, zwangerschapsduur, geboortegewicht, BPD, leeftijd en seizoen. Met deze maandelijkse risico's kan de arts passieve immunisatie tegen RSV toepassen bij kinderen in de maanden waarin het risico op ziekenhuisopname het hoogst is.

Invloed van het seizoensbeloop van RSV-infecties op de validiteit van het besliskundig model

RSV-epidemieën vertonen een wisselend beloop tijdens verschillende seizoenen. Er zijn verschillen in het totaal aantal opgenomen kinderen per seizoen, de maand waarin de piek van de epidemie plaatsvindt en de verdeling van opnames over de verschillende maanden van het seizoen. Hierdoor kan de prestatie van het besliskundig model, dat gebruikt maakt van een gemiddeld seizoenspatroon, per seizoen duidelijke schommelingen laten zien. Men zou zelfs kunnen overwegen om een alternatief model te ontwikkelen, zonder seizoen als voorspeller.
Om de invloed van het seizoensbeloop van RSV-infecties op de validiteit van het besliskundig model te onderzoeken werd het oorspronkelijke model met vijf klinische voorspellers en seizoen als voorspeller (seizoensmodel) vergeleken met een model met alleen de vijf klinische voorspellers (klinisch model) (**hoofdstuk 2.2**). De prestaties van de modellen werden bepaald aan de hand van discriminatie (hoe goed kan het model kinderen met een hoog risico op ziekenhuisopname door RSV onderscheiden van kinderen met een laag risico) en calibratie (hoe goed komen de door het model geschatte risico's overeen met de in de groep kinderen waargenomen risico's). Verder bepaalden we de validiteit van de modellen in de verschillende seizoenen en de invloed van het wisselende seizoensbeloop. Zowel de discriminatie per seizoen als de gemiddelde discriminatie over drie seizoenen van het seizoensmodel was significant beter dan van het klinisch model. De calibratie van zowel het seizoensmodel als het klinisch model was echter matig in de verschillende seizoenen.
Wij concludeerden dat een besliskundig model met de voorspeller seizoen een beter discriminerend vermogen had dan een besliskundig model zonder de voorspeller seizoen. Echter, zowel het model met seizoen als het model zonder seizoen vertoonde verschillen in calibratie tussen de verschillende RSV-seizoenen, hetgeen mogelijk verbeterd kan worden door gebruik te maken van een dynamisch seizoen.

De kosten van ziekenhuisopname voor RSV bij jonge kinderen in Nederland

Werkelijke directe medische kosten van ziekenhuisopname met een ernstige RSV-infectie (polikliniekbezoek, diagnostiek, en therapie) werden bepaald vanuit een maatschappelijk perspectief (**hoofdstuk 3.1**). Hiervoor werden gegevens verzameld van alle jonge kinderen die in verband met een RSV-infectie waren opgenomen in de regio Zuidwest Nederland tijdens de RSV-seizoenen 1996-1997 t/m 1999-2000 (n=3.458), De gemiddelde kosten van ziekenhuisopname voor alle kinderen bedroegen €3.110. De kosten van ziekenhuisopname vertoonden een sterke relatie met de duur van ziekenhuisopname. Daardoor waren de kosten van ziekenhuisopname beduidend hoger voor ex-prematuren (tot €5.555 bij zwangerschapsduur ≤28 weken), kinderen met een laag geboortegewicht (tot €3.895 bij een geboortegewicht ≤2500 g), kinderen met BPD (€5.785), en zeer jonge kinderen (€4.730 bij kinderen opgenomen in de eerste levensmaand).

Op basis van deze gegevens werd een model ontwikkeld dat op basis van vier kenmerken van het kind (leeftijd, zwangerschapsduur, geboortegewicht en aan/of afwezigheid van BPD), voor een individueel kind schat wat de te verwachten kosten van ziekenhuisopname zouden zijn indien dit kind opgenomen zou worden met een RSV-infectie. De R^2 van dit model was 0.08, hetgeen aangeeft dat de kosten van ziekenhuisopname op individueel niveau sterk kunnen variëren rond de gemiddelde schatting.

Wij concludeerden dat de kosten van ziekenhuisopname voor RSV met name voor ex-prematuren, kinderen met BPD en jonge kinderen aanzienlijk waren en sterk samenhingen met de duur van ziekenhuisopname. Alhoewel het niet goed mogelijk was om verwachte individuele kosten van ziekenhuisopname voor RSV voor kinderen te voorspellen, was het wel mogelijk om verwachte gemiddelde kosten van ziekenhuisopname voor RSV voor kinderen te voorspellen op basis van vier kenmerken van het kind (leeftijd, zwangerschapsduur, geboortegewicht en aan/of afwezigheid van BPD). Deze voorspelde kosten kunnen gebruikt worden voor economische analyses van preventie van ernstige RSV-infecties.

Kosteneffectiviteitsanalyse van passieve immunisatie tegen RSV bij jonge kinderen in Nederland

Om de kosteneffectiviteit van passieve immunisatie tegen RSV in Nederland te bepalen werden schattingen van het risico op ziekenhuisopname (**hoofdstuk 2.1**), de effectiviteit van passieve immunisatie, en de directe medische (**hoofdstuk 3.1**) en

niet-medische kosten van ziekenhuisopname en immunisatie aan elkaar gerelateerd in een besliskundig model (**hoofdstuk 3.2**). Kosteneffectiviteit werd primair uitgedrukt in meerkosten per voorkomen ziekenhuisopname. In de analyse werd rekening gehouden met kenmerken die het risico en de kosten van ziekenhuisopname bepalen (geslacht, zwangerschapsduur, geboortegewicht, aan/of afwezigheid van BPD, leeftijd en seizoen). Hoewel de analyse resulteerde in kosteneffectiviteitratio's voor verschillende patiëntprofielen per seizoensmaand, werden specifieke kosteneffectiviteitratio's alleen gerapporteerd voor een kind dat gedurende het hele RSV-seizoen het hoogste risico liep om te worden opgenomen met een RSV-infectie; de referentiecasus (jongen, zwangerschapsduur ≤28 weken, geboortegewicht ≤2500 g, aanwezigheid van BPD, en een leeftijd van nul maanden bij aanvang van het RSV seizoen). De meerkosten per voorkomen ziekenhuisopname varieerden tussen €15.520 in december en €883.930 in oktober. Iedere maand waren de meerkosten per voorkomen ziekenhuisopname hoger voor kinderen met langere zwangerschapsduur en kinderen zonder BPD. De meerkosten per voorkomen ziekenhuisopname waren ook hoger voor meisjes en namen toe met een toename van het geboortegewicht en de leeftijd. Passieve immunisatie was altijd het meest kosteneffectief in december, maar was nooit kostenbesparend. Er werden univariate sensitiviteitsanalyses uitgevoerd voor de kosteneffectiviteitratio van de referentiecasus in de maand waarin passieve immunisatie het meest kosteneffectief was (december), om de gevoeligheid van de kosteneffectiviteitratio's te bepalen voor veranderingen in de risico-, effectiviteit- en kostenschattingen. De schattingen werden veranderd tussen een half en twee maal hun waarden in de hoofdanalyse en tussen de 95% betrouwbaarheidsintervallen van de waarden in de hoofdanalyse. Tevens werd onderzocht voor welke waarden van de schattingen de meerkosten per voorkomen ziekenhuisopname gelijk waren aan €0 (kostenneutraliteit). De kosteneffectiviteit van passieve immunisatie tegen RSV was het meest gevoelig voor veranderingen in de kosten van passieve immunisatie, gevolgd door variatie in het risico op ziekenhuisopname, effectiviteit van passieve immunisatie en kosten van ziekenhuisopname. Voor de referentiecasus kon passieve immunisatie tegen RSV leiden tot kostenbesparing indien de inkoopprijs van passieve immunisatie lager was dan €335, het maandelijks risico op ziekenhuisopname hoger was dan 20%, of een ziekenhuisopname meer dan €25.750 kostte. Zelfs als passieve immunisatie alle RSV ziekenhuisopnames zou voorkomen, zouden de meerkosten per voorkomen ziekenhuisopname van passieve immunisatie meer dan €2.645 zijn.

Wij concludeerden dat de kosteneffectiviteit van passieve immunisatie tegen RSV weliswaar sterk varieerde tussen kinderen met verschillende eigenschappen, maar dat de meerkosten per voorkomen ziekenhuisopname altijd hoog waren. Daarom adviseerden wij een beperkt gebruik van passieve immunisatie tegen RSV. De

inkoopprijs van passieve immunisatie dient verlaagd te worden om tot acceptabele kosteneffectiviteitsratio's te komen voor hoog risico kinderen in hoog risico maanden.

Kwaliteit van leven na ziekenhuisopname voor een RSV-infectie

De meeste economische analyses van passieve immunisatie tegen RSV laten zien dat de behaalde effecten gepaard gaan met hoge kosten. Op basis van deze resultaten lijkt een beperkt gebruik van passieve immunisatie aan te raden. Bij het maken van keuzes omtrent het gebruik en de vergoeding van passieve immunisatie dient men echter niet alleen te kijken naar kosten en klinische effecten, maar ook naar de effecten op het lichamelijk, psychisch en sociaal functioneren van kinderen en hun ouders. Hiervoor kan men gebruik maken van gezondheidsgerelateerde kwaliteit van leven (KvL).

Bij 50 kinderen hebben wij de KvL gemeten, ongeveer vier maanden nadat ze opgenomen waren geweest met een RSV-infectie. Hiervoor gebruikten wij de TNO-AZL Preschool Children Quality of Life (TAPQoL) methode (**hoofdstuk 4**). De gemiddelde TAPQoL scores van de RSV-kinderen werden vergeleken met gezonde, niet opgenomen kinderen. De RSV-kinderen scoorden significant lager op de meeste TAPQoL schalen, hetgeen een lagere KvL impliceert. Matig tot sterk negatieve effecten (Cohen's effect size: het relatieve verschil tussen de gemiddelden van twee groepen, gedeeld door de grootste standaarddeviatie van de twee groepen) werden gevonden voor de schalen 'longen' en 'motoriek'. Een sterk positief effect werd gevonden voor de schaal 'probleem gedrag'.

Verschillen in de ernst van de RSV-infectie (gebaseerd op de duur van opname, het gebruik van extra zuurstof, intensive care opname of beademing) hadden geen effect op de KvL. Kinderen die na het doormaken van de RSV-infectie longklachten (benauwdheid en/of piepen) bleven houden scoorden significant lager op de TAPQoL schalen 'slapen', 'eetlust' en 'longen', in vergelijking tot kinderen die geen longklachten hadden na de RSV-infectie. Met name voor de schaal 'longen' werd een groot negatief effect gevonden.

Wij vonden significante negatieve correlaties tussen de leeftijd van de RSV-kinderen bij het invullen van de TAPQoL lijst en scores op de schalen 'slapen', 'eetlust', 'longen', 'probleem gedrag' en 'angst'. Jongens scoorden significant lager op de schalen 'probleem gedrag' dan meisjes. De tijd tussen het doormaken van de RSV-infectie en het invullen van de TAPQoL, het geslacht en het opleidingsniveau van de ouder die de TAPQoL invulde, hadden geen effect op de scores.

Er werd een multivariate analyse verricht om bij de RSV-kinderen te kijken naar de associatie van geslacht en leeftijd van het kind, blijvende longklachten en duur van

ziekenhuisopname met de scores op de verschillende TAPQoL schalen. Jongens scoorden significant lager op de TAPQoL schaal 'probleem gedrag' dan meisjes en oudere kinderen scoorden significant lager op de TABQoL schaal 'angst' dan jongere kinderen. Kinderen met blijvende longklachten scoorden significant lager op de TAPQoL schalen 'slapen', 'eetlust' en 'longen' dan kinderen zonder blijvende longklachten. Kinderen die langer in het ziekenhuis waren opgenomen scoorden significant hoger op de TABQoL schalen 'slapen', 'eetlust' en 'maag' dan kinderen die korter in het ziekenhuis waren opgenomen.
Wij concludeerden dat enkele maanden na een ziekenhuisopname voor RSV kinderen een lagere KvL hadden dan gezonde, niet opgenomen kinderen. Negatieve effecten op de TABQoL schalen 'slapen', 'eetlust' en 'longen' werden primair veroorzaakt door blijvende periodes van benauwdheid en/of piepen.

Associatie van ernstige RSV-infectie met IL-4 en IL-4 receptor α polymorfismen

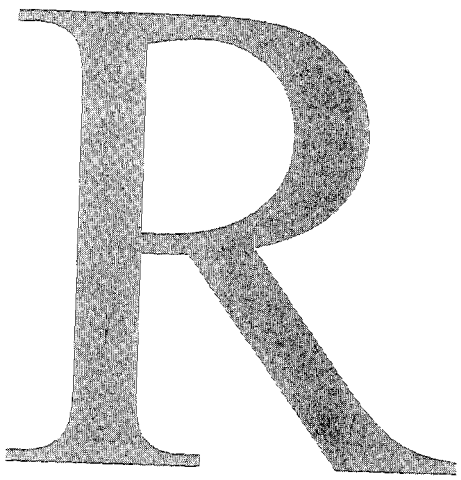
De ernst van RSV-infecties bij jonge kinderen varieert van een milde bovenste luchtweginfectie tot een ernstige lagere luchtweginfectie leidend tot respiratoire insufficientie en soms zelfs overlijden. Hoewel er meerdere factoren beschreven zijn die geassocieerd zijn met een ernstiger ziektebeloop, kunnen deze de variatie in ziekte-ernst niet geheel verklaren. Wellicht kan een gedeelte van de variatie in ziekte-ernst worden verklaard door genetische heterogeniteit van de immuunrespons van geïnfecteerde kinderen. De door T-cellen gemedieerde immuunpathologie, in het bijzonder een meer uitgesproken T helper type 2 (Th2) reactie, kan een belangrijke rol spelen bij de ontwikkeling van een ernstige RSV-infectie. Er wordt ook gesuggereerd dat er een relatie bestaat tussen het doormaken van een ernstige RSV-infectie en het krijgen van astma op latere leeftijd.
Wij bestudeerden de associatie tussen ernstige RSV-infecties en gain-of-function mutaties (veranderingen die leiden tot een verhoogde werking) in de genen die coderen voor het Th2 cytokine interleukine (IL)-4 (C-590T) en de IL-4 receptor α keten (IL-4Rα) (I50V en Q551R) (**hoofdstuk 5**). De resultaten van 207 kinderen die opgenomen zijn geweest met een ernstige RSV-infectie werden vergeleken met twee controle groepen. Een groep bestond uit de ouders van de opgenomen kinderen (voor de transmission/disequilibriumtest), de ander groep (n=447) bestond uit een gerandomiseerde steekproef uit de algemene populatie (voor de case-controltest). De resultaten werden ook geanalyseerd in een combinatie van deze twee testen (combinatietest).

Het IL-4 590T allel werd vaker dan verwacht gevonden bij kinderen die opgenomen zijn geweest met een ernstige RSV-infectie in de case-control en combinatietest. Zowel het IL-4 590T als het IL-4Rα R551 allel werden vaker gevonden bij kinderen die ouder waren dan 6 maanden ten tijde van hun RSV opname in vergelijking met kinderen die jonger waren dan 6 maanden ten tijde van opname en de controle groepen.
Wij concludeerden dat gain-of-function varianten van Th2 cytokinegenen een rol kunnen spelen bij ernstige RSV-infecties, met name na de zesde levensmaand.

Veiligheid en immunogeniciteit van een nieuw vaccin tegen RSV bij gezonde jong volwassen vrijwilligers

Omdat ernstige RSV-infecties veel ziektelast veroorzaken bij zuigelingen en jonge kinderen, is preventie van ernstige RSV-infecties zeer wenselijk. Wij bepaalden de veiligheid en immunogeniciteit van BBG2Na, een nieuw recombinant RSV subunit vaccin, in een gerandomiseerde, enkel-blind, placebo gecontroleerde studie bij 108 gezonde jonge vrijwilligers (**hoofdstuk 6**). De vrijwilligers werden gerandomiseerd om 1, 2 of 3 intramusculaire injecties (met een interval van vier weken) te krijgen van 10, 100 of 300 µg BBG2Na in aluminiumfosfaat, of fysiologisch zout (placebo). Bloedafnames werden verricht voor iedere injectie en vier en acht weken na de laatste injectie. Lokale en sytemische verschijnselen werden geregistreerd tot zes dagen na de injectie. De vrijwilligers werden ook gecontroleerd op verschijnselen van mogelijk RSV-gerelateerde bovenste luchtweginfecties (BLWI).
Er werden alleen milde lokale en systemische verschijnselen geobserveerd. Humorale IgG responsen met RSV-A, G2Na, BBG2Na en BB waren het hoogst in de 100 µg en 300 µg groepen. Van alle vrijwilligers die 100 µg of 300 µg BBG2Na ontvingen, werd een tweevoudig of hogere en viervoudig of hogere toename in G2Na-specifieke ELISA eenheden geinduceerd bij respectievelijk tot 100% en tot 57%. De daarmee overeenkomende RSV-A-specifieke responen waren respectievelijk 89% en 67%. Eén injectie was meestal voldoende om de maximale antilichaam respons op te wekken, die vervolgens gedurende acht weken stabiel bleef. Er werden geen RSV-B-specifieke antilichaam responsen gevonden. Na vaccinatie werd een significante toename in virus-neutraliserende (VN) antistoffen gevonden bij de 100 µg en 300 µg groepen. Tot 71% van de vrijwilligers had een tweevoudig of hogere toename van de VN antistoftiters. Er werden geen RSV-B-specifieke VN antistoffen gevonden na vaccinatie. De antilichaam responsen op twee muis long beschermende epitopen werden sterk gestimuleerd na vaccinatie. Vier vrijwilligers hadden een RSV infectie doorgemaakt, waarvan er één gepaard ging met BLWI verschijnselen.

Wij concludeerden dat BBG2Na veilig was, goed werd verdragen en zeer immunogeen was in gezonde jonge RSV-seropositieve vrijwilligers.



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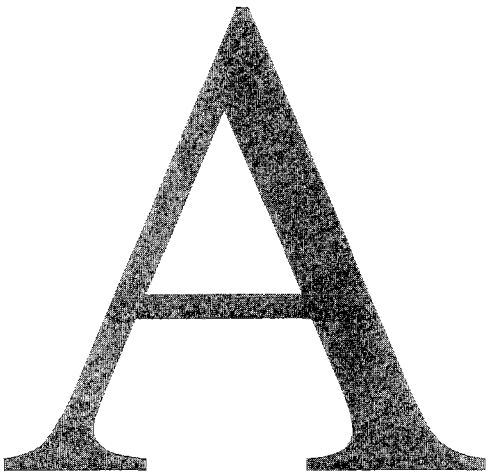


Appendix A:
prediction rule for the monthly RSV hospitalisation risk

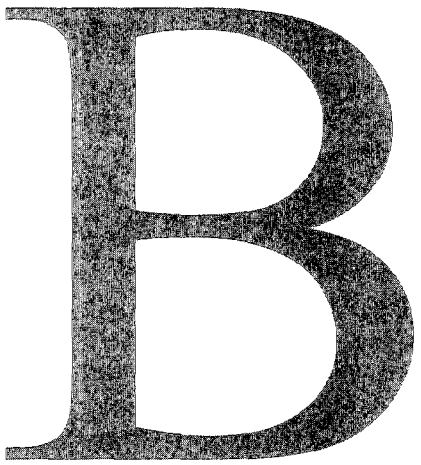
Prediction rule for the monthly RSV hospitalisation risk

The monthly RSV hospitalisation risk can be calculated as:

Risk = (1 / [1 + exp(-lp)]),

with lp = -7.013 + 0.335**gender* + 0.545**bw1* + 0.234**bw2* + 1.150**ga1* + 1.042**ga2* + 0.849**ga3* + 0.447**ga4* + 1.398**aged* - 0.075**age***bpd* - 0.207**age***(1-bpd)* + 0.851**s*,

where *gender* is 1 if male, 0 if female; *bw1* is birth weight ≤ 2500 g, *bw2* from 2501 to 3000 g; *ga1* is gestational age ≤ 28 weeks, *ga2* from 29 to 32 weeks, *ga3* from 33 to 34 weeks, *ga4* from 35 to 36 weeks; *aged* is 0 if age = 0 months, 1 if age > 0 months; *age* in months (age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks); *bpd* is 1 if BPD yes, 0 if BPD no; *s* is seasonal month at risk with the values: -1.40 for October, 0.53 for November, 1.36 for December, 0.83 for January, 0.53 for February, -0.22 for March and -1.63 for April.



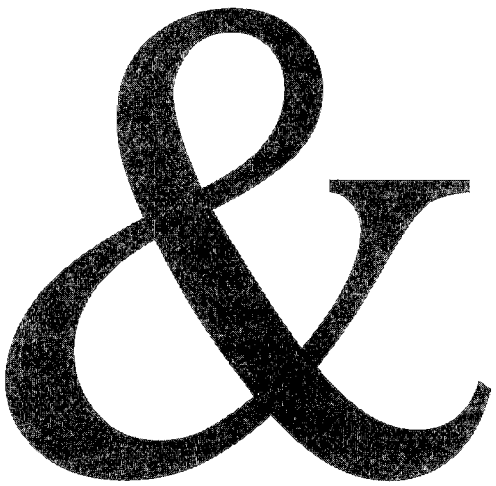
Appendix B: linear regression model for anticipated RSV hospitalisation costs

Regression model for anticipated RSV hospitalisation costs

The anticipated hospitalisation costs of groups of children at-risk for RSV hospitalisation can be estimated as:

$$\text{Costs} = 2274 + 1226*ga1 + 313*ga2 + 591*bw1 + 284*bw2 + 2192*(1-bpd)*(1/age+1) + 14344*bpd*(1/(age+1),$$

where *ga1* is gestational age ≤ 28 weeks, *ga2* from 29 to 34 weeks; *bw1* is birth weight ≤ 2500 g, *bw2* from 2501 to 3000 g; *bpd* is 1 if BPD yes, 0 if BPD no; *age* in months (age of children born prior to 38 weeks of gestation was corrected to a postconceptional age of 38 weeks).



Dankwoord
Curriculum Vitae
List of co-authors

Dankwoord

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Curriculum Vitae

Edwin Rietveld was born in Rotterdam, on April 3, 1969. He passed his secondary school exam (VWO) at the 'OSG Van Oldenbarnevelt' in Rotterdam in 1987. From 1987 to 1988 he studied medical information technology at the University of Leiden. In 1988 he started his medical training at the medical faculty of the Erasmus University, Rotterdam. During his study he participated in the Netherlands Medical Students' International Committee and completed a clinical internship on infectious diarrhoeal disease at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh (supervisor: dr. O. Massee Bateman). He also participated in studies at the department of Neonatology of the Erasmus MC-Sophia, Rotterdam (supervisor: dr. C. Koopman-Esseboom; head: prof. dr. P.J.J. Sauer) and the department of Obstetrics of the Erasmus MC, Rotterdam (supervisor: dr. W. Visser; head: prof. dr. H.C.S. Wallenburg). After obtaining his medical degree in 1996, he worked as a resident at the department of Internal Medicine of the Ikazia Ziekenhuis Rotterdam (head: dr. R.J.Th. Ouwendijk) until 1998. From 1998 to 2003 he worked as a research fellow at the department of Paediatric Infectious Diseases and Immunology of the Erasmus MC-Sophia, Rotterdam (supervisor: dr. H.A. Moll; head: prof. dr. R. de Groot). During this period all research presented in this thesis was performed in close collaboration with the department of Public Health of the Erasmus MC, Rotterdam, the department of Paediatrics of the Wilhelmina Childrens' Hospital, Utrecht, and the Laboratory of Health Effects Research of the National Institute for Health and the Environment, Bilthoven. Since July 2003 he is working as a resident at the department of Paediatrics of the Erasmus MC-Sophia, Rotterdam (head: prof.dr. H.A. Büller; prof.dr A.J. van der Heijden).

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